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Experimental Evaluation of Analgesic and Antioxidant Effects of Hydromethanolic Extract of *Dioscorea dumetorum* Tuber

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Authors' contributions

This work was carried out in collaboration among all authors. Author MIE designed the study and wrote the protocol. Author SOO managed the animal, collected the data and performed the statistical analysis. Author COU collected data, did literature search and wrote the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: The hydromethanolic extract of *D. dumetorum was* evaluated for antinociceptive and antioxidant activities to validate its uses in traditional medicine for these purposes.
Methods: The antioxidant activity of *D. dumetorum* was done in vitro using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) photometric assay. The antinociceptive effect of the extract were investigated using acetic acid-induced writhing reflex and tail flick models with aspirin and pentazocine as reference drugs respectively.

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Results: The extract (200 mg/kg) produced a comparable effect to aspirin (100 mg/kg) in the acetic acid induced abdominal writhing reflex. In tail flick respond model, the extract produced significant ($P<0.05$) dose-dependent increase in pain reaction time (PRT) in the treated rats when compared to the distilled water treated rats. The extract (200 mg/kg) and pentazocine (3 mg/kg) produced 62.86 and 62.52% increase in PRT, respectively when compared to the negative controls. Similarly, DPPH radical scavenging activity of *D. dumentorum* extract produced concentration-dependent increase in the percentage antioxidant activity in the DPPH photometric assay. The extract at 400 µg/ml concentration produced antioxidant activity comparable to the standard.
Conclusion: The results of our study confirmed the uses of *D. dumetorum* in traditional medicine and provided evidence that tuber extract of *D. dumetorum* might indeed be potential sources of natural antinociceptive and antioxidant agents.

Keywords: Dioscorea dumetorum; antinociceptive; antioxidant; pentazocine; aspirin.

1. INTRODUCTION

Food is very essential to man and animal as it prevents and reduces several risk factors of diseases and enhances mental and physical well-being [1]. One of such food is *Dioscorea dumetorum* (Bitter yam or trifoliate yam). *Dioscorea dumetorum* occurs in both wild and cultivated forms. It is mainly cultivated in Tropical Africa with Nigeria and Cameroon as the major producers [2]. It is a tuber crop with fleshy edible parts which can be yellow or white and occurs naturally in clusters hence its name cluster yam. It has different names among different ethnic groups in Nigeria. It is called *"una*" by the Igbos of Southeast Nigeria, *Kosanrogo* in Hausa and *Esuru* in Yoruba [1,2,3]. The *D. dumetorum* has high nutritive value, containing high crude protein (11.4%), crude fibre (2.03%), carbohydrate (77.55%) and energy (361.90 kcal/100 g), vitamins, minerals and amino acid [4,5]. The phytochemical profile has been reported containing, fatty acids, phenols, sterols, aldehydes, ketones, alcohols, hydrocarbons, esters, amines and alkaloid [3,6].

There are edible and non edible *D. dumetorum.* The edible varieties are cultivated while non edible varieties grow in wild and are not normally eaten except at times of food scarcity. However, the non edible *D. dumetorum* are highly utilized in traditional medicine treatment of some illness such diabetes mellitus, schistosomiasis, jaundice, malaria, as a topical anesthetic and also applied to suppurating abscesses [1,7,8]. The antidiabetic, antioxidant and antimicrobial effects of *D. dumetorum* have been reported [2,8-11]. These effects have been attributed to some compounds found in the tuber such as 9, 12, Octadecadienoic acid (Linoleic acid) and, 3, 5-Di-t-butyl phenol [6].

However, the analgesic property of the *D. dumetorum* has not been extensively studied owning to the little scientific information on the antinociceptive effects of the plant. This necessitated the design of this study to evaluate the antinociceptive effects of the hydromethanolic extract of *D. dumetorum* in order to validate its uses in traditional medicine for this purpose.

2. MATERIALS AND METHODS

Fresh tubers of non edible strain of *D. dumetorum* were harvested in the wild from Umuida in Enugu-Ezike, Enugu State, Nigeria.

2.1 Preparation of the Plant Extract

The tuber was sliced into smaller pieces, dried and reduced into a coarse powder using the manual grinder (Corona China). The cold maceration method was used for the extraction of coarse powder of *D. dumetorum* (86 g) using 80% methanol in a Winchester bottle for period of 48 h at room temperature. Filtration was done using Whatman No. 1 filter papers, funnel and conical flask. The filtrate was kept in a hot oven at 40ºC to enhance the evaporation of the methanol and concentrate the filtrate. The *D. dumetorum* extract (DDE) was stored in a refrigerator at 4°C throughout the duration of this study [12]. The weight of *D. dumetorum* recovered was 13.3 g and the percentage yield was 15.5% w/w dry matter. The extract was dark brown in color and solid in consistency.

2.2 Experimental Animals

Thirty five male Wistar Albino rats (120-140 g) and thirty male mice (28-35 g) were used in the study. The animals were obtained from the

laboratory animal unit of the Department of Veterinary Physiology, Pharmacology, Biochemistry, Animal Health and Production; Michael Okpara University of Agriculture, Umudike. They were maintained in accordance with the recommendations of the "guide for the care and use of laboratory animals" [13]. They were kept in clean dusted aluminum cage at 6 animals per cage and were fed *ad libitum* with standard commercial pelleted feed (Vital Feed®) with free access to clean drinking water, kept at normal environmental temperature and natural light/darkness daily cycle. They were allowed 2 weeks to acclimatize before the commencement of the experiment. The animal experiment protocol was approved by the Michael Okpara University of Agriculture Animal Ethics Committee.

2.3 Oral Acute Toxicity Study

The oral acute toxicity test of *D. dumetorum* extract (DDE) was determined according to the OECD guideline No. 425 (acute oral toxicity-up-and-down-procedure). Briefly, a group of five rats were dosed orally with 2000 mg/kg of *D. dumetorum* and were observed for 48 h for signs of toxicity and death [14].

2.4 *In vitro* Antioxidant Activities of the *D. dumetorum* Extract

The free radical scavenging activity of DDE was analyzed by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) photometric assay [12]. This was done using 2 ml of the extracts at different concentrations (25, 50, 100, 200 and 400 µg/ml) each mixed with 0.5 mM DPPH (in 1 ml of methanol) in a cuvette and incubated for 30 min in the dark at room temperature. The absorbance at 517 nm was taken after the incubation. The concentrations were prepared in triplicates and the percentage antioxidant activity (AA) 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 ml of the extract was used as the blank while 1 ml of the 0.5 mM DPPH solution plus 2 ml of methanol was used as a negative control. Ascorbic acid (vitamin C) was used as a reference standard [15].

2.5 Effect of *D. dumetorum* on Acetic Acid-induced Abdominal Writhing in Mice

The acetic acid-induced writhing test measures abdominal constrictions together with stretching of the hind limbs resulting from intraperitoneal (i.p.) injection of 0.7% acetic acid (10 mL/kg). This was carried out according to the procedures described by Vale et al. [16]. Five Groups (A-E) of mice consisting of 6 mice each were fasted for 12 h but given adequate amount of water. Group A mice received distilled water (10 ml/kg) and served as negative control. Group B mice received aspirin (100 mg/kg) orally (positive control), while Groups C-E mice received 50, 100 and 200 mg/kg of DDE by oral administration, respectively. After 45 min, the mice received 10 ml/kg of 0.7% acetic acid intraperitoneally. Then, the number of writhing or abdominal stretches produced in each mouse was counted for 30 min.

2.6 Effects of *D. dumetorum* on Tail Flick Response in Rats

The experiment was carried out by measuring tail withdrawal time from hot water [17]. Thirty rats were randomly divided into 5 Groups (A-E) of 6 rats each and fasted for 12 h. The rats were treated as follows; Group A served as negative control and received 10 ml/kg distilled water (10 mg kg) orally, Group B served as positive control and received pentazocine (3 mg/kg) intraperitoneally while Group C-E received DDE (50, 100 and 200 mg/kg, respectively) orally. One hour post-drug treatment about 3 cm of the tail of each rat was dipped into a water bath containing hot water maintained at temperature of 50±1ºC. The time taken for the rat to flick the tail, known as pain reaction time (PRT) was recorded for all the rats.

2.7 Statistical Analysis

Statistical analysis was done using SPSS, version 17.0. Data obtained were presented as mean ± standard error of the mean and analyzed using one-way Analysis of Variance. 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

3.1 Oral Acute Toxicity Study

Neither clinical signs of toxicity nor death was recorded following oral administration of the

D. dumetorum extract at higher dose of 2000 mg/kg at the end of 48 hours period.

3.2 Effects of *D. dumetorum* on Acetic Acid-induced Writhing reflex in Mice

The D. *dumentorum* methanolic extract and aspirin produced high inhibition to acetic acid – induced writhing reflex in mice. The DDE (50 mg/kg, 100 mg/kg and 200 mg/kg) produced significant (P<0.05) dose-dependent decrease in the mean number of abdominal constriction in the treated group of mice when compared to the negative control. However, The DDE (200 mg/kg) produced a comparable effect (P<0.05) to aspirin (100 mg/kg) in the acetic acid induced abdominal writhing reflex (Table 1).

3.3 Effects of *D. dumetorum* on Tail Flick Response in Rats

In tail flick respond model, the extract produced significant (P<0.05) dose-dependent increase in pain reaction time (PRT) in the treated rats when compared to the distilled water treated rats. The extract (200 mg/kg) and pentazocine (3 mg/kg) produced 62.86 and 62.52% increase in PRT, respectively when compared to the negative controls (Table 2).

3.4 Effect of *D. dumetorum* on DPPH Radical Scavenging

The result of DPPH radical scavenging activity of DDE is showed in Fig. 1. The DDE produced concentration-dependent increase in the percentage antioxidant activity (AA) in the DPPH photometric assay. The increase in concentration from 25 µg/ml to 400 µg/ml produced an appreciable increase in the percentage AA.

Table 1. Effects of *Dioscorea dumetorum* extract on acetic acid induced writhing reflex in mice

Treatment	Mean number of writhing ± SEM	% inhibition
Distilled water 10 ml/kg	125.5±0.78	-
aspirin 100 mg/kg	30.75±0.62*	75.5
DDE 50 mg/kg	44.44±0.94*	64.59
DDE 100 mg/kg	39.14±1.40*	68.81
DDE 200 mg/kg	18.75±0.81*	85.06

**P<0.05 compared with the negative control (distilled water), SEM: standard error of mean DDE: Dioscorea dumetorum extract*

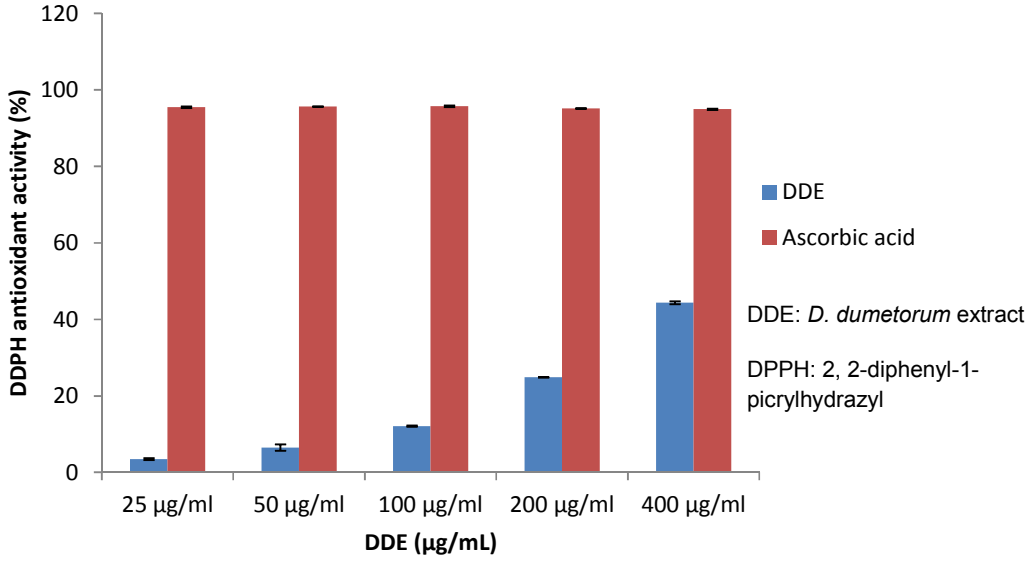

Fig. 1. Effect of DDE on DPPH radical scavenging

Table 2. Effects of *Dioscorea dumetorum* extract on tail flick response in rats

Treatment	Mean PRT ± SEM (Sec)	% inhibition of pain
Distilled water 10 ml/kg	5.87±0.20	-
pentazocine 3 mg/kg	9.54±2.36*	62.52
DDE 50 mg/kg	5.80±0.56	-1.19
DDE 100 mg/kg	8.22±0.35	40.03
DDE 200 mg/kg	9.56±1.07*	62.86

**P<0.05 compared with the negative control (distilled water), SEM: standard error of mean DDE: Dioscorea dumetorum extract, PRT: pain reaction time*

4. DISCUSSION AND CONCLUSION

The DDE was well tolerated by the rats, as no death and clinical signs of toxicity were recorded at the high dose of 2000 mg/kg. This indicates that the LD50 is greater than 2000 mg/kg dose.

The acetic acid-induced writhing reflex is a sensitive procedure in screening peripherally acting analgesics [18]. Intra-peritoneal injection of acetic acid induces an abdominal constriction response which is thought to involve local peritoneal receptors [18,19]. Acetic acid induces writhing by direct stimulation of acid sensitive receptors or through the release of pain mediators such as histamines, serotonin and prostaglandins [20]. Prostaglandins sensitize the pain receptor [12]. In this study, the number of writhing movements during a 30 min observation in the control group was 125.5±0.78. The aspirin (reference drug) gave a 75.5% inhibition of writhing in the animals while the 200 mg/kg dose gave 85.06% inhibition showing that its effect is higher and comparable to that of the reference drug. Active substances against pain may either interfere with pain mediator systems or act on the central nervous system (CNS) by blocking the pain influx transmission [20]. Aspirin (acetylsalicylic acid) inhibit the production of prostaglandin in CNS and body by inhibiting the cyclooxygenase (COX) enzymes [21]. The observed effects in the present study suggest that the extract may have good peripheral pain relieving effect through inhibitory effect on pain mediator systems.

In addition, the effect of the extract on deep pain was tested using tail flick method, a nociception model for the evaluation of the deep analgesic effects of drugs [12]. The extract caused a significant dose-dependent increase in the PRT which is higly comparable to that of reference drug. The reference drug, Pentazocine is a synthetic opioid agonist-antagonist that produces narcotic analgesia and sedation by an interaction with k-receptors [12,22]. The DDE may have acted on the central nervous system (CNS) by blocking the pain influx transmission and provide relief to deep pain sensation which may have been mediated through an increase in the pain threshold in the central nervous system [12,20].

The efficacies of antioxidants are often associated with their ability to scavenge free radicals [23]. A dose- dependent relationship was found in DPPH radical scavenging effect of DDE. The effect increased as the concentration increased (Fig. 1). It suggested that further increase in the concentration of the extract may compare well with the standard drug. Sakthidevi and Mohan [23] reported high DPPH radical scavenging effect of *Dioscorea alata* at concentration of 1000 g/kg. The positive DPPH test suggested that DDE *was* scavenger of free radicals and potential antioxidant. This can be attributed to some of *D. dumetorum* phytoconstituents, such as phenols, flavonoids and tannin. Phenolic compounds are well known as antioxidant and scavenging agents for free radicals associated with oxidative damage [7,23].

In conclusion, this study has shown that, the methanol extract of *D. dumetorum* showed significant antinociceptive effect and moderate antioxidant activity. This justified its use in traditional medicine as a topical anaesthetic. However, more work is required for the isolation and characterization of the active principles responsible for these activities.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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