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Evaluation of antidiarrheal activity of ethanolic leaf extract of *Ficus sur* (Moraceae)

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Abstract

Ficus sur is a widely distributed tropical African tree that belongs to the Morceau. The research aims at evaluating the anti-diarrheal activity of the ethanolic leave extract of *Ficus sur*. Collected leave samples were washed and air-dried to constant weight; pulverized and extracted via cold maceration with ethanol. Preliminary phytochemical analysis was conducted testing for different plant secondary metabolites. In the in vivo studies, mice were used in the castor oil induced diarrhea. They were divided into four groups having five animals in each, Group one received distilled water, Group two standard loperamide, Group three low dose of the extract (200 mg/kg), Group four received high dose of the extract (400 mg/kg), after one hour of administration, the castor oil 0.05 ml was given to all of them and they were placed in the beaker having it's floor lined with pre-weighed filter paper. Then, parameters like onset of diarrhea, number of wet and dry feces and total weight of feces were observed. The phytochemical analysis qualitatively validates the presence of alkaloid, flavonoids, tannins, glycosides, phenols, and the absence of steroids and saponins. The extract possess antidiarrheal effect which is not statistically significant as was presented using one-way ANNOVA method of analysis, when compared to the control, having the low dose to be more active than the high dose. The results obtained shows that ethanolic leave extract of *Ficus sur* has effect on diarrhea, especially at dose of 200 mg/kg.

Keywords: *Ficus sur*; Anti-Diarrheal Activity; Phytochemicals; Ethanolic Leave Extract

1. Introduction

Traditional medicine has remained the most affordable and easily accessible source for the management of diseases. It allows one utilize natural means as a way of improving health and finding relief from numerous diseases. Approximately 80% of the world`s population relies mainly on traditional medicine, predominantly originated from plants, for their primary healthcare, this was estimated by the World Health Organization (WHO). In recent years, considerable researchers have discovered that the medicinal values of many plants, composed of bioactive compounds with proven efficacy as the basic raw material of drugs a [1,5].

In developing countries where orthodox medicines are quite expensive, traditional medicine is widely practiced thus screening ethnomedical plants for active compounds such as antimicrobial [2,5,10], antioxidant [1,2,3,7,19], immunostimulatory [8, 18]. Dental [12] active compound is vital so as to ascertain genuine active plants and active compounds.

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Diarrhea can be defined as increased frequency, fluidity, or volume(weight) of fecal material. The fecal material may include the presence of abnormal constituents such as blood, mucus or pus in the bowel. In diarrhea, the mean daily fecal weights may increase from the normal 100-150 g to 150-300 g per day and the fluid content of the fecal material may increase from normal 60% to 90% [6]. Castor oil can be used to induce diarrhea. After castor oil is administered, lipase in the upper portion of the small intestine hydrolyzes it to ricinoleic acid, the active form, which produces inflammation of the gut, local irritation, the release of prostaglandin that promotes intestinal hyperactivity, and an excess of fluid secretion. This will stop the body from absorbing water and electrolytes, reduce the amount of sodium potassium-ATPase that is active in the intestine, and ultimately cause diarrhea [13].

Ficus sur Forssk. (Moraceae) is a medicinal plant species found in Africa and the leaves are used in traditional medicine for various ailment [7]. Almost all the parts of *Ficus sur* plant have been found useful. Its leave, bark and roots are used to treat conditions like malaria [4], diarrhea, wound [7,11], hemorrhoids, epilepsy, as a blood builder to boost iron levels for the treatment of anemia, skin disorders and sexually transmitted diseases.

This study aimed to investigate the antidiarrheal activity of ethanol extract of the leaves of *Fiscus sur* (Forssk) on experimental animal models, and also to determine the phytochemicals present in the leave extract.

2. Materials and methods

2.1. Equipment's

Milling machine (local miller), Analytical weighing balance (OHAUS Model 2610), pocket weighing balance, Rotary evaporator, Water bath.

2.2. Solvents and Reagents

Distilled water, Concentrated ethanol, Wagner's reagent, Piric acid, Dragendoff's reagent, Mayer's reagent, Ferric chloride, 0.1% ferric chloride, Dilute ammonia, Chloroform, Conc. H_2SO_4, Acetic anhydride, Acetic acid, Glacial acetic acid, Animal feeds, Loperamide, Castor oil.

2.3. Apparatus

Bama bottles, Filter papers (radius 7 cm), Beakers (50, 250 and 500 ml), Measuring cylinders (10, 50 and 100 ml), Test tubes and test tube racks, Hand gloves, Sieve clothes, Rat cages, 5 ml syringe, Aluminum foil, Cotton wool, Water cans, 1 kg stones, Permanent makers (blue, red and black), Masking tape, Test tube holder, Cutlass, Plastic and glass funnels, Stirrers, Spatula.

2.4. Plant collection, identification and preparation

The leaves of *Ficus sur* were collected on a sunny evening from Awka south Local Government Area, Anambra state. Nigeria. The plant was authenticated by a plant taxonomist, Miss Eucharia Ikeh, Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences Nnamdi Azikiwe University Awka, with a voucher Herbarium Number: PCG/474/M/019 and was deposited in the herbarium for future reference. After authentication, the plant was washed to remove dust and was air dried in a shade to a constant weight. The dried leaves were then powdered in a mixer grinder. The powdered plant material (900 g) was divided and placed in 10 different bottles and 500 ml of ethanol was added on bottle containing 90 g and stirred, the mixture was kept for twenty-four hours, and the supernatant was decanted off, another 300 ml of ethanol was added, stirred and left for twenty-four hours, the supernatant was decanted off, and the last batch of the solvent, 200 ml was added to the bottle. After twenty-four hours, the supernatant was decanted off. The three batches of the supernatant were filtered and mixed thoroughly to achieve homogenization. The ethanolic extract was dried to 1/6 of its original volume using rotary evaporator at temperature of 45 degrees Celsius and revolving speed 72 cycles per minute to obtain the concentrated ethanolic extract.

2.5. Animal selection

Wistar albino mice weighing between 20 and 35 g were chosen for the study. To ensure they were free of any diseases, the animals underwent examinations. The rats were taken from the Chukwuemeka Odumegwu Ojukwu University Igbariam Campus's animal house at the Department of Pharmacology and Toxicology.

They were acclimatized for one week, having free access to water and a normal laboratory mouse chow meal. A 12-hour light-dark cycle was observed, and a room temperature of 28 °C was maintained.

2.6. Preliminary phytochemical screening

The test was carried out on the ethanol extract using standard procedures as described by [1]

2.6.1. Antidiarrheal assay

Animals were allocated into groups (control, standard, test 1 and test 2) and fasted for 18 hours. Loperamide (2–3 mg/kg, per oral.) was used as a positive control. Distilled water used as negative control. Test 1 was given the extract 200 mg/kg and test 2 was given 400 mg/kg.

- GROUP ONE: Normal control (distilled water), oral
- GROUP TWO: Standard (Loperamide 3 mg/kg), oral
- GROUP THREE: Ethanolic Extraction of *Ficus sur* (200 mg/kg), oral
- GROUP FOUR: Ethanolic Extraction of *Ficus sur* (400 mg/kg), oral

One hour after treatment, castor oil (5 ml/kg) is administered via the oral route and animals were individually placed in metabolic cages whose floors are lined with clean white filter paper. Onset of diarrhea should be noted. Observation should be done for 5 hours. Animals are removed from their cages and weight and number of dry and wet feces were obtained by subtracting the weight of filter paper from the weight of feces and filter paper.

2.7. Method of data analysis

Results were presented as mean ± Standard deviation (n=5). Comparison of mean values among groups was carried out using one way analysis of variance (ANOVA) followed by post-hoc turkey's test for multiple comparison. $p<0.05$ was considered to be statistically significant, while $p>0.05$ was considered to be statistically non-significant. Statistical package for social science (SPSS-20, for windows) was the software used for data analyses.

3. Results

Table 1 Percentage Yield of Ethanolic Extraction of *Ficus sur*

Extract	Weight of powder (g)	Yield(g)	Percentage yield (%)
Ethanol Extract	900	186.3	20.7

Table 2 Phytochemical Analysis of Ethanolic Leaf Extract *Ficus sur*

Phytochemical	Result
Alkaloid	+
Phenol	+
Glycoside	+
Tannins	+
Flavonoid	+
Terpenoid	–
Saponin	_
Steriod	_

N: B Positive signs indicate presence, while negative sign indicates absence

3.1. Castor oil induced diarrhea result

Table 3 Effects of Ethanolic leaf extract of *Ficus sur* on albino mice

Group	Total number of feces	Number of wet feces	Number of dry feces	Weight of diarrhea feces	Diarrhea onset
Control	7.20 ± 1.30	5.20 ± 1.79	2.00 ± 1.22	0.74 ± 0.25	85.40 ± 16.76
Loperamide, 3 mg/kg	3.40 ± 2.79*	2.80 ± 2.28^{ns}	1.00 ± 0.00 ^{ns}	0.35 ± 0.24^{ns}	195.00 ± 90.60*
Extract, 200 mg/kg	5.40 ± 1.34^{ns}	4.20 ± 1.30 ^{ns}	1.20 ± 0.45 ^{ns}	0.70 ± 0.17^{ns}	136.80 ± 77.03^{ns}
Extract, 400 mg/kg	6.80 ± 1.10 ^{ns}	5.20 ± 0.84^{ns}	1.60 ± 0.55^{ns}	0.90 ± 0.24^{ns}	94.40 ± 14.31^{ns}

Values are presented as mean ± standard deviation, n =5. ^{ns}P>0.05: Not statistically significantly different from control. *P<0.05: Statistically significantly different from control.

4. Discussion

Phytochemicals are responsible for the biological activities of medicinal plants, and their presence in any plant extract is an indication of activity [6]. Previous studies showed that anti-dysenteric and antidiarrheal properties of medicinal plants were mostly due to tannins, alkaloids, flavonoids, saponins, terpenes and sterols [15].

One of the most prevalent health issues in underdeveloped nations has long been recognized to be diarrhea. The main feature of the small intestine is to absorb and excrete materials. An imbalance in the absorptive and secretory mechanisms in the intestinal tract accompanied by intestinal hurry results in frequent loose stools or diarrhea [16]. Adenylatecyclase or mucosal cAMP-mediated active secretion is activated, prostaglandin formation and platelet activating factor are stimulated, and intestinal sodium/potassium ATPase activity is inhibited, which reduces normal fluid absorption. These are some of the mechanisms that have been proposed to explain the diarrheal effect of castor oil. The findings of this study support the traditional applications of *Ficus sur*, including the prolonged onset of diarrhea as seen in table 3; the low dose extract delayed the onset of diarrhea up to 136 minutes, which is 51 minutes later on the control's onset, also inhibition of prostaglandin synthesis, and reduction in number and weight of diarrhea feces. From table 3, loperamide significantly (*p<0.05) reduced the total number of feces when compared to control. Though 200 mg/kg dose of the extract slightly reduced to total number of feces, it was not statistically significant (nsp>0.05), considerable increase in water and feed intake weight of fecal matter. The notable reduction in mice's castor oil-induced diarrhea demonstrates that *Ficus sur* ethanolic leaf extract relieves diarrhea through spasmolytic activity [17]. The general mechanism involved is the inhibition of autocoids and prostaglandins released, which in turn inhibits castor oil-induced secretion. The weight and quantity of the diarrheal feces were the parameters that were tracked in this model. The study's findings demonstrated the presence of alkaloids, phenol, glycosides, flavonoids, tannins, and other components in *Ficus sur* leaf extract. These phytoconstituents could be the reason behind the herb's traditional medical uses, which include managing various bacterial and fungal illnesses with its leaves. The main anti-diarrheal ingredients, tannins, denature proteins to generate tannates, which lessen intestinal mucosa permeability, increasing its resistance and decreasing secretion [14].

In this work, loperamide was used as a positive control to assess the anti-diarrheal properties of *Ficus sur* ethanolic leaf extract in albino mice that had castor oil-induced diarrhea. The reason castor oil is used in the model of diarrhea is because autocoids and prostaglandins play a role in the etiology of diarrhea. Castor oil's ricinoleic acid causes the intestinal mucosa to become irritated and inflamed, which causes prostaglandins to be released and increase motility and secretion. Based on the parameters observed and the results analyzed, there was a strong anti-diarrheal impact. When compared to normal animals, the castor oil-treated rats exhibited an oil-treated animals had a noticeably higher weight of intestinal content. The weight and moisture of the fecal matter of the rats treated with ethanolic extract of *Ficus sur* at doses of 200 and 400 mg/kg significantly decreased. From table 3, loperamide significantly (*p<0.05) reduced the total number of feces when compared to control. Though 200 mg/kg dose of the extract slightly reduced to total number of feces, it was not statistically significant (nsp>0.05). The higher dose 400 mg/kg has the total mean number of 6.8 feces which is not far from that of the control (group that was administered distilled water) that have total mean number of feces to be 7.2.

Also, in the onset of Diarrhea, the loperamide group was able to delay diarrhea till 195 minutes, the low dose extract was also to delay diarrhea till 136 minutes (though still not statistically significant, but the higher dose did less as it delays diarrhea only till 94 minutes which is very close to that of the negative control that delayed diarrhea onset for 85 minutes. This was not the case according from the work done by Ayinde B. A. and Owolabi O. J. [17] using the same leave and the same model which produced dose dependent antidiarrheal both in total number of feces and onset of diarrhea., Maybe because they used water as solvent which may have greater yield of the phytoconstituents responsible for antidiarrheal, also the location of and time of collection may contribute to the variation between the results of the two research.

The anti-diarrheal activity of the plant was compared with that of the standard drug. Loperamide reduces the diarrhea induced by castor oil, prostaglandins effectively. It also slows the intestinal transit, colon flow rate and colonic motility. Reduction in the intestinal motility is associated with inactivating calcium ion influx through voltage gated calcium channels. Loperamide being a synthetic drug which is widely used in the treatment and management of diarrhea, although it is relatively safe at therapeutic doses, life threatening loperamide toxicity can result in new clinical syndrome of loperamide induced toxicity. Others may be dizziness, stomach pain, discomfort or enlargement, vomiting, dry mouth and dizziness. The plant extract of *Ficus sur* is equally used in the treatment of diarrhea as per the present as it may have lesser side effects than those of the synthetic drug loperamide.

5. Conclusion

The anti-diarrhea activity of the current study, "Evaluation of anti-diarrheal activity by using ethanolic leaf extract of *Ficus sur*," was found to be minimally effective in experimental animal models.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors confirm that there are no known conflicts of interest.

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