



Preliminary Investigation of the Possible Anti-inflammatory and Antioxidant Activities of Extract of *Callichilia subsessilis* Stapf. (Apocynaceae)

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ARTICLE INFO

Article history:

Received 06 December 2017

Revised 26 December 2017

Accepted 01 January 2018

Published online 07 January 2018

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ABSTRACT

Callichilia subsessilis (Apocynaceae) is commonly found along the coast of West Africa. In South Eastern Nigeria, it is believed to be useful for medicinal purposes and mostly used by traditional bone setters especially in Ozuitem of Abia state. The antioxidant, anti-inflammatory and antinociceptive potential of extract of *Callichilia subsessilis* leaf were investigated. Cold maceration method was used in the extract preparation. The scavenging of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was used in the evaluation of antioxidant activity. Formalin- and egg albumin-induced paw oedema was used in the anti-inflammatory test while acetic acid-induced writhing and tail flick test were used in the antinociceptive test. The extract was dissolved in 5 % dimethyl sulfoxide (DMSO) and was tested at doses of 50, 100 and 200 mg kg⁻¹. The negative control group received 5 mL kg⁻¹, 5 % DMSO (vehicle) while acetyl-salicylic acid (aspirin) (200 mg kg⁻¹) and pentazocine, 5 mg kg⁻¹ (tail flick test) were used as the reference drugs. The extract produced concentration-dependent antioxidant activity that competes with ascorbic acid. The extract significantly ($P < 0.05$) inhibited formalin- and egg albumin-induced paw oedema in a dose- and time-dependent manner when compared with the vehicle-treated group. The extract significantly ($P < 0.05$) reduced pain sensation in both antinociceptive models used. The activities of the extract (100 mg kg⁻¹) were comparable with the reference drugs used in all the models. In conclusion, this study provided the pharmacological basis for the use of *Callichilia subsessilis* in the traditional management of inflammatory conditions.

Keywords: *Callichilia subsessilis*, anti-inflammatory, antinociceptive, paw oedema, pentazocine, writhing reflex.

Introduction

Medicinal plants are used worldwide in the management of diseases and raw materials for pharmaceutical companies.¹ Medicinal plants and its derivatives represent about 50% of drugs used in clinical practice.² World Health Organization (WHO) estimated that 80% of the human populations in developing countries of the world rely on traditional medicine for their primary healthcare and significant number of traditional medicine preparations consist of plant extract.^{3,4} The use of medicinal plants has formed part of the national health care system of several countries, such as China, Nigeria, India, Cameroon, Congo, Gambia, etc.^{1,5} Inflammation is one of the clinical conditions that is commonly managed in traditional medicine with herbal products.⁶

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Citation: Onoja SO, Ikwuagwu EO, Ezeigbo II, Madubuike KG, Omeh YN, Anaga AO, Ezeja MI. Preliminary Investigation of the Possible Anti-inflammatory and Antioxidant Activities of Extract of *Callichilia subsessilis* Stapf. (Apocynaceae). Trop J Nat Prod Res. 2018; 2(1):34-37. doi.org/10.26538/tjnpr/v2i1.7

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Callichilia subsessilis (Benth.) Stapf is a member of the family Apocynaceae. It is called “Aba-nua” in Ghana, “Vaimi” in Sierra Leone and “Mkpiri” in most parts of South Eastern Nigeria.⁷ It is commonly found along the coast of West Africa. In South Eastern Nigeria, it is believed to be useful for medicinal purposes and mostly used by traditional bone setters especially in Ozuitem of Abia state. The leaves infusion or decoction is luxuriously applied topically to the fracture site in the traditional management of bone fractures (oral communication). It is believed to alleviate any inflammatory processes involved with injuries such as bone fractures. However, till date, there is dearth of information on the medicinal values and chemical composition of *C. subsessilis* leaves. Though, other member of the genus like *C. stenopetala* contain bioactive compounds such as alkaloids, saponins, flavonoids and glycoside and its antimalarial, antinociceptive and antioxidant activities have been documented.⁸ Therefore, this study investigated the antioxidant and anti-inflammatory activities of extract of *Callichilia subsessilis*.

Materials and Methods

Plant collection and extract preparation

Fresh leaves of *Callichilia subsessilis* were collected from Ozuitem, Bende Local Government Area of Abia State, Nigeria in April 2016 and authenticated by Mr. Ndukwe Ibeh, a taxonomist with the College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike (MOUUAU). A voucher specimen (MOUUAU/VPP/2016/20) was deposited at the herbarium of the Department of Veterinary Physiology and Pharmacology, MOUUAU.

The leaves were briskly washed in tap water and sorted for any debris. Thereafter, they were initially dried for 24 h, in a laboratory oven at 40 °C, then air-dried on the laboratory bench before grinding into coarse powder with a manual blender. The coarse powder was soaked in 80% methanol in distilled water at room temperature for 48 h and vigorously shaken every 3 h. Thereafter, the extract was filtered with Whatmann No.1 filter paper into a beaker and filtrate concentrated in a hot air oven at 40°C, and labeled 'CSE' and stored in a refrigerator at 4°C until required for the experiment.

Animals

Matured male albino Wistar rats (100 – 117 g) obtained from the Laboratory Animal Unit of Department of Veterinary Physiology and Pharmacology, MOUAU were used for this study. The rats were kept in a well-ventilated laboratory animal house, under environmental temperatures of 27 ± 2°C, with 12 h natural light/darkness cycle. The rats were allowed access to drinking water and feed (Vital Feed, Nigeria) *ad libitum*, except when fasting was desired. The experimental procedures were carried out in accordance with the Institute for Laboratory Animal Research ethical guidelines governing the use of laboratory animals for experiments.⁹ The experimental procedures were approved by the institutional ethical committee.

Acute toxicity

The up and down procedure with dose limit of 2000 mg kg⁻¹ was adopted. Ten (10) rats were used for the study.¹⁰ Five rats were given 2000 mg kg⁻¹ of CSE and the rest were given an equal volume of 5% dimethyl sulfoxide (DMSO) orally. The rats were served feed and water *ad libitum* and observed for signs of acute toxicity and death.

Phytochemical screening

The extract was subjected to phytochemical screening using standard methods.¹¹

Antioxidant study

Free radical scavenging assay

The free radical scavenging activity of the extract was analyzed by the 1,1-diphenyl-2-picryl hydrazyl (DPPH) Assay using spectrophotometer as described by Ezeja *et al.*¹² The extract was tested at 25, 50, 100, 200 and 400 µg mL⁻¹ and were compared with ascorbic acid.

Anti-inflammatory study

Formalin-induced paw oedema

The modified formalin-induced paw oedema as described by Ezeja *et al.*,¹² was used in this experiment. Twenty-five (25) male albino Wistar rats were assigned to 5 groups (A-E) (n=5) and were fasted for 16 h. The rats were treated as follows: group A received 5 mL kg⁻¹ of 5% DMSO (Vehicle), group B received 200 mg kg⁻¹ acetylsalicylic acid (aspirin) while groups C, D and E received 50, 100 and 200 mg kg⁻¹ CSE, respectively. One-hour post-treatment, 0.1 mL of 1% formalin in normal saline was injected into the subplantar space of the right hindlimb. Volume displacement method was used in the measurement of paw volume. The left paw was used as the control for each animal. The right paw volume was measured at 1, 2, 3 and 24 h post oedema induction.

Change in paw vol. (ΔV) = *Right paw volume* – *Left paw volume*

$$\% \text{ inhibition} = \frac{\text{control } \Delta V - \text{test } \Delta V}{\text{control } \Delta V} \times \frac{100}{1}$$

Egg albumin-induced paw oedema

The modified egg albumin-induced paw oedema as described by Udo *et al.*¹³ was used in this experimental protocol. Another set of 25 male albino Wistar rats were randomly assigned to 5 groups (A-E), (n = 5) and were treated as earlier described. One-hour post-treatment, 0.1 mL of raw chicken egg albumin was injected into the subplantar spaces of the right paw. The right paw volumes were measured at 1, 2, 3, 5 and 24 h post oedema induction. The change in paw volume and percent inhibition were calculated as earlier stated.

Acetic acid-induced writhing

The peripheral antinociceptive activity of the test extract was evaluated with acetic acid-induced writhing. Another set of 30 male mice were randomly assigned to 5 groups (A-E), (n = 6) and were treated as earlier described. One-hour posttreatment, 10 mL/kg of 0.7% acetic acid solution

in normal saline was administered intraperitoneally to all the mice. Thereafter the writhing reflex was counted for 30 min.¹⁴

$$\% \text{ inhibition} = \frac{\text{mean writhing of control} - \text{mean writhing of test}}{\text{mean writhing of control}} \times \frac{100}{1}$$

Tail-flick test

The tail-flick test was used to determine the central antinociceptive response of the test extract. Another batch of 30 male albino Wistar rats were assigned to 5 groups (n = 6). The rats were fasted for 16 h and treated as earlier stated, but 5 mg kg⁻¹ pentazocine was used as reference drug. One-hour post treatment, 3 cm of the tip of the rat tails were submerged in a water bath maintained at 55 ± 1°C. The pain reaction time (PRT) of each rat was recorded with the aid of a stopwatch.¹⁵

$$\% \text{ increase in PRT} = \frac{\text{PRT of test} - \text{PRT of control}}{\text{PRT of control}} \times \frac{100}{1}$$

Statistical analysis

Results were expressed as mean ± standard deviation and were analyzed using one-way analysis of variance (ANOVA) and p-values < 0.05 were considered statistically significant.

Results and Discussion

Callichilia subsessilis extract (CSE) possessed strong activity against inflammatory processes in all the models employed in this study. These significant activities against inflammatory stimuli were both dose- and time-dependent, and compared favorably with commercially available anti-inflammatory medications used as controls. Furthermore, it was observed to possess appreciable antioxidant activity using the DPPH assay; and could be linked to the presence of the phytochemicals in *C. subsessilis* extract. The extract was well tolerated by the rats; no clinical signs of toxicity or death were observed. The LD₅₀ of the extract was greater than 2000 mg kg⁻¹. The extract showed the presence of alkaloids, saponins, flavonoids, terpenes, glycosides and tannins. CSE produced significant (P < 0.05) concentration-dependent increase in antioxidant activity. The antioxidant activity of CSE at 400 µg mL⁻¹ concentration was comparable with ascorbic acid (Figure 1). Some flavonoids, saponins and glycosides of herbal origin have been previously associated with both anti-inflammatory and antioxidative activities.^{16,17}

In both the formalin- and egg albumin-induced paw oedema, aspirin and CSE (50, 100 and 200 mg kg⁻¹) elicited significant (P < 0.05) dose- and time-dependent inhibition of paw oedema in treated groups when compared with vehicle treated group. At 3 h post induction, aspirin and CSE 50, 100 and 200 mg kg⁻¹ produced 44, 40, 36 and 58% inhibition of paw swelling, respectively when compared with vehicle-treated group (Table 1). At 5 h post induction, the mean increase in paw volume of the vehicle, aspirin and CSE (50, 100 and 200 mg kg⁻¹) treated groups were 0.81, 0.46, 0.51, 0.44 and 0.51 mL, respectively (Table 2). Aspirin and CSE (50, 100 and 200 mg kg⁻¹) treatment produced a significant (P < 0.001) decrease in the writhing when compared with vehicle-treated group. The percentage inhibition of writhing of the aspirin and CSE 50, 100 and 200 mg kg⁻¹ treated groups were 86.44, 26.69, 69.05 and 50.00%, respectively, when compared with the vehicle treated group in the acetic acid-induced writhing (Table 3). In the tail flick test, pentazocine (5 mg kg⁻¹) and CSE (50 and 200 mg kg⁻¹) elicited significantly (P < 0.05) increased pain reaction time (PRT) in the treated groups when compared with vehicle-treated group. The pentazocine (5 mg kg⁻¹), and CSE 50 and 200 mg kg⁻¹ increased the PRT by 34.74, 37.89 and 34.21%, respectively in the treated group when compared to vehicle-treated group (Table 4).

The extract elicited both peripheral and central antinociceptive activities. The acetic acid-induced writhing reflex evaluated the peripheral antinociceptive activity while tail flick model evaluated the central antinociceptive potentials.^{18,19} The possible mechanism of the central antinociceptive effect could be the elevation of the pain threshold in the hypothalamus and the inhibition of substance P.²⁰ The anti-inflammatory and antinociceptive activities of CSE might be through the inhibition of cyclooxygenase activity and the migration of inflammatory mediators; substance P and cytokines into the sites of inflammation.¹⁵ Acetyl salicylate irreversibly inhibits the activities of cyclooxygenase which catalyze the biosynthesis of prostaglandins from arachidonic acid.²¹

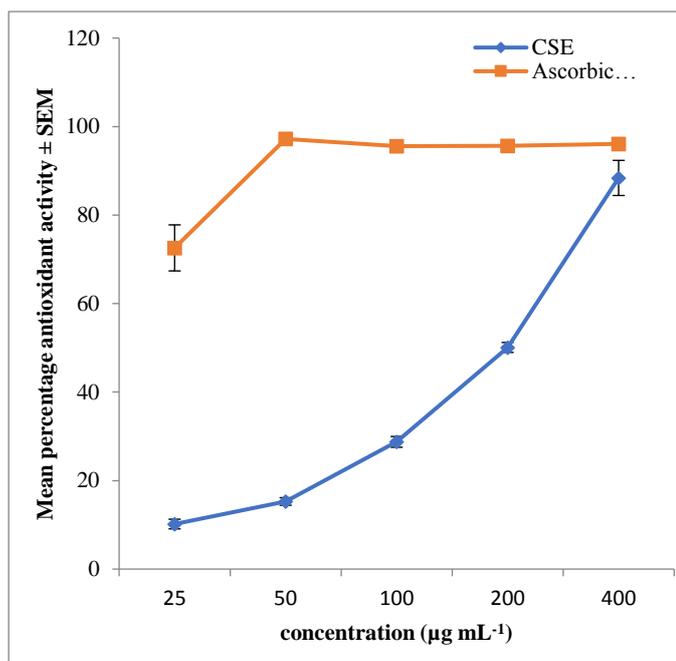


Figure 1: DPPH radical scavenging activity of *Callichilia subsessilis* extract.

The antioxidant activity could be a possible mechanism of the anti-inflammatory and antinociceptive properties of CSE. Free radicals are liberated during inflammatory processes by macrophage and also stimulate cyclooxygenase and lipoxygenase-mediated production of pro-inflammatory mediators from arachidonic acids.²² Antioxidants mop up free radicals and as such have been acclaimed to exhibit anti-inflammatory effects.^{22,23} The finding of this study corroborates the report of Orabueze and co-workers on the antinociceptive and antioxidant activities of *Callichilia stenopetala*.²⁴

Conclusion

Although this investigation is preliminary, it however, lends credence to the traditional use of *Callichilia subsessilis* in the management of inflammatory processes involved with bone fractures, as well as unearths a basis for further investigations. Isolation and identification of bioactive compound(s) as well as unravelling the mechanism of anti-inflammatory activity of the plant extracts would be major contributions to the phytochemistry and pharmacological activity of this plant.

Table 1: Effect of *C. subsessilis* extract on Formalin-induced paw oedema

Treatment	Mean increase in paw volume (mL) ± SEM (% inhibition)			
	1 h	2 h	3 h	24 h
5 % DMSO 5 mL kg ⁻¹	0.33 ± 0.05 (-)	0.42 ± 0.05 (-)	0.50 ± 0.07 (-)	0.27 ± 0.07 (-)
Aspirin 200 mg kg ⁻¹	0.29 ± 0.04 (12.12)	0.30 ± 0.05 (28.57)	0.28 ± 0.05* (44.00)	0.18 ± 0.08 (33.33)
CSE 50 mg kg ⁻¹	0.31 ± 0.04 (6.16)	0.31 ± 0.05 (26.12)	0.30 ± 0.02* (40.00)	0.33 ± 0.05 (14.80)
CSE 100 mg kg ⁻¹	0.29 ± 0.04 (12.12)	0.31 ± 0.03 (26.12)	0.32 ± 0.03* (36.00)	0.16 ± 0.03 (40.7)
CSE 200 mg kg ⁻¹	0.27 ± 0.05 (18.18)	0.27 ± 0.04* (35.71)	0.21 ± 0.06* (58.00)	0.22 ± 0.09 (18.50)

*p < 0.05 when compared with 5% DMSO treated group.

Table 2: Effect of *C. subsessilis* extract on egg albumin-induced oedema

Treatment	Mean increase in paw volume (mL) ± SEM				
	1 h	2 h	3 h	5 h	24 h
5 % DMSO 5 mL kg ⁻¹	1.22 ± 0.06	1.07 ± 0.10	1.02 ± 0.13	0.81 ± 0.08	0.24 ± 0.02
Aspirin 200 mg kg ⁻¹	0.81 ± 0.09***	0.64 ± 0.04***	0.57 ± 0.04***	0.46 ± 0.05***	0.15 ± 0.02***
CSE 50 mg kg ⁻¹	0.90 ± 0.03**	0.84 ± 0.04*	0.64 ± 0.03**	0.51 ± 0.02***	0.09 ± 0.02***
CSE 100 mg kg ⁻¹	0.90 ± 0.10**	0.75 ± 0.09**	0.64 ± 0.06**	0.44 ± 0.06***	0.03 ± 0.01***
CSE 200 mg kg ⁻¹	0.99 ± 0.08	0.84 ± 0.08*	0.74 ± 0.10*	0.51 ± 0.04***	0.04 ± 0.01***

***p < 0.001; **p < 0.01; *p < 0.05 when compared with 5% DMSO treated group.

Table 3: Effect of *C. subsessilis* extract on Acetic acid-induced writhing reflex.

Treatment	Number of writhing reflex	% inhibition
5 % DMSO 5 mL kg ⁻¹	47.20 ± 0.73	0.00
Aspirin 200 mg kg ⁻¹	6.40 ± 0.93***	86.44
CSE 50 mg kg ⁻¹	34.60 ± 1.03***	26.69
CSE 100 mg kg ⁻¹	14.6 ± 0.51***	69.06
CSE 200 mg kg ⁻¹	23.60 ± 1.23***	50.00

***p < 0.001; **p < 0.01; *p < 0.05 when compared with 5% DMSO treated group.

Table 4: Role of *C. subsessilis* extract on Tail flick test.

Treatment	(PRT) (Sec) ± SEM	% inhibition
5% DMSO 5 ml/kg	1.90 ± 0.04	-
Pentazocine 5 mg kg ⁻¹	2.56 ± 0.23	34.74
CSE 50 mg kg ⁻¹	2.62 ± 0.26*	37.89
CSE 100 mg kg ⁻¹	2.02 ± 0.03	6.31
CSE 200 mg kg ⁻¹	2.55 ± 0.36*	34.21

*p < 0.05 when compared with 5% DMSO treated group.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors appreciate the contribution of Mr Ndukwe Ibeh of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike towards plant identification.

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