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Research Article
Phytochemical Screening, Analgesic Effect and Anti-inflammatory Activity of Crude Methanolic Stem Bark Extract of *Acacia nilotica* (Linn.)

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Abstract

Background and Objective: Inflammation is increasingly found to be involved in the development of several chronic diseases. Although steroidal anti-inflammatory drugs and NSAIDs are currently used to treat inflammation, these drugs have not been entirely successful and are accompanied by unexpected side effects. The aim of this study was to carry out phytochemical screening and to evaluate analgesic effect and anti-inflammatory activities of the methanolic stem bark extract of *Acacia nilotica*. **Materials and Methods:** Phytochemical screening was done using standard methods, analgesic effect was evaluated using acetic acid induced writhing test, while the anti-inflammatory activity of the extract was done using formalin induced hind paw edema model. **Results:** The results for the phytochemical screening showed the presence of all the phytochemicals screened except steroids. The stem bark extract at the tested doses produced a significant percentage inhibition of the acetic acid induced abdominal constriction of 41.38, 55.17 and 67.24%, respectively. The three doses of the extract also significantly ($p<0.05$) inhibited formalin induced paw edema in a time and dose dependent manner. **Conclusion:** The result of the present study signified the potential of *Acacia nilotica* stem bark as a source of therapeutic agents, which might provide leads in the ongoing search for analgesic and anti-inflammatory agents from plants.

Key words: *Acacia nilotica*, anti-inflammatory activity, therapeutic agents, analgesic effect, anti-inflammatory drugs, paw edema

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Inflammation is an orchestrated biological process, induced by microbial infection or tissue injury. A major trigger of inflammation is the recognition of microbes by specific receptors of the innate immune system, which play a crucial role in the induction of early signals initiating and establishing the inflammatory setting[1]. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue.

The activities of a number of regulatory enzymes (e.g., protein tyrosine kinases, protein kinase C, phosphodiesterase, phospholipase A_2, lipoxygenases and cyclo-oxygenase) are essential to inflammation and the immune response. These enzymes are central to the activation of endothelial cells and numerous other specialized cells involved in inflammation. Cyclo-oxygenase (COX) is an enzyme that plays a very important role as inflammatory mediator and is involved in the release of arachidonic acid, which is a precursor for biosynthesis of eicosanoids like prostaglandins and prostacyclin[2].

Inflammation is increasingly found to be involved in the development of several chronic diseases such as arteriosclerosis, obesity, diabetes, neurodegenerative diseases and even cancer[3-6]. Although steroidal anti-inflammatory drugs and NSAIDs are currently used to treat acute inflammation, these drugs have not been entirely successful in curing chronic inflammatory disorders while such compounds are accompanied by unexpected side effects. Therefore, there is an urgent need to find safer anti-inflammatory compounds[7]. Traditional medicine has used extracts of different plants for the treatment of a wide variety of disorders including acute and chronic inflammation.

Acacia nilotica (L.) Willd. ex Del. also known as Gum Arabic tree, Babul, Egyptian thorn or prickly Acacia is multipurpose tree. Acacia species are widespread in Africa and Asia and occurs in Australia and Kenya. Indian gum Arabic tree is found in well-watered Sahelian and Sudanian savannas to the southern Arabian Peninsula, East Africa and in the Gambia, the Sudan, Togo, Ghana and Nigeria. It is widely cultivated in the Indian sub-continent and also found on lateritic soil in the Himalayan foothills in India[8]. It is a pioneer species, relatively high in bioactive secondary compound and is important for a variety of functions. It is economically used as a source of tannins, gums, timber, fuel and fodder. In Nigeria, the plant is traditionally used to treat infections such as diarrhoea, dysentery, oxidative stress, intestinal pains, ulcer, cold, haemorrhages, tuberculosis, congestion, coughs and fever[9,10].

Although steroidal anti-inflammatory drugs and NSAIDs are currently used to treat inflammation, these drugs have not been entirely successful and are accompanied by unexpected side effects. Therefore, there was an urgent need to find safer anti-inflammatory compounds. The aim of this study was to carry out phytochemical screening and to evaluate analgesic effect and anti-inflammatory activities of the methanolic stem bark extract of *Acacia nilotica* (Linn.).

MATERIALS AND METHODS

Plant collection and authentication: The fresh disease-free stem bark of *Acacia nilotica* was carefully and separately collected from Bodinga, Sokoto state, Nigeria around May, 2016. It was identified and authenticated by a Botanist at the Biological Sciences Department, Usmanu Danfodiyo University, Sokoto, Nigeria. The plant sample was identified as *Acacia nilotica* (Linn.) with voucher number UDUH/ANS/0247. The sample was shed-dried, ground and kept in air-tight containers till further use.

Location and duration of the research: The research was carried out at Pharmacognosy and Pharmacology Laboratories of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The research was conducted in a 9 months period, from May 2016 to March, 2017.

Preparation of plant extracts: The methanolic crude extract was prepared by soaking a sample (50 g) of powdered leaves material in 90% methanol (300 mL) for 72 h. The extract was filtered using clean cloth and Whatman No. 1 filter paper. The filtrate was concentrated in vacuum at 30°C and stored in sterile sample containers at 4°C until further use.

Phytochemical screening: The extract was screened for the presence of major phytochemicals using standard qualitative methods as described previously[11-13]. The plant extract was screened for the presence of saponins, tannins, alkaloids, flavonoids, terpenoids, glycosides and steroids.

Experimental animals: Female albino rats, weighing 160-200 g were used in this toxicity study. They were obtained from the Animal Research Centre (ARC) of the Ahmadu Bello University (ABU), Zaria, Nigeria. The rats were kept in the Animal House of the Department of Pharmacology, Faculty of Pharmacy, Usmanu Danfodiyo University, Sokoto, Nigeria, where they were acclimatized to standard laboratory conditions for 7 days. They were housed in groups and

maintained on 12 h light:dark cycle, with standard pellet diet and water *ad libitum.* An approval was obtained from the Animal Ethics Committee, Usmanu Danfodiyo University, Sokoto, Nigeria. The approval number assigned was UDUS/AEC/0436. The institutional animal ethical guidelines were strictly observed.

Analgesic studies: The acetic acid induced writhing test in rats was used to test for analgesic effect[14]. The rats were divided into five groups of four rats each. The groups were treated thus: Group 1 received 10 mL kg^{-1} b.wt., distilled water (control) intra-peritoneal, group 2 received 10 mg kg^{-1} b.wt., of Piroxicam intra-peritoneal, group 3, 4 and 5 received 150, 300 and 600 mg kg^{-1} b.wt., of crude methanolic extract of *Acacia nilotica*, respectively. About 30 min later, the rats in the five groups were treated with 1% acetic acid intra-peritoneal. About 5 min later, the rats were placed in individual cage and the number of abdominal contractions counted for each rat in a 10 min period. Inhibition of writhing (%) was calculated using the expression:

$$\text{Inhibition}(\%) = \frac{\text{Mean number of writhing (control)} - \text{Mean writhing (treated)}}{\text{Mean number of writhing (control)}} \times 100$$

Anti-inflammatory studies:acute inflammation: The formalin (2.5%) induced inflammation was used in this study, as described by Winter *et al.*[15]. The increase in paw diameter (edema) was measured using vernier calliper. The difference in weight of the right hind paw and the left hind paw indicates inflammation. The hind paw volume (edema) was measured immediately before 0 h and after 1, 2, 3, 4 and 5 h following formalin injection using vernier caliper. The percentage inhibition was calculated from the expression:

$$\text{Inhibition}(\%) = \frac{\text{Mean oedema volume (control)} - \text{Mean oedema volume (treated)}}{\text{Mean oedema volume (control)}} \times 100$$

Statistical analysis: The results were expressed as the mean±SD (n = 4). The results were statistically analyzed using version 20 of the IBM-SPSS statistical program (IBM Corp., Armonk, NY, USA). One-way ANOVA was used followed by Duncan's test for parametric multiple comparisons between the control and the treatment groups. Differences were considered significant at $p<0.05$.

RESULTS

Phytochemical screening: The result of the phytochemical screening of the methanolic stem bark extract of *Acacia nilotica* is presented in Table 1. The result revealed the presence of all the phytochemicals screened except steroids.

Analgesic effect of crude methanolic stem bark extract of *Acacia nilotica*: The results of the inhibition of the acetic acid induced abdominal constriction (writhes) in rats are presented as mean±SD. The extract at the three doses of 150, 300 and 600 mg kg^{-1} b.wt., showed a significant ($p<0.05$) percentage inhibition of 41.38, 55.17 and 67.24%, respectively compared to negative control (Table 2).

Anti-inflammatory activity of crude methanolic stem bark extract of *Acacia nilotica*

Effect of the extract on edema volume in rats: The results of the formalin induced anti-inflammation activity showed a significant ($p<0.05$) inhibition of hind paw edema in a dose and time dependent manner compared with the control (Table 3). Piroxicam as a positive control showed significant ($p<0.05$) reduction of hind paw edema in a time dependent manner compared to negative control (Distilled water).

Anti-inflammatory (inhibition %) effect of the extract on experimental animals: The extract at the three doses of 150, 300 and 600 mg kg^{-1} b.wt., showed a significant ($p<0.05$)

Table 1: Phytochemical constituents present in the methanolic stem bark extract of *A. nilotica*

Phytochemicals	Results
Flavonoid	+
Tannins	+
Saponins	+
Glycosides	+
Alkaloids	+
Steroids	ND
Terpenoids	+

+: Present, ND: Not detected

Table 2: Effect of stem bark extract of *A. nilotica* on acetic acid induced writhing response

Treatment (mg kg^{-1})	No. of abdominal writhing	Inhibition (%)
Dist. water (10 mL kg^{-1})	14.5±0.58	0.00
Piroxicam (10 mg kg^{-1})	4.00±0.82*	72.41
Extract		
150 mg kg^{-1}	8.50±0.58*	41.38
300 mg kg^{-1}	6.50±0.58*	55.17
600 mg kg^{-1}	4.75±0.50*	67.24

Data presented as mean±SD (n = 4), Dist. Water: Distilled water, *Significantly different from the control ($p<0.05$)

Table 3: Effect of the extract of *Acacia nilotica* on edema volume in rats

	Volume (edema)					
	0	1st	2nd	3rd	4th	5th
Treatments (mg kg^{-1})	Time (h)					
Dist. Water	8.75±0.36	8.83±0.33	8.99±0.24	9.12±0.27	9.20±0.11	9.38±0.23
Pirox. (10)	8.65±0.06	7.97±0.51*	7.85±0.11*	7.61±0.38*	7.51±0.34*	7.11±0.10*
150	8.70±0.27	8.23±0.10	8.00±0.10*	7.91±0.07*	7.79±0.31*	7.70±0.23*
300	8.61±0.20	8.21±0.16	7.90±0.22*	7.47±0.36*	7.26±0.50*	6.93±0.32*
600	8.61±0.22	8.16±0.21*	7.86±0.30*	7.29±0.26*	6.93±0.62*	6.36±0.42*

Data presented as mean±SD (n = 4), Dist. Water: Distilled water, Pirox.: Piroxicam, *Significantly different from the control (p<0.05)

Table 4: Anti-inflammatory activity (inhibition %) of extract on experimental animals

	Inhibition (%)				
	1st	2nd	3rd	4th	5th
Treatments (mg kg^{-1})	Time (h)				
Dist. Water	0.00	0.00	0.00	0.00	0.00
Pirox. (10)	9.74	12.68	16.56	18.37	24.20
150	6.80	11.01	13.27	15.33	17.91
300	7.02	12.12	18.10	21.10	26.12
600	7.59	12.56	20.10	24.67	32.20

Values mentioned (17.91, 26.12 and 32.20%) were that of the final percentage inhibition i.e., at the final (5th) hour level of inhibition

percentage inhibition of formalin induced hind paw edema of 17.91, 26.12 and 32.20% (at final 5th h level), respectively compared to negative control (Table 4). The extract at the three doses (150, 300 and 600 mg kg^{-1} b.wt.) showed strong anti-inflammatory activity similar to the effect of piroxicam in rats, especially at the 5th hour following formalin injection.

DISCUSSION

The result of the phytochemical screening of the crude methanolic stem bark extract of *A. nilotica* (Table 1) revealed the presence of all the screened phytochemicals except steroids. Several other studies have reported similar phytochemicals from this plant samples[16,17], these supported the data reported in this research. These compounds are known to be biologically active[18,19] and thus may contributed to the observed analgesic and anti-inflammatory activities in this plant.

For instance, flavonoids posses a wide range of biological activities which include anti-microbial, anti-inflammatory, analgesic, anti-allergic effects, cytostatic and anti-oxidant properties[20]. Flavonoids' ability to scavenge hydroxyl radicals, superoxide anion radicals and lipid peroxyl radicals signifies many of the health-promoting functions of flavonoids in organisms which are important for prevention of diseases associated with oxidative damage of membranes, proteins and DNA[21]. Tannins act by iron deprivation, hydrogen bonding or specific interaction with proteins such as enzymes, cell envelopes and complex formation with polysaccharides[22,23]. Herbs that have tannins as their component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery[22].

Saponins are known to produce inhibitory effects on inflammatory processes. They were also reported to possess antibacterial property. Their mode of action was attributed to their ability to cause leakage of proteins and certain enzymes from bacterial cells[24,25]. Alkaloids have been associated with medicinal uses for centuries. In addition, alkaloids possess anti-inflammatory, anti-asthmatic and anti-anaphylactic activities with consequences of altered immunological status *in vivo*[26].

The acetic-acid induced writhing test is one of the widely used tests in analyzing drugs and plants extracts for their analgesic effects. It is widely used for analgesic evaluation[27,28]. The administration of acetic acid intra-peritoneally induces the release of prostaglandins and sympathomimetic system midiators like PGE_2 and $PGF_{2\alpha}$ and their levels will be increased in the peritoneal fluid of the acetic acid induced rats. Protanoids and lipoxygenase compounds have also been found in the peritoneal fluid following acetic acid injection[29]. The results of the present study revealed that the stem bark extract of *A. nilotica* significantly ($p<0.05$) reduced the number of acetic acid induced writhing in rats in dose dependent manner. The highest inhibition of 67.24% was observed at the highest dose of 600 mg kg^{-1} b.wt. which is almost closer to the percentage inhibition of 72.41% observed in peroxicam (a standard analgesic drug) at 10 mg kg^{-1} b.wt. (positive control). This indicated an analgesic effect of the

extract. This effect may be due to either the action of the extract on visceral receptors sensitive to acetic acid, inhibition of the synthesis of algogenic substances or the inhibition of the transmission of painful stimuli[29,30].

Formalin-induced rat paw oedema is a suitable model commonly used for the anti-inflammation studies in experimental animals. Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants[31]. There are two major stages in an inflammatory reaction that contribute to the associated symptoms and tissue injury. In general, the first (early) phase involves inflammation mediated by the release of histamine and serotonin and increased synthesis of prostaglandins in the vicinity of the damaged tissues. The second (late) phase is the result of the release of kinins mainly prostaglandins[32].

There were also evidences that compounds inhibiting the formalin induced oedema were effective in inhibiting the enzyme cyclo-oxygenases[33]. Based on these reports, the inhibitory effect of *A. nilotica* extract on formalin-induced inflammation could be mediated via this mechanism. Similar anti-inflammatory effects were reported with other species of the Acacia genus[34,35]. Chemical studies on other Acacia species had led to the isolation of triterpenoidal saponin from *Acacia auriculiformis*[36] and phenolic compounds from the flowers and leaves of *A. nilotica*[37,38]. Saponins and flavonoids have previously been reported to have anti-inflammatory activities[39,40].

Such compounds may be responsible in part for the described anti-inflammatory activity of *A. nilotica* extract. On the other hand it was reported that free radicals are involved in the inflammatory process[41]. Different parts of *A. nilotica* were reported to possess anti-oxidant properties[42,43]. Therefore the anti-oxidant property of *A. nilotica* might have a beneficial role in its anti-inflammatory activity.

CONCLUSION

According to the present study, it can be concluded that the crude methanolic extract of *A. nilotica* stem bark possesses analgesic and anti-inflammatory effects, justifying its wide use in folklore medicine for the treatment of inflammation conditions. Further investigations are required to isolate the active principles present in the extract and to determine their exact mechanism of action.

SIGNIFICANCE STATEMENT

The screening of plant extracts has had an impressive history of identifying active agents. The choice of the stem bark of *Acacia nilotica* as the plant part of interest in this work was based on its vast medicinal importance among traditional medicine practitioners in northern Nigeria to treat infections such as intestinal pains, ulcer, cold, haemorrhages, coughs and fever. The bioactivity results of *Acacia nilotica* stem bark provided preliminary scientific justification for the traditional medicinal uses of this ethno remedy, an important step towards its acceptance and development as alternative therapeutic agent in the management of pain and inflammation. The findings would help researchers in the area of isolation and purification of anti-inflamatory compounds important for possible anti-inflammatory drug development.

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