Journal of Intercultural Ethnopharmacology
www.jicep.com
DOI: 10.5455/jice.20161229055924

Original Research

Antinociceptive and anti-inflammatory effects of aqueous extract of *Chenopodium opulifolium* schrad leaves

Abayomi M. Ajayi[1,2], Julius Khidzee Tanayen[1], Albert Magomere[3], Joseph O. C. Ezeonwumelu[3]

[1]Department of Pharmacology and Toxicology, Kampala International University, Western Campus, Ishaka, Bushenyi, Uganda, [2]Ethnopharmacology and Inflammation Unit, Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Nigeria, [3]School of Pharmacy, Kampala International University, Western Campus, Ishaka, Bushenyi, Uganda

Address for correspondence:
Abayomi M. Ajayi, Ethnopharmacology and Inflammation Unit, Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Nigeria.
Email: yomexj@yahoo.com.

Received: October 20, 2016
Accepted: December 07, 2016
Published: December 22, 2016

ABSTRACT

Aim: *Chenopodium opulifolium* is a specie of the Chenopodiaceae commonly used as vegetables in local diet and for treating different ailment in Uganda. This study was conducted to evaluate the antioxidant, antinociceptive and anti-inflammatory effects of the aqueous extract of *C. opulifolium* leaves (AECO). **Materials and Methods:** The dried leaf of the plant was extracted by maceration in water. Qualitative and quantitative phytochemical analysis, antioxidants, and membrane stabilizing effects were determined in the extract. The extract was then investigated for acute toxicity, antinociceptive (writhing, hot plate and open field test), and anti-inflammatory (egg albumin-induced paw edema) effects in rodents. **Results:** Phytochemical analysis revealed the presence of alkaloids, tannins, phlobatannins, flavonoids, and saponins in AECO. Total caffeic acid derivatives and total flavonoids content were 91.7 mgCAE/g sample and 94.7 mgRE/g sample, respectively. AECO demonstrated antioxidant effects in both 1,1-diphenyl-2-picryl-hydrazyl and NO assays. Significant membrane stabilizing activity was observed in both the heat and hypotonic solution-induced lysis of erythrocytes. The acute toxicity test showed that AECO (5000 mg/kg) did not cause any significant change in behavior or death in rats. AECO (100-400 mg/kg) produced a significant antinociceptive effect in both the writhing and hot plate tests, but no significant reduction in the locomotory activity in mice. Furthermore, the extract significantly ($P < 0.05$) reduced egg albumin-induced rat paw edema by 44.2%, 44.5%, and 51.2%, respectively, after 120 min. **Conclusion:** The results showed that *C. opulifolium* extract possesses significant antioxidant, antinociceptive and anti-inflammatory effects, and these affirm the reasons for its folkloric use.

KEY WORDS: Anti-inflammatory, caffeic acid, *Chenopodium opulifolium*, erythrocytes, writhing

INTRODUCTION

Medicinal plants represent unique biodiversity resources on the African continent contributing immensely to the health and wellness of its population over the centuries. The contributions of the variety of medicinal plants to local economies, cultural integrity, and health care and ultimately the well-being are increasingly being discussed [1]. In Sub-Saharan Africa for example, households in rural areas rely heavily on plant resources for food, fodder, and herbal medicine. The World Health Organization decade old statistics estimated that about 80% of the African population depends on herbal medicines for some aspect of primary health care needs [2]. Inaccessible and unaffordable modern medicines are major factors that still promote the use of medicinal plants among rural Ugandans for their daily health care needs [3]. Medicinal plants being a dominant component of the traditional medicine practice in Uganda, is used for the prevention, diagnosis, and treatment

of social, mental and physical illness [4]. One of such plants used is *Chenopodium opulifolium* Schrad. ex Koch & Ziz, with a synonym *Chenopodium ugandae* (Aellen) Aellen, a member of the Chenopodiaceae family that grows in Uganda [4].

The Chenopodiaceae is a large family comprising about 102 genera and 1400 species. The genus chenopodium includes a variety of weedy herbs (more than 200 species) that are native to Europe, Africa, Asia, North and South America [5]. The importance of the Chenopodium species was due to their variety of medicinal properties, commercially used as spices or drugs because of the presence of useful secondary metabolites. The most characteristic constituents are flavonoids, essential oils, and terpenes [6]. The reported biological activities of the chenopods include the antiviral, antimicrobial, antifungal, anti-inflammatory, analgesic, and immunomodulatory effects [7].

C. opulifolium known locally as "Kavumbavumba" in central or "Omwentago" in Western Uganda has been reported to have several ethnobotanical uses [8,9]. The leaf is used as vegetable in local diets in East Africa [10]. The leaves or root decoction is drunk to induce menstruation and hasten childbirth and to relieve irregular painful menstruation [11]. The leaves can be used as eye ointment or macerated leaf used for female asthenia, and abdominal colic for newborns [12]; and for managing pediatrics cases of febrile illnesses [13]. *C. opulifolium* leaf is reported to be used in the management of Herpes simplex viral infection in HIV/AIDS [14]. The water decoction or infusion of its leaves has been reported to be used for the treatment of malaria and cough, either alone or in combination with *Azadirachta indica*, *Momordica foetida*, *Ocimum gratissimum*, *or Vernonia amygdalina* [15].

The ethnobotanical information on this plant did not match the pharmacological or toxicological information. *C. opulifolium* medicinal properties have not been extensively studied in biological systems, and no published data are available in scientific literatures. This study was, therefore, carried out in an attempt to screen for the antioxidant, antinociceptive, and anti-inflammatory effects of the aqueous extract of *C. opulifolium* (AECO) leaves.

MATERIALS AND METHODS

Materials

Chemicals and drugs

Glacial acetic acid, indomethacin, sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), sodium nitroprusside, and quercetin were all product of Sigma-Aldrich, USA.

Animals

Adult Wister rats (120-180 g) or Swiss mice (18-25 g) of either sex were used throughout this study. The animals were kept under environmentally controlled laboratory room conditions at 21-27°C. The animals were housed in cages lined with wood shavings and were fed with standard pelletized feed (Nuvita® Animal Feeds Ltd., Jinja Uganda) and water *ad-libitum*. The animal experiments were conducted according to the National Institute of Health Guide for the care and use of laboratory animals [16] and ethical guidelines for investigation of experimental pain in conscious animals [17].

Plant Material

The leaves of *C. opulifolium* locally known as "omwentago" were collected in Ishaka, Bushenyi District, in Western Uganda. The plant was authenticated by Assoc Prof Dominic Byarugaba, a botanist at Kampala International University; a voucher specimen was prepared as herbarium sample and deposited at the school of pharmacy herbarium (KIU-WC/10/001). Fresh leaves of the plant were dried by placing them under shade, and then, reduced to fine powder by grinding using a metallic mortar and pestle in Kampala International University pharmacy laboratory.

Extraction

Extraction of *C. opulifolium* powdered leaf was done by maceration method. Briefly, powdered leaf (100 g) was soaked in 1 L of distilled water and shaken for 48 h on a laboratory rotator (Nuve SL 350 Quality System, Digisystem Laboratory Inc., Taiwan). The extract was filtered and evaporated over water bath at 50°C and was finally dried in the oven set at 40°C. The extract obtained was darkish brown in color denoted as AECO and was stored in the refrigerator at 4°C.

Qualitative Phytochemical Screening

The aqueous extract used for pharmacological screening was subjected to preliminary qualitative phytochemical analysis using standard protocols [18,19]. The presence of the compounds to be tested was rated as positive (+) or negative (−). These compounds included tannins, phlobotannins, saponins, terpenoids, flavonoids, alkaloids, and reducing sugars.

Determination of Total Caffeic Acid Derivatives Content (TCAD)

TCAD in the aqueous extract was determined using the spectrophotometric method with Arnow's reagent [20]. 0.2 mL of AECO (1 mg/mL), 1 mL HCl (0.5 N), and 1 mL Arnow's reagent and 1 mL NaOH (1 M) were mixed in test tubes and allowed to stand for 5 min. The volume was made up to 5 mL and after 30 min, the absorbance was read at 500 nm with a spectrophotometer (Spectronic 21D Milton Roy, USA). Caffeic acid content was determined from caffeic acid calibration curve with a linear regression curve ($R^2 = 0.999$).

Determination of Total Flavonoids Content in AECO Leaves

The total flavonoid content (TFC) in the extract was determined by the $AlCl_3$ spectrophotometric method as

described by Sultana *et al*. [21]. 1 mL of AECO (1 mg/mL) was dispensed into three separate test tubes. After that, 0.3 mL of 10% (w/v) $NaNO_2$ was added to the test tubes, and left to react for 5 min, 0.3 mL of 10% (w/v) $AlCl_3$ was added and left for 1 min to react. Thereafter, 2 mL of 1M NaOH was added and the mixtures shaken. Aliquots of the mixtures were transferred to a cuvette, and the absorbance values measured with a spectrophotometer (Spectronic 21D Milton Roy) at 510 nm. A mixture of 1 mL of 80% (v/v) methanol, 4 mL of deionized water, 0.3 mL of 10% (w/v) $NaNO_2$, 0.3 mL of 10% (w/v) $AlCl_3$, and 2 mL of 1 M NaOH was prepared and used as the blank. Rutin was used as a standard for the calibration curve ($R^2 = 0.985$).

Evaluation of Free Radical Scavenging Effects of AECO

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay

The free radical scavenging properties of AECO were assessed using DPPH spectrophotometric assay by Aderogba *et al*. [22] with slight modifications. To 2 mL of AECO (12.5, 25, 50, 100, 200 and 400 μg/mL in methanol), quercetin (25 μg/mL) or methanol (2 mL) were added 3 mL of freshly prepared DPPH solution (0.1 mM) in methanol. The mixture was incubated in the dark cupboard for 30 min at room temperature, and the absorbance was measured at 514 nm on an ultraviolet (UV)/vis spectrophotometer (INESA). The percentage free radical scavenging activity was calculated accordingly:

% inhibition=[(absorbance of control–absorbance of test sample)/absorbance of control] ×100%

Nitric oxide free radical scavenging assay

Nitric oxide radical scavenging activity assay according to the method described by Kumaran and Karunakaran [23] was used. Briefly, sodium nitroprusside (10 mM in 0.1 M sodium phosphate buffer, pH 7.4) was mixed with AECO (12.5, 25, 50, 100, 200 and 400 μg/mL) or quercetin (25 μg/mL) and incubated at room temperature for 150 min. The control contained the same reaction mixture except the extract. After incubation period, 0.5 mL of Griess reagent (equal volume of 1% sulfanilamide in 5% phosphoric acid and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance was read at 546 nm in a UV/vis Spectrophotometer (INESA).

Evaluation of Membrane Stabilizing Properties

Preparation of 10% erythrocytes suspension

Blood was collected by cardiac puncture from healthy male Wistar rats lightly anesthetized with ether. The collected blood was mixed with equal volume of sterilized Alsever solution; packed red cells were obtained by repeated centrifugation (3000 rpm for 10 min), in isotonic phosphate buffer solution (154 mM NaCl in 10 mM sodium phosphate buffer, pH 7.4). The packed cell was re-suspended to 10% (v/v) in the isotonic buffer.

Effect of AECO on heat-induced rat erythrocytes hemolysis

The effects of AECO on hemolysis of rat red blood cell (RBC) induced by heat was evaluated according to the modified method by Anosike *et al*. [24]. Briefly, AECO and indomethacin were dissolved in isotonic phosphate buffer solution (154 mM NaCl in 10 mM sodium phosphate buffer, pH 7.4). The assay mixture consisted of 5 mL graded doses of extract (100, 200, 400, 800 and 1000 μg/mL); and 0.5 mL of 10% RBC suspension. The control was prepared as above without the extract, while RBC was omitted from the extract control tubes. Each sample was prepared and arranged in quadruplicate sets (4 sets per dose). A pair of the tubes was incubated at 54°C for 20 min in a regulated water bath. The another pair was maintained at −10°C in a freezer for 20 min. Afterward, the tubes were centrifuged at 4000 rpm for 3 min, and the hemoglobin contents of the supernatant were estimated at 540 nm using Spectronic 21 D (Milton Roy) Spectrophotometer.

Effect of AECO on hypotonic solution induced rat erythrocytes hemolysis

The effects of AECO on hemolysis of rat RBC induced by hypotonic solution was evaluated according to the method described by Shinde *et al*. [25] with some modifications. The extract was dissolved in hypotonic sodium phosphate buffer solution (50 mM NaCl in 10 mM sodium phosphate buffer, pH 7.4). The hypotonicity-induced hemolysis assay mixture consisted of 5 mL graded concentration (100, 200, 400, 800 and 1000 μg/mL), in hypotonic solution, and 0.5 mL of 10% RBC suspension.

The control was prepared as above except that the extract was omitted, while the extract controls lacked erythrocyte suspension. Each sample was prepared and arranged in quadruplicate sets (4 sets per dose). The mixture was incubated for 1 h at room temperature (23 ± 2°C), and afterward, centrifuged for 3 min at 4000 rpm. The absorbance of the hemoglobin content of the supernatant was estimated at 540 nm using Spectronic 21D (Milton Roy) spectrophotometer.

Acute Toxicity Study

Acute toxicity refers to adverse effects occurring within a short time of administration of a single dose of a substance or multiple doses given within 24 h. The safety of the leaf extract was tested as described by Adzu and Haruna [26]. Rats were grouped into five groups of three rats each. The extract was administered orally to four groups in increasing doses of 250, 500, 1000 and 5000 mg/kg body weight, and the fifth group (control group) received distilled water at 10 mL/kg body weight. All the rats were kept in the same conditions. They were observed for pharmacotoxic signs continuously for 4 h and at the 24 h post administration to score for mortality. Thereafter, they were observed for up to 7 days post administration. Pharmacotoxic signs include salivation, diarrhea, piloerection, sedation, restlessness, sensitivity to sound and touch, gasping, cyanosis, blood in urine and edema.

Evaluation of Antinociceptive Properties of AECO

Acetic acid-induced writhing in mice

The method as described by Koster *et al*. [27] was used. Five groups of mice ($n = 5$) were used in this study comprising the vehicle (0.1 mL/10 g distilled water), indomethacin (10 mg/kg), or AECO (100, 200, 200 mg/kg). Each mice were placed singly inside the plexiglass observation chamber immediately after oral treatment for 1 h before injection of 0.6% acetic acid (0.1 mL/10 g body weight). The frequency of writhing occurring between 5 and 20 min was counted. Writhing movement was accepted as a contraction of the abdominal muscles accompanied by stretching of hind limbs.

Hot Plate Test

Thermal noxious stimulus was produced in mice by placing them on the hot plate (UgoBasile hot/cold plate 35100, Italy) according to a method described by Turner [28]. Five groups of mice ($n = 5$) were used, in this study, comprising the vehicle (0.1 mL/10 g distilled water), pentazocine (5 mg/kg), or AECO (100, 200, 200 mg/kg). The mice were placed singly on the hot plate which was maintained at 55 ± 1°C. Reaction time was recorded when the animals licked their fore and hind paws or jumped; at before (0) and 30, 60, 90 and 120 min after administration of test drugs. The mice which reacted within 20 s were selected for the study. The mean percentage maximum possible effect (% MPE) was calculated as:

$$\%\text{MPE} = \frac{\text{Post-drug latency} - \text{Pre-drug latency}}{\text{Cutt-off time} - \text{Post drug latency}} \times 100$$

Assessment of Effect of AECO on Locomotory Activity in Open-field

To assess the possible nonspecific muscle relaxants or the sedative effects of AECO, the motor performance of the mice was evaluated on the open-field apparatus (Archer, 1973). Groups of mice ($n = 5$) were treated with vehicle (0.1 mL/10g, p.o.), AECO (100, 200, 400 mg/kg, p.o.) 1 h before the performance of the test. The mice were placed in the center of the UgoBasile activity cage apparatus and allowed to have free ambulation for 5 min of observation of the locomotion frequency (horizontal activity and vertical activity).

Evaluation of *In Vivo* Anti-inflammatory Activity in Egg Albumin-induced Edema in Rat Paw

The phlogistic agent employed in this study to induce acute inflammation was fresh egg albumin according to the method described by Akah and Nwambie [29]. The rats were fasted overnight prior to the experiment and were divided into five groups of five rats each. Group 1 (negative control) received distilled water (10 mL/kg body weight), Groups 2-4 received AECO (100 -400 mg/kg body weight), while Group 5 (positive control) received indomethacin (10 mg/kg). All treatments were orally administered using oral canula.

Edema was induced 30 min later in all the rats by a single subplantar injection of 0.1 mL of raw egg albumin to the left hind paw. Edema formation was taken as increase in paw circumference measured by wrapping a white cotton thread around the injected paw and measuring the circumference on a meter rule [30]. Paw circumference was taken at 30, 60, 90 and 120 min after egg albumin injection. The percentage inhibition of edema was calculated using the formula:

$$\text{Percentage inhibition} = \frac{(C_t - C_o)_{\text{control}} - (C_t - C_o)_{\text{treated}}}{(C_t - C_o)_{\text{control}}} \times 100$$

Where C_t - paw (edema) circumference at time (t) after albumin injection and C_o - paw (edema) circumference before egg albumin injection.

Statistical Analysis of Data

Results were expressed as mean ± standard error of the mean and statistical comparison of means was performed using analysis of variance, all levels of significance were set as $P < 0.05$. Significant main effects were further analyzed by *post-hoc* test using Bonferroni's multiple comparison test. Graphs and statistical analysis were performed using Microsoft Excel® (Microsoft Corporation, Redmond, WA, USA) and GraphPad Prism® software version 5.01 (GraphPad Software, Inc. La Jolla, CA 92037 USA).

RESULTS

Result of Phytochemical Analysis of AECO

The screening for the secondary metabolites revealed the presence of alkaloids, tannins, phlobotannins, flavonoids, and saponins (data not shown). Steroids, terpenoids, and reducing sugars were not detected in AECO. The TCAD determined by the Arnow's reagent method showed that AECO contained an amount equivalent to 91.7 ± 0.01 mgCAE/g of sample. The amount of TFC in the extract estimated from the rutin calibration curve was 94.7 ± 0.03 mgRE/g of the sample (Figure 1).

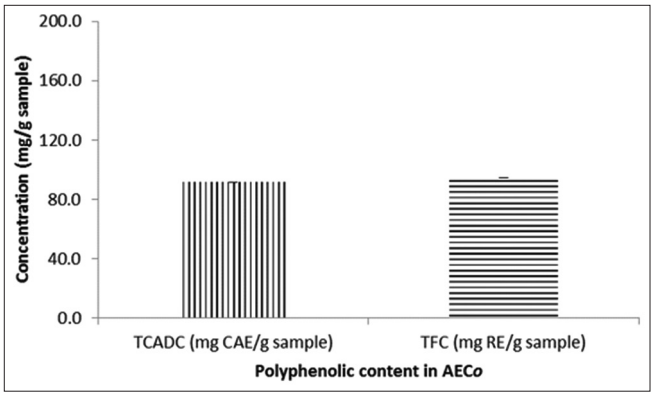

Figure 1: Total caffeic acid derivatives content and total flavonoid content in aqueous extract of *Chenopodium opulifolium*

In Vitro Antioxidant Effects of AECO

The results as shown in Figure 2 below revealed that AECO possesses *in vitro* antioxidant activity by mopping free radicals generated in both the DPPH and NO free radical scavenging assays. There was a corresponding increase in antiradical activity with an increase in concentration. The antiradical activity was higher in the NO than in the DPPH scavenging assay. Quercetin (25 μg/mL) activity was better than AECO (400 μg/mL) in the DPPH assay but lower in the NO assay.

Membrane Stabilizing Activity

AECO (100 -1000 μg/mL) demonstrated *in vitro* anti-inflammatory activity by its ability to inhibit heat-induced rat erythrocytes hemolysis (Table 1). The high percentage inhibition of hemolysis (36.5%, 44.0% and 49.6%) obtained for doses 400, 800 and 1000 μg/mL, respectively, was statistically significant ($P < 0.05$). Similarly, results, as shown in Table 2, revealed that AECO (100-1000 μg/mL) inhibited hypotonic solution-induced rat erythrocytes hemolysis in a concentration-dependent manner. The high percentage inhibition of hemolysis (37.5% and 40.3%) obtained for concentrations 800 and 1000 μg/mL, respectively, was statistically significant ($P < 0.05$).

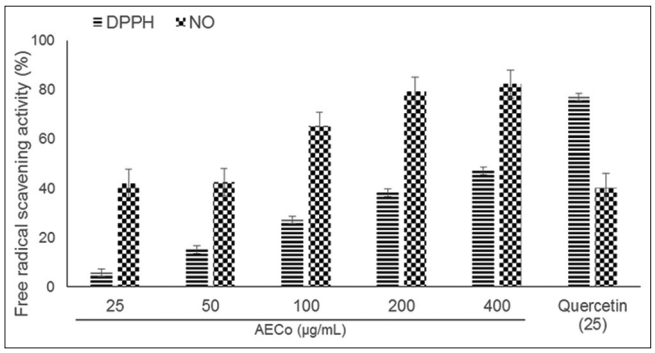

Figure 2: *In vitro* antioxidant activity of aqueous extract of *Chenopodium opulifolium*

Acute Toxicity

In the acute toxicity test of the AECO, there was no mortality or any signs of behavioral changes or pharmacotoxicity observed after oral administration of extract of the different doses even to the highest dose administered (5000 mg/kg body weight) in rats. No noticeable sign of toxicity was observed in all test doses until the end of the study period.

Anti-nociceptive Effect of AECO in Writhing Test

The pretreatment of mice with AECO 100, 200 and 400 mg/kg caused a significant ($P < 0.001$) and dose-dependent decrease in number of abdominal constriction in mice compared to control (Table 3). The percentage of inhibition of constriction was calculated as 49.1%, 52.2%, and 74.6% by AECO at 100, 200, and 400 mg/kg, respectively. Indomethacin (10 mg/kg) showed significant ($P < 0.001$) inhibition of abdominal constriction (75.9%).

Antinociceptive Effect of AECO in Hot Plate Test

In this model of thermally-induced nociception, pretreatment of mice with AECO (100-200 mg/kg) significantly prolong reaction time significantly ($P < 0.05$) compared to control as shown in the percent maximal possible effect curve (Figure 3). Dose-dependent maximal effect was observed at 60 min following administration. Pentazocine (5 mg/kg) showed a significant ($P < 0.001$) maximal possible effects when compared to control.

Table 1: Membrane stabilizing effect of AECO on heat-induced hemolysis of red blood cell

Treatment	Concentration (μg/mL)	Mean absorbance±SEM[a]		Percentage inhibition of hemolysis
		Heated solution	Unheated solution	
Control	-	1.034±0.062	0.036±0.011	-
AECO	100	0.795±0.013	0.038±0.001	24.1*
AECO	200	0.794±0.022	0.067±0.013	27.2*
AECO	400	0.759±0.003	0.124±0.024	36.5*
AECO	800	0.703±0.005	0.144±0.004	44.0*
AECO	1000	0.702±0.010	0.199±0.011	49.6*
Indomethacin	500	0.791±0.022	0.038±0.001	28.5*

[a]Values are triplicate measurements expressed as mean±SEM. *Denotes $P<0.05$ compared with control (Newmann Keuls *post-hoc* test).
AECO: Aqueous extract of *Chenopodium opulifolium*, SEM: Standard error of the mean

Table 2: Membrane stabilizing effect of AECO on hypotonicity - induced hemolysis of red blood cell

Treatment	Concentration (μg/mL)	Mean absorbance±SEM[a]	Percentage inhibition of hemolysis
Control	-	0.072±0.004	-
AECO	100	0.058±0.005	19.4
AECO	200	0.052±0.002	27.7
AECO	400	0.047±0.005	34.7
AECO	800	0.045±0.001	37.5*
AECO	1000	0.043±0.003	40.3*
Indomethacin	500	0.059±0.005	18.1

[a]Values are triplicate measurements expressed as mean±SEM. *Denotes $P<0.05$ compared with control (Newmann Keuls *post-hoc* test).
AECO: Aqueous extract of *Chenopodium opulifolium*, SEM: Standard error of the mean

Effects of AECO on Mice in the Open Field Test

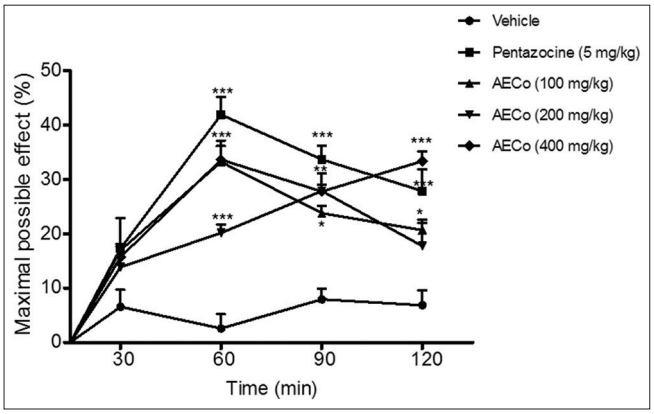

Figure 3: The effect of orally administered aqueous extract of *C. opulifolium* on the hot plate test. Result expressed as mean±standard error of the mean (*n*=5). The statistical significance was calculated by two-way analysis of variance followed by Bonferroni's test. **P*<0.05, ***P*<0.01, ****P*<0.001 when compared to vehicle

Table 3: Antinociceptive activity of AECO in acetic acid-induced abdominal constriction test in mice

Treatment	Dose (mg/kg)	No of writhing[a]	% inhibition
Vehicle	10 mL/kg	45.60±4.95	
AECO	100	23.20±1.53***	49.1
AECO	200	21.80±2.71***	52.2
AECO	400	11.60±1.63***	74.6
Indomethacin	10	11.00±1.14***	75.9

[a]Values are expressed as mean±SEM (*n*=5). Denotes *P*<0.001 compared with control (Newmann Keuls *post-hoc* test). AECO: Aqueous extract of *Chenopodium opulifolium*, SEM: Standard Error of Mean, ***: *P* < 0.001 compared with control (Newmann Keuls post hoc test),

Table 4: The effects of orally administered AECO in the open field test in mice

Treatment	Dose (mg/kg)	Parameters[a]	
		SMA[b]	Rearing
Vehicle	10 mL/kg	778.75±69.19	75.25±13.28
AECO	100	679.75±38.99	47.75±8.78
AECO	200	708.75±64.71	68.75±4.29
AECO	400	810.00±94.31	68.00±8.57

[a]Values are expressed as mean±SEM (*n*=5), [b]SMA. AECO: Aqueous extract of *Chenopodium opulifolium*, SEM: Standard error of the mean, SMA: spontaneous motor activity

Evaluation of the effect of oral administration of AECO on the spontaneous motor activity and rearing in mice did not show any significant alteration ($P > 0.05$) in those activities when compared with control (Table 4).

Anti-inflammatory Effect of AECO in Egg Albumin - Induced Edema in Rat Paw

The results obtained from this experiment are shown in Table 5. Egg albumin induced edema in all experimental animals at different time points, and maximum edema formation was observed at 30 min post albumin injection. Pre-treatment with AECO (100, 200 and 400 mg/kg) produced statistically significant ($P < 0.001$) and time-dependent inhibition of the edematous response during the 120 min duration measurement. The percentage inhibition of edema formation was 44.2%, 44.5%, and 51.2% by AECO 100, 200, and 400 mg/kg, respectively, while indomethacin administered at 10 mg/kg inhibited edema by 51.2%.

DISCUSSION

The findings of this study showed the antioxidants, antinociceptive and anti-inflammatory activities of AECO leaves. The phytochemical screening of AECO used in the pharmacological tests revealed the presence of alkaloids, tannins, phlobotannins, flavonoids, and saponins. Quantification of caffeic acid derivatives and TFC in the extract showed an appreciable amount of polyphenolics. The species of the family Chenopodiaceae are widely distributed weedy herbs often used commercially as spices or drugs because of the presence of useful secondary metabolites [31]. Phenolics, flavonoids, saponins, and triterpenoids were reported to be the major phytoconstituents of the *Chenopodium* genus, and about 300 and 79 compounds have been reportedly isolated [7]. AECO showed weak antiradical activity in the DPPH and potent activity in NO free radical scavenging assays. DPPH free radical scavenging assay is a primary assay for investigating the antioxidant properties of extracts. Antioxidant potentials usually correlate well with the phenolic composition of extract.

In a concentration-dependent manner, the extract shows membrane stability activity in both the heat and hypotonic solution-induced hemolysis of rat erythrocytes. The ability to protect the erythrocytes from stress-induced lytic effect of heat and hypotonic solution demonstrate the *in vitro* anti-

Table 5: Anti-inflammatory effects of AECO on egg albumin-induced rat paw oedema

Treatment groups	Dose (mg/kg)	Changes in paw sizes (cm)[a]			
		30	60	90	120
Vehicle	10 mL/kg	1.03±0.07	0.65±0.03	0.55±0.07	0.43±0.02
AECO	100	0.88±0.04 (14.6)	0.52±0.07 (25.0)	0.38±0.04 (30.9)	0.24±0.02 (44.2)*
AECO	200	0.64±0.04 (37.9)***	0.40±0.05 (38.5)**	0.32±0.05 (41.8)**	0.24±0.04 (44.5)*
AECO	400	0.54±0.09 (47.6)***	0.38±0.07 (41.5)***	0.32±0.05 (41.8)**	0.21±0.02 (51.2)*
Indomethacin	10	0.58±0.05 (43.7)***	0.38±0.04 (41.5)***	0.36±0.04 (34.5)*	0.21±0.02 (51.2)*

[a]Results expressed as mean±SEM (*n*=5). The statistical significance was calculated by two-way ANOVA followed by Bonferroni's test. **P*<0.05, ***P*<0.01, ****P*<0.001 when compared to vehicle. Percentage inhibitions of oedema formation are in parenthesis. AECO: Aqueous extract of *Chenopodium opulifolium*, SEM: Standard error of the mean, ANOVA: analysis of variance

inflammatory properties of the extract. During inflammation, there are lyses of lysosomes which release their component enzymes that produce a variety of disorders. The lysosomal enzymes are implicated in the pathogenesis of articular tissue degradation in several rheumatic diseases. Drugs by stabilizing the membrane can prevent the rupture of the lysosomes and inhibit the release of lysosomal enzymes [32]. The RBC membrane stabilization test serves as a model for lysosomal membrane stabilization since several agents capable of releasing hydrolytic enzymes from lysosomes also injure erythrocytes [24]. The presence of polyphenols such as flavonoids and tannins in AECO may contribute to the anti-inflammatory effects observed. Flavonoids are known to exert profound stabilizing effects on lysosomes membrane which is often attributed to their free radical scavenging properties [33].

Results of the acute toxicity test of the AECO showed that there was no mortality or any significant change in the behavior of the rats recorded up to the dose of 5000 mg/kg body weight. Acute toxicity test provides preliminary information on the toxic nature of a material for which no other toxicological information is available [34]. Based on the results of the preliminary toxicity testing on AECO, the highest dose was found to be non-lethal, hence we can safely conclude that median lethal dose (LD_{50}) is >5000 mg/kg body weight. The high safety margin of AECO may partly explain the historical use of *C. opulifolium* decoction or infusion in managing fever associated with malaria parasite infection in Uganda [13]. The information from acute toxicity studies is often used in dose setting for other studies, thus the set dose for anti-inflammatory screening of AECO was lower than 1/10th fraction of the highest tolerated dose [35,36].

C. opulifolium extract showed similar antinociceptive effect to Indomethacin in the acetic acid-induced abdominal writhing test. This model characterized by stereotypical behavior of abdominal constriction involves peripheral nociceptive mechanisms. Most importantly is the release of biogenic amines (e.g., histamine and serotonin), bradykinin, prostaglandin E_2 and $PGF_2\alpha$ which activates visceral nociceptors [37].

Thermally-induced nociception in the hot plate is considered to be selective for centrally acting analgesic compounds (morphine-like drugs). AECO showed positive activity in this test. In order to rule out the chances of false positive effect of AECO, we evaluated its effect on spontaneous locomotor function in the activity meter cage. AECO did not alter the ambulatory and rearing activities. The results revealed that the observed antinociceptive effect of AECO was not as a result of sedation or impairment of motor activity in mice. Taken together, effect in acetic acid-induced writhing and hot plate test revealed that AECO antinociceptive activity might involve peripheral, spinal and supraspinal inhibition of pain.

The AECO leaves exhibited an anti-inflammatory effect by inhibiting the edema induced by egg albumin in rat paw. This model is used as an *in vivo* model of acute inflammation that can be used to screen agents for anti-inflammatory effect [29,30]. The results showed that AECO suppressed inflammation at the early phase, an indication that it may be blocking the release of the early phase mediators. Histamine, bradykinin, serotonin, and prostaglandins are inflammatory factors released from damaged cells, and they are mediators of inflammation and pain. Prostaglandins are products of arachidonic acid metabolism via the cyclooxygenase pathway [29].

CONCLUSION

This study clearly revealed that the AECO leaf contains polyphenolics (flavonoids, caffeic acid, and tannins) that showed antioxidant, antinociceptive, and anti-inflammatory activities. This result provides a scientific credit for the use of *C. opulifolium* in traditional medicine as a remedy against inflammatory conditions and thus a candidate for further studies.

ACKNOWLEDGMENTS

We acknowledge the kind assistance of Associate Professor Dominic Byarugaba for plant authentification and Mr. Pascal Mwembembezi and Ronald, in the school of Pharmacy, Kampala International University, Western Campus for technical assistance.

REFERENCES

1. Okigbo RN, Eme UE, Ogbogu S. Biodiversity and conservation of medicinal and aromatic plants in Africa. Biotechnol Mol Bio Rev 2008;3:27-134.
2. WHO. Traditional Medicine Strategy 2002-5. 2002, WHO/EDM/TRM. Geneva: World Health Organization; 2002.
3. Kamatenesi-Mugisha M, Oryem-Origa H. Medicinal plants used to induce labour during childbirth in western Uganda. J Ethnopharmacol 2007;109:1-9.
4. Tabuti JR, Kukunda CB, Kaweesi D, Kasilo OM. Herbal medicine use in the districts of Nakapiripirit, Pallisa, Kanungu, and Mukono in Uganda. J Ethnobiol Ethnomed 2012;8:35.
5. Smith BD. Eastern North America as an independent center of plant domestication. Proc Natl Acad Sci U S A 2006;103:12223-8.
6. Bylka W, Kowalewski Z. Flavonoids in *Chenopodium album* L and *Chenopodium opulifolium* L. Herba Pol 1997;43:208-13.
7. Kokanova-Nediakova Z, Nedialkov PT, Nikolov SD. The genus *Chenopodium*: Phytochemistry, ethnopharmacology and pharmacology. Pharmacogn Rev 2009;3:280-306.
8. Mugisha-Kamatenesi M, Deng AL, Ogendo JO, Omolo EO, Mihale MJ, Otim M, *et al*. Indigenous knowledge of field insect pests and their management around Lake Victoria basin in Uganda. Afr J Environ Sci Technol 2008;2:342-8.
9. Esezah K, Godwin A, Fredrick A, Ogwal-Okeng J. Antifungal medicinal plants used by communities adjacent to Bwindi impenetrable National Park, South Western Uganda. Eur J Med Plants 2015;7:184-92.
10. Ruffo CK, Birnie A, Tengnas B. Edible Wild Plants of Tanzania. RELMA Technical Handbook Series. Nairobi, Kenya: RELMA; 2002. p. 27.
11. Mugisha-Kamatenesi M, Oryem-Origa H, Odyek O. Medicinal plants used in some gynaecological morbidity ailments in Western Uganda. Afr J Ecol 2007;45:34-40.
12. Ssegawa P, Kasenene JM. Medicinal plant diversity and uses in the Sango bay area, Southern Uganda. J Ethnopharmacol 2007;113:521-40.
13. Nalumansi P, Kamatenesi-Mugisha M, Godwin A. Medicinal plants used in Paediatric ealth care in Namungalwe Sub County, Iganga District, Uganda. Nova J Med and Biol Sci 2014;2:1-14.
14. Kisangau DP, Lyaruu HV, Hosea KM, Joseph CC. Use of traditional medicines in the management of HIV/AIDS opportunistic infections in Tanzania: A case in the Bukoba rural district. J Ethnobiol Ethnomed 2007;3:29.
15. Yeap SK, Ho WY, Beh BK, Liang WS, Ky H, Yousr AH, *et al*.

Vernonia amygdalina, an ethnoveterinary and ethnomedically used green vegetable with multiple bioactivities. J Med Plants Res 2010;4:2787-812.
16. National Institute of Health. Guide for the Care and Use of Laboratory Animals. Washington, DC: National Academic Press, 1996.
17. Zimmermann M. Ethical guidelines for investigations of experimental pain in animals. Pain 1983;16:109-10.
18. Sofowara A. Medicine Plants and Traditional Medicine in Africa. Ibadan, Nigeria: Spectrum Books Ltd.; 1993.
19. Evans WC. Trease and Evans Pharmacognosy. 15th ed. Edinburgh: Habid, W.B Saunders; 2002.
20. Benedec D, Vlase L, Hanganu D, Oniga I. Antioxidant potential and polyphenolic content of Romanian *Ocimum basilicum*. Digest J Nanomat Biostruct 2012;7:1263-70.
21. Sultana B, Farooq A, Muhammad A. Effect of extraction solvent/ technique on the antioxidant activity of selected medicinal plant extracts. Molecules 2009;14:2167-80.
22. Aderogba MA, Okoh EK, Adelanwa TA, Obuotor EM. Antioxidant properties of the *Nigerian piliostigma* species. J Bio Sci 2004;4:501-3.
23. Kumaran A, Karunakaran RJ. Antioxidant and free radical scavenging activity of an aqueous extract of coleus aromaticus. Food Chem 2006;97:109-14.
24. Anosike CA, Obidoa O, Ezeanyika LU. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanum aethiopicum).* DARU J Pharm Sci 2012;20:76.
25. Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Sarsf MN. Membrane stabilization activity - A possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. Fitoterapia 1989;70:251-7.
26. Adzu B, Haruna AK. Studies on the use of *Zizyphus spina-christi* against pain in rats and mice. Afr J Biotechnol 2007;6:1317-24.
27. Koster R, Anderson M, DeBeer EJ. Acetic acid for analgesic screening. Fed Proc 1959;18:412.
28. Turner RA. In: Turner RA, Hebban P, editors. Analgesics: Screening Methods in Pharmacology. New York: Academic Press; 1965. p. 100.
29. Akah PA, Nwambie IA. Evaluation of Nigerian traditional medicinal plants used for rheumatoid disorders. J Ethnopharamacol 1994;42:179-82.
30. Ajayi AM, Tanayen JK, Balogun SO, Ibrahim A, Ezeonwumelu JO, Kiplagat D, *et al.* Anti-inflammatory and analgesic properties of ethanolic stem bark extract of *Ficus trichopoda* in rats. Pharmacogn J 2011;2:43-7.
31. Dembitsky V, Shkrob I, Hanus LO. Ascaridole and related peroxides from the genus *Chenopodium.* Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2008;152:209-15.
32. Ignarro LJ. Effects of anti-inflammatory drugs on the stability of rat liver lysosomes *in vitro*. Biochem Pharmacol 1971;20:2847-60.
33. Oyedapo OO, Akinpelu BA, Akinwunmi KF, Adeyinka MO, Sipeolu FO. Red blood cell membrane stabilizing potentials of extracts of *Lantana camara* and its fractions. Int J Plant Physiol Biochem 2010;2:46-51.
34. Asare GA, Addo P, Bugyei K, Gyan B, Adjei S, Otu-Nyarko LS, *et al.* Acute toxicity studies of aqueous leaf extract of *Phyllanthus niruri*. Interdiscip Toxicol 2011;4:206-10.
35. NC3RS. National Centre for Replacement, Refinement, and Reduction of Animals in Resaerch; 2007.
36. Bala A, Kar B, Haldar PK, Mazumder UK, Bera S. Evaluation of anticancer activity of *Cleome gynandra* on Ehrlich's Ascites Carcinoma treated mice. J Ethnopharmacol 2010;129:131-4.
37. Duarte ID, Nakamura M, Ferreira SH. Participation of the sympathetic system in acetic acid-induced writhing in mice. Braz J Med Biol Res 1988;21:341-3.

Source of Support: Nil, Conflict of Interest: None declared.