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Research Article

Effects of aqueous and ethanolic extracts of *Tridax procumbens* leaves on gastrointestinal motility and castor oil-induced diarrhoea in wistar rats

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ABSTRACT: The antidiarrheal as well as the phytochemical properties of the aqueous and ethanolic leaf extract of *Tridax procumbens* was carried out in this study. Forty (40) albino Wistar rats weighing between 150 and 200 g were purchased for used. The 40 rats were divided into two sets for the different experiments. The animals were acclimatized to room temperature (28±5 °C) in a standard wire meshed plastic cages for 7 days prior to commencement of the experiment. During the entire period of study the animals were supplied with standard pellet diet and water *ad libitum*. Phytochemical studies carried out on aqueous and ethanol extract of *Tridax procumbens* leaves revealed the presence of twelve bioactive compounds which are alkaloid, saponin, phenol, tannin, flavonoid, cardiac glycoside, steroid, phytosterol, triterpenoid and phlobatannin. Tannins, phenols, phytosterol, triterpenoids and phlobatannins were detected in trace amount for the aqueous extract compared to the ethanol extract. Both aqueous and ethanol leaf extracts of *Tridax procumbens* showed significant (p<0.05) antidiarrheal activity on gastrointestinal motility with barium sulfate milk model, while with the castor oil-induced diarrheal model, the aqueous extract showed no significant reduction (p>0.05) in the number of stool (wet feces) for 2h when compared with Lomotil drug (standard group). However, there was statistical significant difference (p<0.05) in wet stool for the ethanol extract. These result obtained revealed that the leaf extract might possess some pharmacological antidiarrheal activity and this may possibly explain the use of the plant in traditional medicine.

KEYWORDS: Antidiarrheal, Barium sulfate milk, Castor oil, *Tridax procumbens*, Phytochemical screening

INTRODUCTION

Emergence of pathogenic microorganisms that are resistant or multi resistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs (Karaman *et al.*, 2003; Savithramma *et al.*, 2011). In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics (such as hypersensitivity, allergic reactions, immunosuppression etc.) and are major burning global issues in treating infectious diseases (Schinor *et al.*, 2007). Although pharmacological industries had produced considerable number of commercial antibiotics time to time, resistance in pathogens towards these drugs has increased too at high rate and multi drug resistant microorganisms have exacerbated the situation (Nino *et al.*, 2006, Sharma and Kumar, 2009).

As part of the efforts aimed at meeting these challenges, there is an urgent and continuous need of exploration and development of cheaper, effective new plant based drugs with better bioactive potential and least side effects. Hence, recent attention has been paid to biologically active extracts and compounds from plant species used in herbal medicines (Essawi and Srour, 2000).

Diarrheal diseases are one of the leading causes of morbidity and mortality in developing countries and are responsible for the death of millions of people each year (Carlos and Sanieel, 1990). Despite immense technological advancement in modern medicine, many people in the developing countries still rely on the healing practices and medicinal plants for their daily health care needs (Ojewole, 2004). Therefore, the World Health Organization encouraged studies for the treatment and prevention of diarrheal diseases depending on traditional medical practices (Atta and Mouneir, 2004).

Tridax procumbens is a species of flowering plant in the daisy (Asteraceae) family. It is best known as a widespread weed and pest plant. It is native to the tropical Americas but it has been introduced to tropical, subtropical, and mild temperate regions worldwide. *Tridax procumbens* is common grass found in the tropical Southern part of Nigeria, growing primarily during raining season. The plant bears daisy like yellow-centered white or yellow flowers with three-toothed ray florets. The leaves are toothed and generally arrowhead-shaped. Its fruit is a hard achene covered with stiff hairs and having a feathery, plume-like white pappus at one end. Calyx is represented by scales or reduced to pappus (Suseela *et al.*, 2002). *Tridax procumbens* is known from previous researches for several potential therapeutic activities like antiviral, antioxidant, antibiotic efficacies, wound healing activity, insecticidal and anti-inflammatory activities (Saxena and Albert, 2005; Sharma and Kumar, 2009).

This present study attempts to investigate the antidiarrheal properties of the aqueous and ethanol leaf extract of the Nigerian species of *Tridax procumbens* as well as preliminary characterization of its phytochemical constituents that could relate to the antidiarrheal properties.

MATERIALS AND METHODS

Plant Material

Tridax procumbens leaves were collected from the wide growing habitat within Campus III of Delta State University Abraka, within the month of May and June. The leaf was identified to the species level at the Forest Research Institute of Nigeria, Ibadan, Nigeria (FRIN), where samples were deposited. The leaves were removed from the stalk and air dried at room temperature (28 ± 2 °C), to a constant weight after which it was grounded with sterilized machine and sieved to fine powder and made into extracts used for the experiment.

Preparation of the aqueous extract (AE)

The preparation of the aqueous extract was carried out in accordance to method as described by Salahdeen *et al.*, 2004. The plant extract was prepared by blending and macerating 500g of the air-dried leaves of *Tridax procumbens* with 100ml of 0.9% (w/v) NaCl solution and kept at 40°C for 24 hours for extraction to take place. The resulting mixture was filtered and the extract recovered from the filtration by evaporation under reduced pressure in a Rotavapor with water bath set at 40°C. The resulting residue was weighed and reconstituted in 0.9% (w/v) NaCl solution to a final concentration of 2.2g/ml and kept wet-frozen in ampoules until ready for use.

Preparation of the ethanolic extract (EE)

The preparation of the ethanolic extract was carried out as described by Razia *et al.* (2013). The plant materials (leaves of *Tridax procumbens*) were air-dried at room temperature for 2 weeks and grinded to a uniform powder. The ethanolic extract was prepared by soaking 10 g of powdered plant materials in 100 ml of ethanol at room temperature for 48 h. Extract was filtered after 48 h, with Whatmann filter paper No. 42 (125 mm) and then through cotton wool. The extract was concentrated using a rotary evaporator with the water bath set at 40 °C.

Phytochemical Screening

The phytochemical screening for the major constituents on *Tridax procumbens* was undertaken using standard qualitative procedures as previously described (Harborne, 1973; Trease and Evans, 2009; Sofowora, 2008, Sharma and Sharma, 2010). The test for tannins was carried out by dissolving 0.5 g of the dried powdered extract in 20 ml distilled water, then filtered and 0.1% ferric chloride reagents was added to the filtrate. For cardiac glycosides, Keller Killani test (Trease and Evans, 2009) was adopted (0.5 g of extract was added to 2 ml acetic anhydride plus H₂SO₄). The test for alkaloid was carried out by adding 0.5 g aqueous extract in 5 ml 1% HCl, boiled and filtered. Then Mayer's reagent was added (Harborne, 1984; Trease and Evans, 2009). The extract was subjected to frothing test for the identification of saponin. Haemolysis test was further performed on the froth extracts in water to remove false positive result (Sofowora, 1993; Sofowora, 2008). The extract was also tested for free

glycoside bound anthraquinones (Sofowora, 1993; Sofowora, 2008). Five grammes of the extract were added to 10 ml benzene, after which it was filtered and ammonia solution added. The presence of flavonoids was determined using 1% aluminium chloride solution in methanol concentration HCl, magnesium turnings and potassium hydroxide solution (Sofowora, 1993; Sofowora, 2008). The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol. A 10 mg sample of the extract was dissolved in 1 ml chloroform; 1 ml acetic anhydride was added followed by the addition of 2 ml concentrated H₂SO₄. Formation of a reddish violet colour indicates the presence of triterpenoids (Trease and Evans, 2009, Sharma and Sharma, 2010). To test for steroid, the crude extract was mixed with 2 ml of chloroform, after which 2 ml each of concentrated H₂SO₄ and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

Gastrointestinal motility test with barium sulfate milk:

Measurement of colon transit time is the most basic and primary tool in evaluating disorders of colonic motility. In particular, it is helpful in pathologic diagnosis and for planning management in patients with constipation. This experiment was carried out by the method described by Chatterjee (1993) and as adapted by Rahman *et al.* (2013). Twenty albino rats were randomly divided into four groups of five rats each. The groups were labeled and treated as follows: Control group (Group I) received 2 ml distilled water only, while Group II rats received commercially available antidiarrheal drug Lomotil 1 mg/kg body weight. Rats in Group III received 400 mg/kg aqueous leaves extracts of *Tridax procumbens* while Group IV animals were given ethanolic leaves extracts of *Tridax procumbens* of the same dose as those in Group III. Thirty minutes after the last administration, 2 ml of 10% barium sulfate solution were administered to rats in all groups. The animals were sacrificed thirty minute later by cervical dislocation. The abdomen was opened and total length of small intestine and the distance travelled by barium sulphate milk was measured with a calibrated ruler and expressed as a percentage of the total length of small intestine (from pylorus to the ileo-cecal junction). Also the percent inhibition of movement was calculated by subtracting the percentage travelled from 100%.

Castor oil-induced diarrhea

Castor oil-induced diarrhoea model was carried out using the method described by Shoba and Thomas (2001). Twenty albino rats were used for this study. The animals were randomly divided into four groups of five rats each. Group I (Control) and Group II (standard) received 2 ml of distilled water and 1 mg/kg of Lomotil respectively by intubation (orally). Animals in Group III were treated with the aqueous

leaves extracts of *Tridax procumbens* at a dose of 2 mg/kg body weight. Also, rats in Group IV received the same dose as those in Group three and in the same manner ethanolic leave extract of *Tridax procumbens*. In order to induce diarrhea in all animals, rats in each group were administered 1.0 ml of castor oil orally. The Lomotil and the extracts treated groups were given 1 hour before the administration of standard dose of 1.0 ml of castor oil. The faecal matter of each group was collected and observed at the end of the experiment.

The number of both hard and soft pellet was counted at every hour over 6 hour period for each rat. Diarrhea was defined as the presence in the stool with fluid material that stained the paper placed beneath the cages. Percent inhibition (PI) was calculated as follows:

$$PI = \frac{\text{Mean defaecation (Control Group - Treated Group)} \times 100}{\text{Mean defecation of control group}}$$

Table 1. Qualitative analysis of the aqueous and ethanol extract of *Tridax procumbens* Leaves

Phytochemicals	Aqueous extract	Ethanol extract
Alkaloids	++-	+++
Saponins	++-	+++
Tannins	+--	+++
Flavonoids	++-	+++
Phenol	+--	++-
Anthraquinones	++-	++-
Cardiac glycosides	++-	++-
Steroid	++-	++-
Terpenoids	++-	++-
phytosterol	+--	++-
triterpenoids	+--	++-
Phlobatannins	+--	+++

+++ Abundantly present;
 ++ - Moderately present ;
 + - - Present in trace amount

Table 2. Effect of the *Tridax procumbens* extracts on barium sulfate milk transit time

Treatment/ Grouping	Length (stomach-caecum)	Distance Covered	% inhibition
Group I (Control)	91.40 ± 7.78	89.10 ± 3.88	100
Group II (Lomotil)	88.80 ± 1.39	28.60 ± 2.96*	32.20
Group III (ALEMF)	89.40 ± 0.40	85.60 ± 4.03	61.54
Group IV (ELEMf)	92.20 ± 2.30	31.80 ± 1.36*	20.23

Values are expressed as mean ±SEM. The value of significance was set as $p < 0.005$. Values with subscript *are significantly different from control.

ALEMf = AQUEOUS LEAVES EXTRACT OF *Tridax procumbens*
 ELEMf = ETHANOL LEAVES EXTRACT OF *Tridax procumbens*

Data analysis

All the values of antidiarrheal, tests were expressed as mean + SEM (Standard error of the Mean) and were analyzed by one way analyses of variance (ANOVA) using statistical package for social science (SPSS, 20). Difference between the means were tested with post Hoc- LSD test for multiple comparison and significance was considered when $p < 0.005$. Student's dependent t-test was used to analyze the significant difference between the groups.

RESULTS

The result of the qualitative phytochemical analysis carried out on *Tridax procumbens* leaves (Table 1), revealed the presence of twelve bioactive compounds which are phenols, tannins, flavonoids, saponins, steroids, terpenoids, anthraquinones, cardiac glycosides, alkaloids, phlobatannins, phytosterol and triterpenoids. Both the ethanolic and aqueous extract indicated the presence of these active compounds although, the ethanolic extract possessed more quantity than the aqueous in some. Phenols, tannins, flavonoids, saponins, steroids and alkaloid were present in abundant amount in the ethanolic extract than the aqueous extract (Table 1).

For the antidiarrheal properties on *Tridax procumbens* leaves, two models were employed: the gastrointestinal motility using barium sulphate milk model and castor oil-induced diarrhea model. The result of this present study (Table 2) showed that *Tridax procumbens* leaves extract significantly ($p < 0.005$) decreased the distance of gastrointestinal motility of rats. *Tridax procumbens* leaves extracts significantly ($p < 0.05$) decreased the distance of gastrointestinal motility of from 100% (control group) to 61.54% (aqueous group) and 37.39% (ethanolic groups).

However, the administration of Lomotil (1mg/kg) exhibited much more marked reduction of 32.20% with barium sulfate milk at 30 min study than the aqueous extract treated group (Table 2).

The leaves extracts showed a significant ($p < 0.005$) activity against castor oil-induced diarrhea. Although the values obtained were more pronounced with the ethanol extract than with the aqueous extract. The result is comparable to the effect of widely used antidiarrheal drug Lomotil when tested at 1 mg/kg. The severity of the inhibition diarrhoea induced by castor oil was observed to be about 66.10% for the aqueous extract treated group while ethanol treated group was shown to be 39.08%. The number of stools at 2 hours for ethanol extract treated group was significantly ($p < 0.005$) decreased as compared to control group and this was rather not significantly different when compared to the effect of standard anti diarrheal drug Lomotil (20.20%) (Table 3).

DISCUSSION

Resistance in microorganisms to many antibiotics has resulted in morbidity and mortality from treatment failure and increased health care costs. Though a number of antibiotics are available but increasing capability of microbes to develop multidrug resistance has encouraged search for new, safe and effective bioactive agents of herbal origin (Sharma and Kumar, 2009).

The extracts of *Tridax procumbens* have been reported to have various pharmacological effects, antimicrobial activity against both gram-positive and gram-negative bacteria, and

Table 3. Effect of the *Tridax procumbens* extracts on Castor oil-induced diarrhea

Treatment/ Grouping	Faeces (n/rats)	Wet faeces	% Anti-diarrheal activity
Group I (Control)	5.20 ± 0.37	14.80 ± 0.37	84.61
Group II(Lomotil)	19.80 ± 0.37	4.60 ± 0.25*	20.20
Group III (ALEMF)	10.20 ± 0.46	8.60 ± 0.58	66.10*
Group IV (ELEMF)	15.20 ± 0.48	5.20 ± 0.40*	39.08

Values are expressed as mean ±SEM. The value of significance was set as $p < 0.005$. Values with subscript *are significantly different from control.

ALEMF = AQUEOUS LEAVES EXTRACT OF *Tridax procumbens*

ELEMF = ETHANOL LEAVES EXTRACT OF *Tridax procumbens*

stimulate wound healing (Taddel and Rosas, 2000; Udopa *et al.*; 1991, Diwan *et al.*; 1982, and Diwan *et al.*; 1983). Flavones, glycoside, polysaccharide, monosaccharides and asteraceae have been isolated from the leaves of the plant (Ali *et al.*; 2001, Yadawa and Saurabh, 1998, Raju and Davidson, 1994).

Phytochemical analysis conducted on the *Tridax procumbens* extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities similar to previous research studies (Aiyegroro and Okoh, 2001; Razia *et al.*, 2013). Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids (Tables 1). The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh, 2007). They possess biological properties such as anti-apoptosis, anti-aging, anticarcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Del-Rio *et al.*, 1997).

Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Krings and Berger, 2001). Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. (Parekh and Chanda, 2008). The presence of tannins suggests the ability of the plant to possess anti-diarrhea principles while flavonoids which are hydroxylated phenolic substances, are known to be synthesized by plants in response to microbial infection and have been found to have anti-microbial properties against a

wide array of microorganisms *in vitro* (Del-Rio *et al.*, 1997). Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie, 1996). Triterpenoids are terpenoid derivatives of triterpene molecules. They may have useful anticancer properties, (Salah *et al.*, 1995). They also are effective antioxidant and show strong anti-cancer activities (Okwu, 1994; Ojeh *et al.*, 2013).

The plant extracts contain saponins which are known to produce inhibitory effect on inflammation. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al.*, 2000).

Steroids have been reported to have antibacterial properties, (Cowan, 1999) and they have potential physiological effects especially due to their interference with compounds such as sex hormones (Nobori *et al.*, 1994; Sharma and Kumar, 2009).

Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity (Antherden, 1969). Several workers have reported the analgesic (Okwu and Okwu, 2004), antispasmodic and antibacterial (Nyarko and Addy, 1990; Salahdeen, *et al.*, 2004) properties of alkaloids. Glycosides are known to lower the blood pressure according to many reports (Nyarko and Addy, 1990). The results obtained in this study suggest that the identified phytochemical compounds could be the bioactive constituents responsible for the observed effects, and that this plant is proving to be an

increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

The antidiarrheal effect of the aqueous and ethanol leaves extracts of *Tridax procumbens* in rats was evaluated in this study. The result from Table 2 of this study showed that both extract reduced gastrointestinal motility which became more pronounced with the ethanolic extract. The effect of the extract on gastric motility is unclear. Previous studies have suggested that antidiarrheal extracts under investigation may contain certain components having affinity to the μ (μ) receptor, which is an opioid receptor located on the GI mucosa and relieves diarrhea when activated by an agonist (Goodman and Gillman, 1996). Barium sulphate increases the volume of the intestinal content by preventing the reabsorption of water. It also promotes the liberation of cholecystokinin from duodenal mucosa, which increases the secretion and motility of small intestine and also prevents the reabsorption of NaCl and water. Barium sulphate induced diarrhea is presumed to be by osmotic properties and cholecystokinin production (Galvez *et al.*, 1993; Rahman *et al.*, 2013).

The leaves extracts showed a significant ($p < 0.005$) activity against castor oil-induced diarrhea (Table 3). Although values obtained, were more pronounced with the ethanol extract than with the aqueous extract. The result is comparable to the effect of widely used antidiarrheal drug Lomotil when tested at 1 mg/kg. The possible reason for the less effective activity of the aqueous extract over the ethanol extract might be because of its hydrophilic nature. Drugs that are highly hydrophilic tend to be easily excreted from the gastrointestinal tract hence showing less pharmacological activity. Also, less bioactive ingredients were extracted using the aqueous means than with ethanolic extraction.

Castor oil which is made up of 90% ricinoleate (Salahdeen *et al.*, 2004) is metabolized to ricinoleic acid. Ricinoleic acid causes the irritation and inflammation in the intestinal mucosa, leading to release of prostaglandins, which stimulate the net secretion of water and electrolytes into the small intestine (Luderer *et al.*, 1980; Mekeon *et al.*, 1999). We speculate that the antidiarrheal effects of leaves extracts may be due to the inhibition of prostaglandin biosynthesis. The result of this study reveals that the leaves extract of *Tridax procumbens* contains pharmacologically active substances with antidiarrheal properties. These properties could be a potential source of modern pharmaceutical products. Further investigation is necessary for the isolation, identification and characterization of the different individual active compounds from the extract and for elucidating the modes of action responsible for the effects observed in this study.

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