Phytochemical Screening and Anti-diarrheal Activity of the Pulp and Seed Extracts of *Ziziphus mauritiana* in Rats.

Abstract

In the present study, attempt was made to evaluate the phytochemical composition and anti-diarrheal activity of the seed and pulp extract of *Ziziphus mauritania*. The anti-diarrheal activity of the crude seed and pulp were evaluated using castor oil induced diarrheal model, charcoal meal test and anti-fluid accumulation test in rats. The result of phytochemical test indicated that tannins, flavonoids, saponin, cyanogenic glycosides, and terpenoids were present in both seed and pulp. In the castor oil induced model both the seed and pulp extract significantly prolonged diarrheal onset was observed in treated rats compared to the negative control. Similarly, in the fluid accumulation test, the extract of the seed and pulp produced a significant decline in volume of intestinal contents. Results from the charcoal meal test revealed that all the extract produced a significant anti-motility effect. Based on the findings of this work, the pulp extract of this plant possess anti-diarrheal properties and validates its use in traditional medicine for the treatment of diarrhea.

Key words: *Ziziphus Mauritania,* Diarrheal, Charcoal meal, Castor oil.

1.0 Introduction

The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed by UNESCO [1]. Modern pharmacopoeia still contains at least 25% drugs derived from plants and many

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others, which are synthetic analogues, built on prototype compounds isolated from plants. Interest in medicinal plants as a re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and wellbeing and the bioprospecting of new plant-derived drugs. The growing recognition for medicinal plants use is due to several reasons, including escalating faith in herbal medicine [2]. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies [1].

The medicinal properties of plants could be based on the antioxidant, antimicrobial, antipyretic effects of the phytochemicals in them [3]. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy [4].

Medicinal plants produce bioactive compounds used mainly for medicinal purposes. These compounds either act on different systems of animals including man, and/or act through interfering in the metabolism of microbes infecting them. The microbes may be pathogenic or symbiotic. In either way, the bioactive compounds from medicinal plants play a determining role in regulating host-microbe interaction in favour of the host. So the identification of bioactive compound in plants, their isolation, purification and characterization of active ingredients in crude extracts by various analytical methods is important. The instant rising demand of plant-based drugs is unfortunately creating heavy pressure on some selected high-value medicinal plant populations in the wild due to over-harvesting. Several of these medicinal plant species have slow growth rates, low population densities, and narrow geographic ranges [5]; therefore they are more prone to extinction. Conversely, because information on the use of some plant

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species for therapeutic purpose has been passed from one generation to the next through oral tradition, this knowledge of therapeutic plants has started to decline and become obsolete through the lack of recognition by younger generations as a result of a shift in attitude and ongoing socioeconomic changes [5].

Phytochemical screening, evaluation of antimicrobial properties and antidiarrheal status of medicinal plants are today recognized as the most viable methods of identifying new medicinal plants or refocusing on those earlier reported for bioactive constituents [6]. Plants which are observed to be efficacious and frequently prescribed may contain compounds that are potential drug candidates and could rightly be recommended for further examination [7].

The growing resistance of pathogenic organisms against the antibiotics formerly recognized for their efficiency is today a real problem of public health [4]. In human pathology, microorganisms are responsible for many infections including respiratory tract diseases (pneumonia, bronchitis), skin, wound and mucous infections, sinusitis, endocartidis, osteomyelitis, syphilis, gonorrhea, tuberculosis, food poisoning and carbuncles to mention a few. They are also the germs frequently met during surgical wound infections which are often provoked by the use of intravascular catheters or by the spread of bacteria from another source of infection. *Staphylococcus aureus* for instance one of the most pathogenic among the species of *Staphylococcus* is responsible for almost 25 % of septicemias met in hospitals [8]. Generally, the treatment of infections caused by microorganisms is long and expensive. The antimicrobial properties of plants have been investigated by a number of researchers worldwide though thorough biological evaluation of plants extracts is vital to ensure their efficacy and safety. These factors are of importance if plant extracts are to be accepted as valid medical agents for the treatment of infectious diseases especially in the light of the emergence of drug resistance microorganism [4].

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Diarrhea is characterized by increased stool frequency and a change in stool consistency is one of the major health threats to populations in the tropical and subtropical poor countries [9]. The World Health Organization (WHO) 2013 estimated that 3-5 billion cases occur each year and that approximately 5 million deaths are due to diarrhea annually.

Diarrhea is a common symptom of gastrointestinal infections caused by a wide range of pathogens, including bacteria *(Escherichia coli*, *Shigella*, *Campylobacter*, *Vibriocholerae*), viruses (rotavirus), and protozoa (*Cryptosporidium*). However, a handful of other organisms are also responsible for most acute cases of childhood diarrhea. Among these, rotavirus is the leading cause of acute diarrhea, and responsible for about 40% of all hospital admissions due to diarrhea among under five children worldwide. Globally, rotavirus is the most common cause of severe diarrhea in children [10].

Evidence from several experimental studies showed that plant material with antimicrobial activity also possesses significant antidiarrheal activity particularly in infectious diarrhea. In both cases, these activities have been attributed to the presence of bioactive agents such as tannins, alkaloids, saponins, flavonoids, steroids, and terpenoids [11-13].

Ziziphus mauritiana belongs to the family *Rhamnaceae*. It is widely grown in mild-temperate region and is adapted to warm climates. *Ziziphusmauritiana*can grow either as shrublets, shrubs or trees with thorny branches and are used as a hedge to form defensive fences for animals [14]. The plant is known with many local names including jujube, Chinese date, Indian plump [15]. It is called “Magarya” in Hausa or “Huhue” in bura among the northern people of Nigeria.

The plant finds various uses in traditional medicine for instance; the pulp is applied on cuts and ulcers; are employed in pulmonary ailments and fevers; the dried ripe pulp is a mild laxative.

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The seeds are sedative and are taken sometimes with butter, to halt nausea, vomiting and abdominal pains in pregnancy. Mixed with oil, they are rubbed on rheumatic areas. The leaves are helpful in liver trouble, asthma and fever. The bitter, astringent bark decoction is taken to halt diarrhoea and dysentery and relieve gingivitis. A root decoction is given as a febrifuge, taenicide and emmenagogue, and the powdered root is dusted on wounds. Juice of the root bark is said to alleviate gout and rheumatism [15]. The root is also used in the treatment of epilepsy [16]. The dried root is also used to treat diarrhoea in Northern Parts of Nigeria (Personal communication). The leaves are applied as poultices and are helpful in liver troubles, asthma andfever [17].The hepatoprotective activity of ethanol extract of *Ziziphus mauritiana*leaf against CCl_4 - induced liver damage in rats and the antidiarrheal activity of the methanol root extract were reported [18,19]. The antioxidant activity of the aqueous extract of *Ziziphus mauritiana leaf* has also been reported [20].

2.0 Materials and Method

2.1 Experimental Animals

A total of twenty (20) albino rats of either sex with 110-150g body weight were used for this study. The animals were obtained from the animal house of National Veterinary Research Institute, Vom, Plateau State, Nigeria. The rats were housed in clean and disinfected plastic cages and were allowed to acclimatize for one week in the laboratory unit of Biochemistry Department, Ahmadu Bello University Zaria before the start of the experiment. The rats were fed with a standard rat chow and allowed to drink water *ad libitum*. All animals were handle properly and carefully to minimize the effects of experimental stress. All experiments were carried out in accordance with the guidelines of the Institutional Animal Ethics Committee.

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2.2 Collection of Plant Material

Pulp and seeds of *Ziziphus mauritiana* were collected from Hyera village of Hawul local Government Borno State, Nigeria, identified and authenticated at the herbarium of department of Biological Sciences, Ahmadu Bello University with the voucher number 295.

2.3 Preparation of Plant Extracts.

The dry pulp and seed of *Z. mauritiana* were pulverized using pestle and mortar and sieved. About 200g of the coarse powdered fruit and seed were extracted with 1000 ml of methanol by cooled maceration. Methanol was evaporated in water bath at less than 40^0C. Distilled water was used to reconstitute the solid extract to obtain a desired concentration for the studies.

2.4 Preliminary Phytochemical Screening

Preliminary phytochemical screening for the presence of phenols, tannins, flavonoids, alkaloids, terpenoids, anthraquinones, steroids and saponins were carried out using standard test protocols [21].

2.5 Castor Oil Induced Diarrhea

The effect of the pulp and seed extractsof *Z. mauritiana*on castor oil induced diarrhea was evaluated according to the method of Awouter *et al* [22]. Rats were weighed and grouped into 4 groups (n=5). Group 1 receive distilled water, group 2 and 3 were administered 50 and 100 mg/kg extract orally while group 4 received loperamide (2mg/kg) orally. Each animal was then given 0.5 ml of castor oil orally after 30 minutes of treatment and placed in transparent cages. The consistency of faecal matter and frequency of defecation by the animals were recorded during an observation interval of 4 hours.

2.6 Castor Oil Induced Fluid Accumulation

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Rats were weighed and grouped into 4 groups (n=5). Group 1 receive distilled water, group 2 and 3 were administered 50 and 100 mg/kg extract orally while group 4 received loperamide (2mg/kg) orally. 30 minutes later, each rat was administered 2ml castor oil. The rats were anaesthetized 30 minutes later by inhalation with chloroform. The small intestine from the pylorus to caecum was dissected out and its content expelled into a measuring cylinder and the volume of the fluid was measured [23].

2.7 Small Intestinal Propulsion

The effect of *Z. mauritiana* pulp and seeds extracts on Intestinal Propulsion in rats was tested using the charcoal meal method [24]. The rats were fasted for 24 hours but allowed free access to water. Rats were weighed and grouped into 4 groups (n=5). Group 1 receive distilled water, group 2 and 3 were administered 50 and 100 mg/kg extract orally while group 4 received loperamide (2mg/kg) orally. After 30 minutes each rat was administered 1ml of 5% activated charcoal suspended in 10% aqueous tragacanth orally. The rats were sacrificed 30 min later by inhalation with chloroform. The small intestine of each animal was carefully inspected and the distance traversed by the charcoal meal from the pylorus was measured. The length of the whole small intestine was also measured. The distance traversed by the charcoal meal from the pylorus was expressed as a percentage of the distance from the pylorus to the ileocaecal junction.

3.0 Statistical Analysis

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The results of the experiment were expressed as means ± SD. The data were analyzed using one-way ANOVA followed by Duncan Multiple's Range test. Results with $p< 0.05$ were considered significant.

4.0 Results

Result of phytochemical screening (Table 1.0) revealed the presence of tannins, flavonoids, saponins, terpenoids, and cyanogenic glycosides in both the pulp and seed. Phenol was only detected in the seed. Alkaloids and anthraquinones were not detected at all in both seed and pulp. In the castor oil-induced diarrheal model for pulp (Table 2.0), the pulp extracts of *Ziziphus mauritiana* significantly prolonged the time of diarrheal induction and the frequency of stooling. Data from the experiment revealed that the percentage of diarrheal inhibition compared to controls was 63.64%, and 61.36% at the doses of 50 and 100 respectively. The drug showed a higher percentage inhibition (80.64%) compared to the extract as well as the control. In intestinal fluid accumulation test for pulp (Table 3.0), the pulp extract reduced the volume of intestinal fluid. Maximum percentage inhibition of the volume of intestinal contents was observed at 50 mg/kg of the extract, being 27.71% followed by 26.51% at dose of 100mg/kg. However, the differences observed were not statistically significant. The standard drug produced a better result compared to the test doses. The pulp extract significantly inhibited gastrointestinal transit time of charcoal meal at 50 (8.67%) and 100 (20.33%) mg/kg as compared to the control (Table 4.0).

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Table 1.0: **Result of Preliminary Qualitative Phytochemical Screening**

Phytochemical	**Seed**	**pulp**
Phenolics	+	-
Tannins	+	+
Flavonoids	+	+
Alkaloids	-	-
Saponin	+	+
Steroids	-	-
Cyanogenic glycoside	+	+
Anthraquinones	-	-
Terpenoids	+	+

+= Present - = Absent

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Table 2.0: Effect of *Ziziphusmauritiana* pulp extract on castor oil induced diarrhoea in rats

Treatment	Dose	No. of Watery Diarrhoea	% Inhibition
Control	-	11.00±3.46[a]	-
Extract	50 mg/kg	4.00±1.15 [b]	63.64
Extract	100 mg/kg	4.25±1.50[b]	61.36
Loperamide	2 mg/kg	2.13±1.10[c]	80.64

Values are mean±SD (n=5). Results with different superscript differ significantly at $p<0.05$ (One Way ANOVA followed by Duncan Multiple Range Test).

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Table 3.0:Effect of methanolic pulp extract of *Ziziphusmauritiana*on castor oil induced intestinal fluid accumulation

Treatment	Dose	Fluid Volume (ml)	% Inhibition
Control	-	3.32±0.23[a]	-
Extract	50 mg/kg	2.40±0.42 [b]	27.71
Extract	100 mg/kg	2.44±0.46 [b]	26.51
Loperamide	2 mg/kg	1.40±0.12 [c]	57.83

Values are mean±SD (n=5). Results with different superscript differ significantly at $p<0.05$ (One Way ANOVA followed by Duncan Multiple Range Test).

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Table 4.0: Effect of methanolicpulpextract of *Ziziphusmauritiana*on gastrointestinal transit in rats**.**

Treatment	Dose	Length of Intestine (cm)	Distance Travelled by Charcoal meal (cm)	% Inhibition
Control	-	79.28±8.71	60.00±6.67[a]	-
Extract	50 mg/kg	82.10±5.66	54.80±7.89 [b]	8.67
Extract	100 mg/kg	83.40±4.98	47.80±9.07 [b]	20.33
Loperamide	2 mg/kg	84.7±4.41	33.22±3.23 [c]	44.63

Values are mean±SD (n=5). Results with different superscript differ significantly at $p<0.05$ (**One Way ANOVA followed by Duncan Multiple Range Test**).

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Results for the study of antidiarrheal activity of the seed are shown in (Table 5-7). The seed extracts of *Ziziphus mauritiana* significantly prolonged the time of diarrheal induction and the frequency of stooling in the castor oil-induced diarrheal model (Table 5.0). Our findings revealed that 100mg/kg of the seed extract had the maximum percentage diarrheal induction (80.70%) inhibition. The synthetic drug used produced a percentage inhibition of 91.23%. In intestinal fluid accumulation test **(Table 6.0)**, there were no significant differences in percentage reduction of the volume of intestinal fluid of the seed extract. Table 7.0 shows the result of gastrointestinal transit time of charcoal meal. The seed extract significantly inhibited gastrointestinal transit time of charcoal meal by 7.63%, 19.87% and 42.27% at 50mg/kg of extract, 100mg/kg of extract and 2mg/kg of the standard drug respectively.

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Table 5.0: Effect of Ziziphusmauritiana Seed extract on castor oil induced diarrhoea in rats

Treatment	Dose	No. of Watery Diarrhoea	% Inhibition
Control	-	11.40±2.07[a]	-
Extract	50 mg/kg	5.40±1.34 [b]	52.63
Extract	100 mg/kg	2.20±1.10 [b]	80.70
Loperamide	2 mg/kg	1.00±0.0.04 [c]	91.23

Values are mean±SD (n=5). Results with different superscript differ significantly at $p<0.05$ (**One Way ANOVA followed by Duncan Multiple Range Test**).

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Table 6.0: Effect of methanolic Seed extract of *Ziziphusmauritiana*on castor oil induced intestinal fluid accumulation

Treatment	Dose	Fluid Volume (ml)	% Inhibition
Control	-	3.44±0.26[a]	-
Extract	50 mg/kg	2.18±0.15 [b]	36.63
Extract	100 mg/kg	2.08±0.13 [b]	39.53
Loperamide	2 mg/kg	1.52±0.36 [c]	55.81

Values are mean±SD (n=5). Results with different superscript differ significantly at $p<0.05$ (**One Way ANOVA followed by Duncan Multiple Range Test**).

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Table 7.0: Effect of methanolic Seed extract of *Ziziphusmauritiana*on gastrointestinal transit in rats.

Treatment	Dose	Length of Intestine (cm)	Distance Travelled by Charcoal meal (cm)	% Inhibition
Control	-	93.4±4.72 [a]	63.88±12.87[a]	-
Extract	50 mg/kg	101.180±4.19 [b]	59.00±9.67 [b]	7.63
Extract	100 mg/kg	103.94±6.07[b]	51.19±9.07 [b]	19.87
Loperamide	2 mg/kg	96.34±9.11[c]	36.88±3.21 [c]	42.27

Values are mean±SD (n=5). Results with different superscript differ significantly at $p<0.05$ (**One Way ANOVA followed by Duncan Multiple Range Test**)

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5.0 Discussion and Conclusion

The use of castor oil to induce diarrhea has been reported in several studies [25,26]. The diarrhea inducing properties is as a result of ricinoleic acid; a castor oil active metabolite, which is liberated by the action of lipases in the upper part of the small intestine [27]. Ricinoleic acid exerts its effect by production of local irritation and inflammation of the intestinal mucosa, causing the release of prostaglandins that eventually increases gastrointestinal motility and net secretion of water and electrolytes [28]. Tunaru*et al*., [29], opined that this effect could also occur due to the capability of ricinoleic acid to activate the G protein-coupled prostanoid receptor (EP3) on the smooth muscle cell of the intestine. In addition, it forms ricinoleate salts with sodium and potassium in the lumen of the intestine and these salts antagonize sodium-potassium ATPase and increase permeability of the intestinal epithelium, which in turn results in cytotoxic effect on intestinal absorptive cells [27].

From the result of castor oil induced diarrheal model, the seed and pulp extracts at all the tested doses significantly decreased the frequency of defecation. This study is in line with other studies in which the extract of different plants reduced the frequency of stooling [30-32]. Terpenoids such as abietic acid and steroids like phytosterols have been shown to inhibit production of prostaglandin E2 [33,34], which are known to play a crucial role in the stimulation of intestinal secretions [35]. Thus, the antidiarrheal effect of the seed and fruit could be attributed to inhibition of castor oil-induced prostaglandin synthesis. The anti-diarrheal activity might also be due to inhibition of active secretion of ricinoleic acid, resulting in the activation of Na^+, K^+ATPase activity that promotes absorption of Na^+ and K^+ in the intestinal mucosa. This effect could probably be linked to the presence of terpenoids, tannins and flavonoids in the seed and fruit extract, which are shown to promote colonic absorption of water and electrolytes [36].

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In the castor oil induced intestinal fluid accumulation test, treatment of rats with graded doses of the seed and fruit extract produced a significant reduction in the intestinal fluid accumulation. Mascolo*et al*. (1994) reported that ricinoleic acid the active metabolite of castor oil might activate the nitric oxide pathway and induce nitric oxide (NO) dependent gut secretion. A number of studies have revealed that terpenoids (Jang *et al*., 2004) and flavonoids (Kim *et al*., 2004; Messaoudene*et al*., 2011) are implicated in attenuation of NO synthesis. Hence, the pronounced inhibition of castor oil induced intestinal fluid accumulation might possibly be due to the presence of flavonoids and terpenoids that increase the reabsorption of electrolytes and water by hindering castor oil mediated NO synthesis. The fact that intestinal fluid accumulation and Na^+ secretion induced by castor oil is attenuated by pretreatment of rats with NO synthesis inhibitors [37] reinforces the notion that the anti-fluid accumulation effect of both the seed and fruit extract could probably be by interfering with the NO pathway. Alkaloids which are detected in the seed and fruit have also been demonstrated to inhibit NO synthesis [38].

Flavonoids exert their antidiarrheal effect by inhibiting intestinal motility and hydro-electrolytic secretion [39,40]. Flavonoids are also able to inhibit the intestinal secretory response induced by prostaglandins E2 [41]. Moreover, the enteric nervous system stimulates intestinal secretion through neurotransmitters such as acetylcholine and vasoactive intestinal peptide. On the other hand, intestinal absorption can be stimulated with alpha two adrenergic agents, enkephalins, and somatostatin [35]. Secondary metabolites such as flavonoids from plant sources could stimulate alpha two adrenergic receptors in the absorptive cells of the gastrointestinal tract [40]. Hence, the significant anti-secretory activity of the seed and fruit extract could probably be related to the presence of flavonoids that in turn stimulate alpha two adrenergic receptors in the enterocytes and facilitate fluid and electrolyte absorption.

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Results of evaluation of gastrointestinal transit in rats demonstrated that the seed and fruit significantly reduced the intestinal propulsive movement of charcoal meal at all the test doses as compared to the negative control. The findings are in line with other studies in which the plant extracts significantly inhibited the distance travelled by charcoal meal [42]. This observation could be ascribed to the synergistic effects of terpenoids and alkaloids present in the seed and fruit to prolong the time for absorption of water and electrolytes through hampering peristaltic movement of the intestine. Indeed, alkaloids and terpenoids have been demonstrated to have inhibitory effect on gastrointestinal motility [36,43]. Although the phytochemical constituents responsible for the antidiarrheal effect are yet to be identified, the amount of phytochemical constituents that are responsible for impeding gastrointestinal motility such as tannins [44-46] and alkaloids appear to increase with dose [36]. This could possibly the reason why significant anti-motility effect was observed.

Plants that have tannins in their composition can precipitate proteins of the enterocytes, reducing the peristaltic movements and intestinal secretions [44-46]. The layers formed by the precipitate of proteins on the mucosal surface of the enterocytes also inhibit the development of microorganisms, thus explaining the antiseptic action of tannins [44].

The pulp and seed extracts of *Ziziphusmauritiana* have anti-diarrheal activity against castor oil induced diarrhea. The anti-diarrheal activity is thought to be due to the presence of phytochemicals in the plant.

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