African Journal of Pharmacology and Therapeutics Vol. 4 No. 1 Pages 28-32, 2015
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Research Article

Anti-inflammatory Activity of *Cyathula prostrata* (L.) Blume

Olawale H. Oladimeji [a,*], and Cyril O. Usifoh [b]

[a] *Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Nigeria*
[b] *Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, University of Benin, Nigeria*

* **Corresponding author:** Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, P.M.B 1017, Uyo, Akwa Ibom State, Nigeria; **Tel:** +234-70-38916740; **Email**: hakeemoladimeji@uniuyo.edu.ng

Background: *Cyathula prostrata* (L.) Blume which belongs to the Amaranthaceae family is used in traditional medicine for the treatment of chest troubles, dysentery, diarrhea, skin ulcers, scabies, sexually transmitted diseases (STDs), tumours and inflammations amongst many others. The growing concerns associated with the incidence of reactive free-radicals widely implicated in many health conditions prompted this present study.

Objectives: The crude extract, fractions and two previosly isolated compounds (HOO-1and HOO-2) from the plant were to be screened for anti-inflammatory activities with the aim of confirming or disproving its uses such as wound healing, treating skin ulcers and rheumatism amongst many others.

Methodology: The xylene and chorio-allantoic membrane (CAM) models were employed in the determination of the anti-inflammatory activity of the plant.

Results and Discussion: The xylene-model test for anti-inflammatory activity showed that the ethyl-acetate fraction and HOO-2 gave moderately similar anti-inflammatory activity of 53.48 % while HOO-1was comparably less active at 30.20 %. The chorio-allantoic membrane (CAM) model also indicated that both the ethyl-acetate fraction and HOO-2 gave moderate anti-inflammatory activity of 52.00 % while HOO-1 was less active at 44.00 %. However, the other fractions were weakly active. These results obtained from this study were not surprising because the phytochemical screening of the extract indicated the presence of flavonoids and terpenes which have demonstrated anti-inflammatory activities in previous studies. Furthermore, the results show some consistency irrespective of the model used.

Conclusion: The results obtained in this study have lent scientific justification to the folklore uses of the plant in treating and managing inflammatory conditions.

Keywords: anti-inflammatory; xylene-model; chorio-allantoic membrane (CAM); *Cyathula prostrata*

Received: November, 2014
Published: March, 2015

1. Introduction

From time immemorial, man has been dependent on plants and other sources for his food and medicine for relief from illness (Christopherson et al, 1991). Plants owe their value as drugs to the medicinal properties of specific inorganic and organic chemical entities present therein (Larrson et al, 2005). Currently, about twenty to fifty percent (20% to 50%) of medicines /drugs in the world is derived from plants (Cowan, 1999). Therefore, any contribution to the discovery of templates for the treatment and or management of disease conditions such as skin, gastro-intestinal, respiratory tract infections, tumours and especially inflammations will be of particular interest to the scientific world and of immense benefits to mankind. Inflammation just like free radical damage and oxidative stress has become major health issues in recent years. It has been implicated in heart disease, stroke, cancer, pancreatitis, laryngitis, asthma, gastritis, dermatitis, hay fever,

 ISSN 2303-9841

rheumatoid arthritis, wounds, atherosclerosis, emphysema, lung dysfunction, skin lesions, radiation injuries, premature aging and diabetes amongst many other inflammatory conditions (Takasu et al, 1987; Bryan, 1996; Thabrew et al, 1998; Toda and Shirataki, 1998; Speroni et al, 1998; Ashcroft, 1999; Burits and Bucar, 2000; Baxter et al, 2004; Dufeng and Arthur, 2004; Guyton and Hall, 2006; Piper, 2006; Nadier, 2007). Inflammation is a complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells or irritants. In the absence of inflammation, wounds and infections would never heal but the progressive destruction of the tissues would compromise the survival of the organism (Ashcroft, 1999). Anti-inflammatory activity using xylene has been reported to induce inflammation by activating the enzyme, phospholipase A_2 which breaks down phospholipids in cell membrane to arachidonic acid from where inflammatory mediators are formed (Lin et al, 1992). The chorio-allantoic membrane assay employs the use of fertilized eggs because of the potential of the eggs developing into chicks. Inflammation is only possible in live tissues or animals.

Apart from the traditional uses of *C. prostrata* in the treatment of skin, chest, gastrointestinal and sexually transmitted diseases; it is also employed in ethno-medicine in the treatment and management of a number of inflammatory conditions. Consequently, it was considered necessary to confirm or disprove these claims using the xylene and chlorio-allantoic membrane (CAM) models for anti-inflammatory tests.

2. Materials and Methods

2.1 Collection and Processing of Plant

The fresh aerial parts of *C. prostrata* (L.) Blume were collected in the month of July, 2011 on a farmland in Itak Ikot, Ikono Local Government Area, Akwa Ibom State, Nigeria. The plant was identified by Dr. (Mrs) M. Bassey of the Department of Botany and Ecological Studies, University of Uyo, Nigeria. The authentication by comparison was done with herbarium samples of the Forestry Research Institute of Nigeria (FRIN) and the National Institute of Horticulture (NIHORT), both at Ibadan, in Nigeria. A voucher specimen of the plant (No H92) was deposited in the herbarium of the Faculty of Pharmacy, University of Uyo, Nigeria.

2.2 Extraction and Processing

The plant was air-dried and powdered in an electric mill. The resultant coarse powder was then extracted with cold 96% aqueous ethanol at room temperature (27± 2 °C) for 72 h. The filtrate was evaporated to dryness *in-vacuo* on a rotary evaporator (Buchi CH-920, Laboratorium Technic, Flawk/SG, Switzerland) and then stored in an amber bottle. Also, the aqueous extract of the plant was partitioned with organic solvents of increasing polarities namely, hexane, chloroform, ethyl-acetate and butanol. The resultant mixtures were then bulked separately to obtain the hexane (3A), chloroform (3B), ethyl-acetate (3C) and butanol (3D) fractions respectively which were then evaporated to dryness *in-vacuo* and then stored in a refrigerator at –4 °C prior to the anti-inflammatory tests.

2.3 Silica-gel Column Chromatography

The ethyl-acetate fraction (3C) was put through a combination of thin-layer, column and preparative chromatography using silica-gel 254 (Sigma, USA) to obtain the isolates HOO-1and HOO-2 as described previously (Oladimeji, 2012; Oladimeji and Usifoh, 2013).

2.4 Anti-inflammatory Tests

Permission was sought from the College of Health Sciences' Animals Ethics Committee, University of Uyo, Uyo, Nigeria and approval was granted on the 24th, August, 2011 as contained in the reference document (UU/CHS/DP/24). The animals were then subsequently used in the anti-inflammatory studies.

2.5 Preparation of Extracts, Fractions, Isolates and Standard Anti-inflammatory Drug

Stock solutions of the crude extract, fractions, isolates and standard anti-inflammatory drug (betamethasone) were prepared using 15% Tween 80 (Fluka Chemie, AG Switzerland) solvent. Stock solutions of the crude extract and fractions were separately prepared. Serial dilutions were carried out with 15% Tween 80 to obtain a concentration of 10 mg/ml for each of the extract and fractions. HOO-1and HOO-2 were tested at 1mg/ml while betamethasone (Neimeth, Nigeria) was administered at 1µg/ml (Nia et al, 2003; Nwosu, 2009).

2.6 Animals

White albino mice of both sexes weighing 16.80 ± 2.00 g were used. They were purchased from the Animal House, University of Uyo, Uyo, Nigeria. The animals were kept in standard cages, maintained on a standard pellet feed and water *ad libitum*. The mice were divided into ten (10) groups of three (3) per group and were appropriately marked for ease of identification. The mice were then fasted for 24 hours before use. Two (2) of the ten (10) groups were used as positive and negative controls respectively. One group was given betamethasone, a standard anti-inflammatory drug while the other group was administered Tween 80. The remaining eight groups were given the crude extract, fractions, HOO-1 and HOO-2 respectively. The crude extract and the fractions were administered at a dose of 100mg/kg as proposed by Kannappan and Sundaram (2009) while HOO-1 and HOO-2 were tested at 10mg/kg. The standard anti-inflammatory drug was given at a dose of 4mg/kg in line with standard steroidal dose used by Okokon et al, 2008. Administration of extract /fraction / isolate/ standard anti-inflammatory drug was done intraperitoneally, thirty (30) minutes before induction of oedema.

2.7 Xylene Model of Induction of Inflammation in Mice

Inflammation was induced in the mice by topical administration of two (2) drops of xylene to the inner surface of the right ear for 15 minutes. The calculated doses of the extract, fractions, isolates and standard anti-inflammatory drug were administered on animals in the respective groups, thirty (30) minutes before induction of inflammation. Under light anaesthesia

 ISSN 2303-9841

using chloroform-soaked cotton-wool, the mice were sacrificed and both ears were cut off. The difference between the ear weights was taken as the inflammation induced by xylene (Tjolsen et al, 1992).

2.8 Chorio-allantoic Membrane (CAM) Assay

The D'Arcy and Howard's model (1966) was used. The chorio-allantoic membranes of chick embryos of eight-day old fertilized eggs were used. Two controls were used. Betamethasone served as a positive control while the negative one was without any sample.

Day-old fertilized eggs (obtained from a local poultry farm) indigenous to Uyo, were incubated for eight days in an incubator at 36-37 °C. A humid environment was maintained by placing dishes of water on shelves of the incubator. The eggs were turned twice daily.

Filter paper discs (13mm) were cut out of Whatman No. 1 filter paper (Whatman International, England). The discs with rough edges were discarded while the discs with smooth edges were sterilized in well fastened glass plate by autoclave (Medical Instruments, England) method. A disc was inserted in each of the eggs.

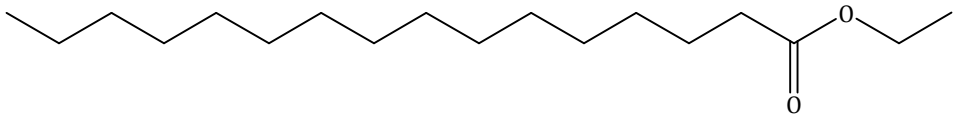
The region of shell where chorio-allantoic membrane (CAM) was best developed was drilled with a scalpel to remove a triangular diameter shell on the eighth day of incubation. Care was taken during drilling to prevent shell fragments from failing on the chorio-allantoic membrane. Also, the shell around the region of the air sac was drilled to form a hole to which a rubber teat was applied to suck out the air. The chorio-allantoic membrane was then dropped from the shell membrane so that a filter paper disc could be implanted. The disc without any sample implantation but Tween 80 was used as a negative control while the one saturated with betamethasone (Neimeth, Nigeria) served as a positive control. Each of the other discs was saturated with extract /fraction/ isolate/standard anti-inflammatory drug and implanted in an egg appropriately labelled. The holes on the shell were sealed using a cello-tape and the eggs were then re-incubated for 4 days. The discs were then removed, dried and weighed at the end of the period of re-incubation.

3. Results and Discussion

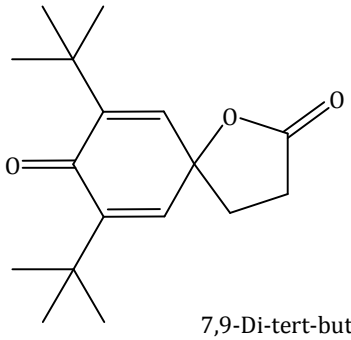
The plant was identified, authenticated and collected observing basic guidelines of plant collection. Also, the rules governing extraction and processing of extracts were kept, thus preventing any changes to the chemical composition of the crude extract (Odebiyi and Sofowora, 1978; Odebiyi and Sofowora, 1979). Studies on the crude extract have revealed the presence saponins, tannins, flavonoids, terpenes and cardiac glycosides while alkaloids, anthraquinones and cyanogenic glycosides were absent (Oladimeji et al, 2005; Oladimeji, 2012; Oladimeji and Usifoh, 2013). Plant metabolites such as saponins, cardiac glycosides, alkaloids, tannins and flavonoids have demonstrated in several previous studies (Hillar et al., 1990; Lamikanra et al, 1990; Burapadaja et al, 1995; Harouna et al, 1995; Aiyelaagbe et al, 1998; Adewunmi et al, 1998; Ibewuike et al, 1998; Adesina et al, 2000) to be responsible for the cure or management of many ailments caused by microbes and different kinds of disease conditions in the ethno-medicine of plants. Prior to this present study, the antimicrobial screening of the extract and fractions indicated that the antimicrobial activity was most pronounced in the ethyl-acetate fraction. Hence, the antimicrobial constituents of the crude extract resided largely in the ethyl-acetate fraction, being the most active. In addition, the ethyl-acetate fraction extracted the largest amount of plant material (Oladimeji, 2012; Oladimeji and Usifoh, 2013). Consequently, the silica-gel 254 chromatographic separation of the ethyl-acetate fraction led to the isolation of HOO-1 and HOO-2 (**Figure 1**) (Oladimeji, 2012; Oladimeji and Usifoh, 2013).

Xylene was used to induce ear oedema (inflammation) in the mice. The irritation characteristic of inflammation was noticed as the mice flipped their ears. Subsequently, inflammation was induced in the right ear so as to allow the left ear (without inflammation) serve as the basis for comparison. Betamethasone was used as the standard anti-inflammatory drug in this test because it is most potent of the anti-inflammatory drugs in clinical practice (D'arcy and Howard, 1966; Okokon et al, 2008) with a known inhibitory activity against phospholipase A_2 and thus can reduce or prevent inflammation. The results of xylene model as presented in **Table 1** show that ethyl-acetate fraction and HOO-2 gave moderately similar anti-inflammatory activity of 53.48 % while HOO-1, butanol and chloroform fractions similarly demonstrated an activity at 30.20 %. However, the hexane fraction was comparably stronger at 45.50 %. The anti-inflammatory activity could be due to the presence of compounds such as flavonoids (Palmer and Ghosh, 1978) or possibly terpenes (Oladimeji et al, 2005; Oladimeji, 2012; Oladimeji and Usifoh, 2013), a chemical group from where ecdysterone (a steroidal terpene) had been isolated from *C. prostrata* (Shah and De Souza, 1971). Filter paper discs made from the Whatman filter paper were sterilized to prevent the introduction of micro-organisms to the sterile environment of the eggs. They were then used to induce inflammation on the chorio-allantoic membranes thereby producing maximal granulation tissue which then become attached to the under-surface of the discs (D'arcy and Howard, 1966; Zwaalo- Klarwasser et al, 2001).

The anti-inflammation process which is to prevent or reduce the formation of granulation tissue is probably effected by the inhibition of the action of chemokines. The results of CAM assay as displayed in Table 2 show that both the ethyl-acetate fraction and HOO-2 demonstrated moderate anti-inflammatory activity of 52.00 %. However, the crude extract, hexane, chloroform and butanol reactions recorded comparably weaker (marginal) activities as highlighted in **Table 2**. Though, the anti-inflammatory activity given by HOO-1 was stronger than that of hexane, chloroform or butanol fractions but was comparably weaker than that given by HOO-2 at 44.00 %. Also, the presence of terpenes or flavonoids (Oladimeji et al, 2005; Oladimeji, 2012; Oladimeji and Usifoh, 2013) or any steroid-like compounds could be responsible for the anti-inflammatory activity given by the plant (Palmer and Ghosh, 1978).



Ethyl hexadecanoate (H001)



7,9-Di-tert-butyl-1-oxaspiro-4,5-deca-6,9-diene-2,8-dione (H002)

Figure 1: The structures of two compounds obtained from the ethyl-acetate fraction of *Cyathula prostrata*

Table 1: Anti-inflammatory Activity of Crude Extract, Fractions and Isolates of *C. prostrate* (Xylene Model)

Dose /mg/kg	Wt. of right (Inflammed) ear (g)	Wt. of left (Un-inflammed) ear (g)	Increase in ear wt. (g)	% Inhibition
Control (15% Tween 80) (10mg/kg)	0.080 ± 0.005	0.036 ± 0.005	0.043 ± 0.010	0.00 %
Crude extract (2A) (100mg/kg)	0.053 ± 0.003	0.027 ± 0.003	0.027 ± 0.006	37.20 %
Hexane fraction (3A) (100mg/kg)	0.047 ± 0.003	0.023 ± 0.003	0.023 ± 0.006	45.50 %
Chloroform fraction (3B) (100mg/kg)	0.053 ± 0.003	0.023 ± 0.003	0.030 ± 0.006	30.20 %
Ethyl-acetate fraction(3C) (100mg/kg)	0.050 ± 0.000	0.030 ± 0.000	0.020 ± 0.000	53.48 %
Butanol fraction (3E) (100mg/kg)	0.053 ± 0.000	0.023 ± 0.000	0.030 ± 0.000	30.20 %
HOO-1 (10mg/kg)	0.047 ± 0.000	0.017 ± 0.000	0.030 ± 0.000	30.20 %
HOO-2 (10mg/kg)	0.053 ± 0.000	0.033 ± 0.000	0.020 ± 0.000	53.48 %
Betamethasone (4mg/kg)	0.036 ± 0.003	0.020 ± 0.003	0.016 ± 0.006	62.80 %

***Key**: The values recorded are the means of the weights from the three mice in each group.*
HOO-1 = Ethyl Hexadecanoate (Ethyl Palmitate)
HOO-2 = 7, 9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione

$$\% \text{ Inhibition} = \frac{\text{Increase in wt. of negative control - Increase in wt. of extract/fraction/isolate/drug}}{\text{Increase in wt. of negative control wt.}} \times 100$$

The results of CAM assay more or less corroborate those obtained from the xylene model, showing some consistency irrespective of the model employed. Hence, results of the anti- inflammatory activity of *C. prostrata* compared favourably with those obtained in previous studies (Palmer and Ghosh, 1978; Ekpendu et al, 1994; Perez et al, 1995; Besra et al, 1996; Forestieri et al, 1996; Zwaalo-Klarwasser et al, 2001; Nia et al, 2003; Ogbole et al, 2007; Oladimeji et al, 2007; Okoli and Akah, 2007; Okokon et al, 2008; Nwosu, 2009).

The anti-inflammatory activity demonstrated by this plant has justified its uses in treating and managing inflammatory conditions such as skin lesions, bronchitis, ulcers, wounds, rheumatoid arthritis and so forth.

Conflict of Interest declaration

The authors declare no conflict of interest.

 ISSN 2303-9841

Table 2: Anti-inflammatory Activity of Crude Extract, Fractions and Isolates of *C. prostrata* (CAM Model)

Dose/mg	Weight of filter disc before implantation (mg)	Weight of filter disc after implantation (mg)	Increase in weight of filter disc (mg)	% inhibition
Control (No sample)	12.00	37.00	25.00	0.00 %
Crude extract (2A) (10mg)	13.00	30.00	17.00	32.00 %
Hexane fraction (3A) (10mg)	11.00	28.00	17.00	32.00 %
Chloroform fraction (3B) (10mg)	11.00	29.00	18.00	28.00 %
Ethyl-acetate fraction (3C) (10mg)	13.00	25.00	12.00	52.00 %
Butanol fraction (3E) (10mg)	13.00	32.00	19.00	24.00 %
HOO-1 (1mg)	13.00	27.00	14.00	44.00 %
HOO-2 (1mg)	12.00	24.00	12.00	52.00 %
Betamethasone (1µg/ml)	12.00	22.00	15.00	60.00 %

***Key**: The values recorded are the means of the weights from the three mice in each group.*
HOO-1 = Ethyl Hexadecanoate (Ethyl Palmitate)
HOO-2 = 7, 9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione,

$$\% \text{ inhibition} = \frac{\text{Weight of filter disc after implantation - Weight of filter disc before implantation}}{\text{Increase in weight of negative control}} \times 100$$

Acknowledgements

The authors warmly appreciate the kind gesture of the Department of Pharmacology and Toxicology for the use of the Animal House in the conduct of the anti-inflammatory tests. The authors also acknowledge the assistance rendered by Mr. N. M. Udoh in the course of this study.

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 ISSN 2303-9841