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Evaluation of the Antioxidant and Analgesic Activities of Hydromethanolic Extract of *Chromolaena odorata* Leaf

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SOO and ECN designed the study, wrote the protocol and executed it, managed literature searches and wrote the first draft of the manuscript. Authors MIE and YNO managed the literature searches and statistical analyses of the data. Author COO collected the plant and managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Objective: The aim of the study was to evaluate the analgesic and antioxidant effects of hydromethanolic extract of *Chromolaena odorata* leaf in rodents.
Methods: The antioxidant activities were evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) Assay. Acetic acid induced writhing reflex and tail flick models were used in the evaluation of the analgesic activities. The rats were grouped into five (A-E) groups of six rats each. The group A and B received 10 ml/kg of water and 200 mg/kg of aspirin respectively. Group C-E received 100 mg/kg, 200 mg/kg and 400 mg/kg of the extract respectively.

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Results: The extract caused a significant ($p<0.05$) decrease in pain sensation in both models. In acetic acid induced writhing, the extract (200 and 400 mg/kg) produced 13.13% and 27.27% reduction in number of writhing in treated rats respectively, when compared to the negative control. In tail flick model, the extract (200 and 400 mg/kg) caused 33.33% and 45.33% increase in pain reaction time (PRT) in the treated group respectively, when compared to the negative control group. In DPPH assay the extract produced a concentration dependent increase in antioxidant activity while in FRAP, there was no concentration dependent increase in antioxidant activity.
Conclusion: Hydromethanolic extract of *Chromolaena odorata* produced a potent analgesic and antioxidant in rodents.

Keywords: Chromolaena odorata; analgesic; antioxidant; acetic acid; writhing reflex; tail flick.

1. INTRODUCTION

Chromolaena odorata commonly called "Saim weed", "Devil weed", "French weed" belong to the family *Asteraceae*. It is native to south and Central America, and has been introduced to many tropical countries of Africa, Asia and other parts of the world [1]. It is a rapidly growing herbaceous perennial shrub that grows up to 2.5 metre high. It has a soft aerial stem; that is woody at the base. The leaves are opposite, arrowhead-shaped, serrated margin and three prominent veins in a "pitchfork" pattern [2]. *Chromolaena odorata* leaves are extensively used in traditional medicine in the management of burns, soft tissue wounds and skin infections [3,4]. In South-Eastern Nigeria, the leaf extract is employed in pain and wound healing. Also, the leaf of the plant is included in the mixture (medicinal plant) for the management of malaria, fever and cough [5]. The antioxidant, antibacterial and antimicrobial, haemostatic, wound healing, anti-inflammatory, astringent and diuretic activities of extracts of *chromolaena odorata* have been reported [4-8]. Akinmoladun et al. [1] reported the presence of alkaloids, saponins, terpenes, steroids, tannins, flavonoids and glycosides in *Chromolaena odorata* leaf. The pharmacological activities of medicinal plants depend on the nature and quantity of their constituent phytochemicals which are affected by the climatic factor and soil conditions of where the plant was grown [9]. This study was aimed at the evaluation of the analgesic and antioxidant activity of hydromethanolic extract of *Chromolaena odorata* leaf collected from South Eastern Nigeria.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

The fresh leaves of *Chromolaena odorata* was collected from Umudike, Abia state Nigeria in June 2015. The leaf was identified by Dr O. Ukpabi of National Root Crop Research Institute, Umudike as *Chromolaena odorata.*

2.2 Preparation of Plant Extract

The leaf of *Chromolaena odorata* was air dried on a laboratory bench at room temperature. The dried leaf was pulverized into a coarse powder with a manual grinder (corona, China). Two hundred (200) gram of the coarse powder was soaked in 80% methanol in a Winchester bottle and was shaken at 3 hours interval for 48 hours. The extract was filtered with Whatmann's No 1 filter paper. The filtrate was concentrated in electric hot air oven (Surgifriend Medicals, England) maintained at 40℃. The *Chromolaena odorata* (COE) obtained was stored in a refrigerator at 4℃ until require for the experiment.

2.3 Animal

Sixty five rats of both sexes were obtained from the laboratory animal unit of Department of Veterinary Physiology, Pharmacology, Biochemistry and Animal Health; Michael Okpara University of Agriculture Umudike, Abia state. The rats were housed in aluminum cage at an average of six rats per cage and were fed *ad libitum* with commercial feed (Grower, Vital feed) and they had free access to clean drinking tap water. The rats were maintained under natural light/dark daily cycle. The rats were acclimatized for 2 weeks prior to the study. The rats were maintained in accordance with the recommendations of the Guide for the care and use of laboratory animals [10].

2.4 Acute Oral Toxicity

The acute oral toxicity was assessed with "Up and Down" method as describe by Organization for Economic Cooperation and Development [11]. Five rats were dosed 2000 mg/kg of COE

and were observed for 48 h for signs of toxicity and death.

2.5 Evaluation of Antioxidant Activities of COE Using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Photometric Assay

The antioxidant activity of COE was evaluated using DPPH photometric assay [12]. The test extract (2 ml) at different concentrations (25, 50, 100, 200, and 400 µg/ml) were mixed with 0.5 mM DPPH (in 1 ml of methanol) in a cuvette. The absorbance at 517 nm was taken after 30 min of incubation in the dark at room temperature. The concentrations were prepared in triplicates and the percentage antioxidant activity calculated as follows.

% antioxidant activity (AA) =
100-({[absorbance of sample —absorbance of blank] ×100}/ absorbance of control)

A volume of 1 ml of methanol plus 2.0 ml of the extract was used as the blank while 1.0 ml of the 0.5 mM DPPH solution plus 2.0 ml of methanol were used as the negative control. Ascorbic acid (vitamin C) was used as reference standard [13].

2.6 Ferric Reducing Antioxidant Power (FRAP) of COE

The ferric reducing antioxidant power was carried out as described by Benzie and Strain [14].

2.7 Reagents

1. Acetate buffer (300 mM), p^H 3.6 (3.1 g sodium acetate. $3H_2O$ and 16 ml glacial acetic acid in 1000 ml buffer solution).
2. 2, 4, 6-triphridyl-s-triazine (TPTZ) (10nM) in 40 mM HCl.
3. $FeCl_3.6H_2O$ (20 mM) in distilled water.

The working solution was prepared by mixing solution 1, 2, and 3 in the ratio of 10:1:1 respectively. The working solution was freshly prepared in test. The aqueous solution of known amount of ascorbic acid was use for calibration.

FRAP reagent (3 ml) and 100 µl sample solution at concentrations of 20, 50,100, 200 and 400 µg/ml was mixed and allowed to stand for 4 minutes. Colometric reading were recorded at 593 nm, at 37℃.The ascorbic acid standard was tested in parallel process. Calculations were made by a calibration curve.

FRAP value of sample (µmol/L) =
{[Changes in absorbance of sample 4 min – 0 min/ Changes in absorbance of standard 4 min - 0 min] × FRAP Value of standard (2 µmol/L)}

2.8 Effect of COE on Acetic Acid-induced Abdominal Writhing in Rats

The modified method of Hosseinzadeh and younesi [15] was adopted in this experiment. Thirty rats were randomly assigned to five groups (A-E) of 6 rats each. The rats were fasted for 12 h but free access to tap water was allowed. Group A served as negative control and received distilled water (10 ml/kg). Group B served as positive control and received acetylsalicylic acid (ASA) (200 mg/kg), while Groups C-E received 100, 200, and 400 mg/kg of COE, respectively. The drug and extract were administered orally. Forty five minutes post treatment the rats received 10 ml/kg of 0.7% acetic acid intraperitoneally. The number of writhing produced in each rat was counted for 30 min.

2.9 Effects of COE on Tail Flick Response in Rats

The modified method as described by Onoja et al. [16] was employed in this experiment. Thirty rats were randomly divided into 5 Groups (A-E) of 6 rats each. The rats were fasted for 12 h with free access to drinking water. The rats were treated as follows: Group A served as negative control and received distilled water (10 ml/kg) orally, Group B served as positive control and received pentazocine (3 mg/kg) intraperitoneally, while Group C-E received COE (100, 200 and 400 mg/kg, respectively) orally. One hour post drug treatment about 3 cm of the tail of each rat was immersed into a water bath containing warm water maintained at a temperature of 55±1℃. The pain reaction time (PRT) was recorded for each rat.

2.10 Statistical Analysis

Data obtained were presented as mean ± standard error of mean and analyzed using one-way Analysis of Variance of SPSS software. The variant mean was separated by least significant difference of the different groups. Significance was accepted at the level of $P < 0.05$.

3. RESULTS

The animals did not show any signs of toxicity during the period of acute toxicity study.

3.1 Effect of COE on Tail Flick Test

The result of tail flick test of COE treated rats is presented in Table 1. The extract at 100, 200 and 400 mg/kg produced a significant ($p < 0.05$) decrease in pain sensation in treated with, 51.33%, 33.33% and 45.33% increase in pain reaction time respectively, when compared to the negative control. At 100 mg/kg, the pain reaction time of the extract (51.33%) was comparable to the positive control (57.33%).

3.2 Effect of COE on Acetic Acid Induced Writhing Test

The result of the acetic acid Induced writhing is presented in Table 2. The aspirin and extract (200 and 400 mg/kg) significantly ($p< 0.05$) reduced the number of the abdominal writhing in the treated rats when compared to negative control groups. The ASA and extract (200 and 400 mg/kg) produced 63.64%, 13.13% and 27.27% reduction in abdominal writhing respectively, when compared to the negative control.

3.3 DPPH Photometric Assay of COE

The result of DPPH Photometric assay of COE is presented in Fig. 1. The Extract produced a concentration dependent increase in antioxidant activity. At 400 mg/ml, the antioxidant activity of the extract (82.20%) was comparable to the antioxidant activity of the ascorbic acid (94.96%).

3.4 Ferric Reducing Antioxidant Power of COE

The result of ferric reducing antioxidant power of COE is presented in Fig. 2. The extract produced no concentration dependent effect. The optimum effect was noticed at 50 µg/ml concentration.

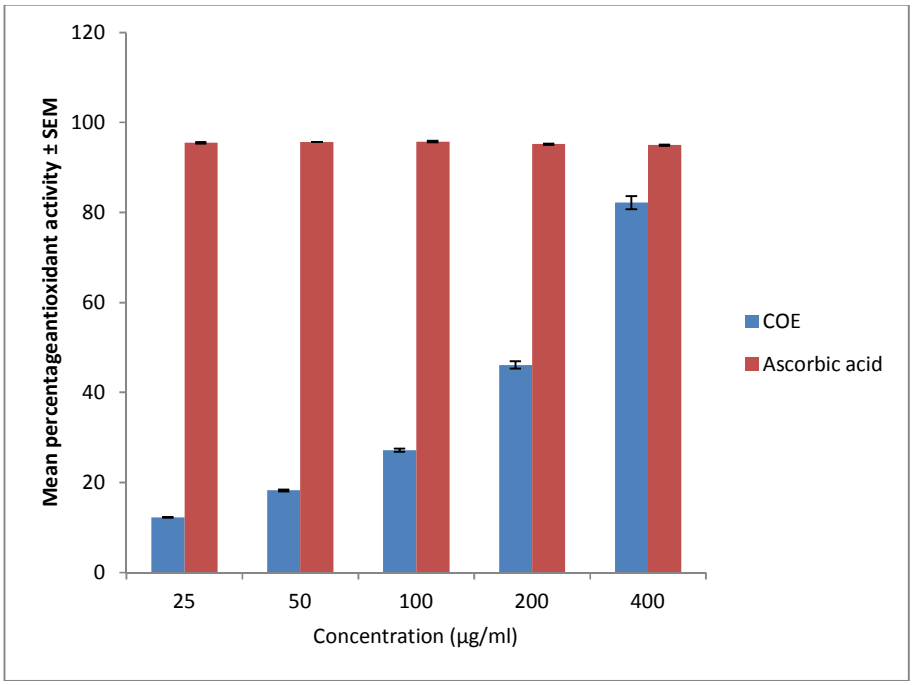

Fig. 1. DPPH photometric assay of COE

Table 1. The effect of COE on tail flick test

Treatment	PRT (sec)	% Increase in PRT
Distilled water 10 ml/kg	1.50±0.17	0.00
Aspirin 200 mg/kg	2.36±0.42*	57.33
COE 100 mg/kg	2.27±0.33*	51.33
COE 200 mg/kg	2.00±0.16*	33.33
COE 400 mg/kg	2.17±0.09*	44.67

**P< 0.05 compared with the negative control*

Table 2. Effect of COE on acetic acid induced writhing

Treatment	Number of writhing	% Inhibition
Distilled water 10 ml/kg	16.50±0.66	-
Aspirin 200 mg/kg	6.00±0.41*	63.64
COE100 mg/kg	16.25±0.85	1.52
COE 200 mg/kg	14.33±0.33*	13.13
COE 400 mg/kg	12.00±0.41*	27.27

**P< 0.05compared with the negative control*

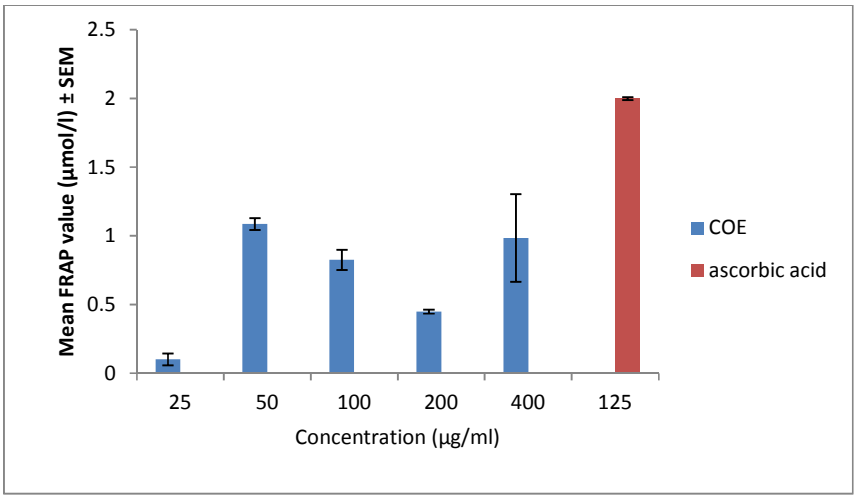

Fig. 2. Ferric reducing antioxidant power of COE

4. DISCUSSION

The antioxidant and analgesic properties of hydromethanolic extract of *Chromolaena odorata* were evaluated in the present study. The extract was well tolerated by the rats as no sign of toxicity was observed. The analgesic property was investigated using acetic acid induced writhing reflex and tail flick models. Acetic acid induced writhing is a commonly used model for peripheral active analgesic [17-23]. Pain sensation in acetic acid induced writhing is mediated by inflammatory response resulting from the release of arachidonic acid from tissue phospholipid and the subsequent biosynthesis of prostaglandins by cyclooxygenase [23,24]. This will lead to increased prostaglandin level in peritoneal fluid which in turn stimulates the peritoneal nociceptors [25]. The tail flick (thermal test) was used as deep pain model, since the pain sensation involves the central nervous system. It is sensitive to strong analgesic and has limited tissue injury [18]. The animal usually flicks the tail in response to the perceived thermal stimuli.

The extract demonstrated potent analgesic and antioxidant potentials which could be mediated by the constituent phytochemical compounds. Akinmoladun et al. [1] reported the presence of alkaloids, saponins, terpenes, steroids, tannins, flavonoids and glycosides in *Chromolaena odorata* leaf. Each of these phytochemicals has been reported to possess analgesic and antioxidant activities [26-28].

The extract produced a greater effect in the tail flick response than in the acetic acid induced writhing reflex and can be attributed to antispasmodic [29] and central nervous system depressant activity of *Chromolaena odorata* [28]. This suggests that the extract has a better effect against deep pain than peripheral pain stimuli. The effects of the extract was comparable to pentazocine; a synthetic opioid agonist-antagonist that produces narcotic analgesia and sedation by an interaction with k-receptors [30]. Our finds is in agreement with the report of Owoyele et al. [31]. The demonstrated antioxidant activity would help in the amelioration of the stress associated with pain sensation.

5. CONCLUSION

In conclusion, this study validates the folkloric uses of *Chromolaena odorata* leaf in management of pain and oxidative stress. Further studies are recommended to isolate and characterize the active principle.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Authors declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the institution's ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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