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Anti-Diarrhoea Property of Crude Aqueous Leave Extract of Red Apple *Psidium guajava* in Castor Oil-Induced Diarrhoea in Rats

Okere O. Shekins[1] and Iliemene Uju Dorathy[1*]

[1]Department of Biochemistry, Bingham University, Karu, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author OOS designed the study, wrote the protocol, manage the laboratory analysis of the study and interpreted the data while author IUD managed the literature searches, performed the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Objective: To investigate the anti-diarrhoea property of aqueous leaves extract of *Psidium guajava* (red apple guava leaves) in castor oil-induced diarrhoea in rats.
Methods: Five groups of five rats each orally received the following treatment; groups II, III & IV received 100, 200 & 400 mg/kg body weight of the extract respectively and group

**Corresponding author: Email: uju220@gmail.com;*

V received a standard drug for diarrhoea (Laperamide), while group 1 received 1ml of castor oil only without treatment. 1 ml of castor oil was given to rats in all groups, one hour after the treatment with extract and standard drug. The rats in each group were then placed singly in a cage with adsorbent paper on the floor of the cage. The diarrhoea episode was observed for 4 hours and the cumulative frequency of the wet and formed stools were noted at the end of the 4th hour. Percentage inhibition of diarrhoea was calculated using the mean stool frequency and the anti-diarrhoea activity determined in terms of percentage protection.

Results: The extract reduced stooling in castor oil induced diarrhoea in rats in group IV which shows no significant difference with the group V treated with standard drug. The rats in group I showed the highest stooling episode which is significantly higher than all other groups.

Conclusion: These findings suggest that an aqueous extract of guava leaves may be used as an effective treatment for non specific diarrhoea in medicine. The anti-diarrhoea action may be linked to direct inhibitory effect of the extract on the propulsive movement of the gastrointestinal tract smooth muscles. It equally shows that the treatment is dose dependent.

Keywords: Psidium guajava; anti-diarrhoea; castor oil; laperamide.

1. INTRODUCTION

Plants are important to us as they provide us with food, clothing, shelter, medicines, even the oxygen we inhale. They also enhance the environment with their beauty and help other living creatures [1]. Any plant that possesses the therapeutic properties or exerts beneficial pharmacological effects on animal body is generally designated as medicinal plant. Equally, it has been established that plants which naturally synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils and contain minerals and vitamins, possess medicinal properties [2].

Psidium guajava commonly known as Guava is a plant in the myrtle family (myrtaceae), genus *Psidium*, which contain about 1000 species of tropical shrubs and small trees. They are found in Mexico, central, northern, South America and Southeast Asia. Due to growing demand, they are also grown in some sub-tropical regions. It is a tropical fruit that is like the shape of pear with green rind and pinkish or white flesh and small seeds. Guava fruit contains vitamin C, appreciable amount of vitamin A. It is a good source of iron and pectin, as well as promoting digestion. It also contains potassium and calcium [3,4]. It has been established that the vitamin C in guava is five times more than that of an orange [3]. It is known to be one of the most popular therapeutic plants in the Philippine. It is used as an antiseptic, astringent and anthelmintic. It kills bacteria, fungi and amoeba. The fresh leaves of the plant are used to facilitate the healing of wounds and cuts and also very effective for toothaches as well as cough and throat pain [3]. The root, leave, bark and immature fruits of guava are used for gastroenteritis as they are astringents. Its fiber content controls blood pressure and cholesterol as such, it is very beneficial for heart and help kidney in eliminating wastes [5]. It contains lycopene which reduces the risk of cancer. The leaf of guava is used traditionally to cure epilepsy and cholera, convulsion in children. The leaves and bark are also used to expel placenta after childbirth traditionally [5]. The leaves are equally used traditionally to stop diarrhoea. *P. guajava* is used for traditional phytotherapy around the world because of various pharmacological activities [6]. Therefore, this study was

undertaken to investigate scientifically the anti-diarrhoea property of crude leaves extract of *Psidium guajava*.

Diarrhoea is the condition of having three or more loose or liquid bowel movements per day. The public health significance of diarrhoea disease cannot be overemphasized. The main aetiology of diarrhoea is related to a wide range of bacteria, enteroparasites and viruses. Diarrhoea diseases are the cause of almost three million deaths annually mainly among children younger than five years of age [7,8]. This is because the loss of fluids through diarrhoea can cause dehydration and electrolyte imbalances. Underlying reasons for the spread of diarrhoea diseases are found in poor hygiene and sanitation, limited access to safe drinking water as well as inadequate education of health care providers and recipients [9,10].

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

The leaves of red apple *P. guajava* was collected from a garden in Abuja, Nigeria and was identified at the Herbarium unit of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria where the Voucher number 3253 (Linn) was given to it.

2.2 Sample Preparation and Extraction

The leaves were cleaned, air-dried at room temperature (16-27℃) and pulverized into fine powder using laboratory mortar and pestle. Two liters of boiled distilled water was added to 180g of the pulverized sample in a flat bottom flask, stirred with a glass rod, shaken, covered and allowed to stand for 48 hours at room temperature. The extract was then filtered and the filtrate was evaporated to dryness on a water bath at 45℃ for 24hours to obtain the aqueous extract of *P. guajava.* The percentage yield was 9.90%.

2.3 Phytochemical Screening

Phytochemical screening was carried out on *P. guajava* using the standard method for detecting the presence of secondary metabolites like; alkaloids, carbohydrates, free reducing sugars, combined reducing sugars, tannins, saponins, glycosides, sterols, terpenes and flavonoids as described by [11,12].

2.4 Animal Management

Wistar strain of male albino rats with body weight ranging from 120-180 g were used for the study. The rats were bred in the Animal House of faculty of Medicine, Bingham University Nasarawa state, Nigeria. They were kept at room temperature and maintained *ad-libitum* on tap water and pelleted commercial growers mash fed (Vital feeds, Jos, Nigeria). They were housed in plastic cages under conditions of 12 h light/12 h dark cycle at 25℃. The study was carried out according to the specifications of the Bingham University Animal Ethical Research Committee.

2.5 Acute Toxicity Study (LD_{50})

Acute toxicity study was carried out according to the modified method described by [13]. The study was carried out in two phases. in the first phase, three groups (1, 2, 3) of three rats per

group were given 50, 100, and 800 mg/kg body weight of the extract orally via cannula respectively. The rats were observed for 24hrs after the administration for any signs of toxicity or mortality. The result of this phase gave rise to the choice of doses for the second phase, in which 1000, 2000, and 5000 mg/kg were given to another set of three groups of rats. They were also monitored for 24hrs. The final LD50 value was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose.

2.6 Induction of Diarrhoea with Castor Oil

Anti-diarrhoea activity of the extract was evaluated using the castor oil induced diarrhea model in rats [4]. Twenty five rats were grouped into five groups of five rats per group. Group A served as negative control group and were administered castor oil only. Groups B, C and D were given 100, 200 and 400 mg/kg of the sample extract orally respectively, while group E served as the positive control and received a standard drug for diarrhoea (loperamide) orally. One hour after the treatment with the sample, rats in all the groups was given 1 ml of castor oil orally.

2.7 Determination of the Anti-diarrhoea Activity of the Sample

Rats in each group were then placed singly in cages with adsorbent paper on the floor of their cages. The diarrhoea episodes were observed for 4hrs and the cumulative frequency of wet and formed stools were noted at the end of the 4^{th} hour. Percentage inhibition of diarrhea was calculated using the mean stool frequency and anti-diarrhoea activity determined in terms of percentage protection.

2.8 Statistical Analysis

The results obtained were statistically analyzed using analysis of variance (ANOVA) followed by Duncan's Multiple range test (DMRT) to separate the means with significant differences. The level of significance was set at $P<0.05$.

3. RESULTS

3.1 Phytochemistry of the Aqueous Extract of *P. guajava* Leaves

The results of the phytochemical analysis shows the presence of phytochemical compunds in the extract as shown in Table 1.

Table 1. Phytochemical analysis of crude extract of *P. guajava*

Phytochemical constituents	Test	Inference
Tannins	Ferric chloride	+++
Saponins	Frothing	++
Carbohydrates	Molish's	+++
Flavonoids	NaOH	-
Alkaloids	Dragendorff's	-
Cardiac glycosides	General test	+

+ = low concentration, ++ = moderate concentration, +++ = high concentration, - = absent

3.2 Acute Toxicity Study

Results of acute toxicity of the leave extract are presented on Table 2. The rats that were administered 50, 100, 800 mg/kg body weight showed no visible adverse reaction after 24 hours of post-administration. Also, there was no death recorded or any visible adverse reaction after 24 hours of post-administration as well as on the rats administered 1000, 2000 and 5000 mg/kg body weight of the extract (Table 2).

Table 2. Acute toxicity evaluation of crude extract of *P. guajava*

Doses of extract (mg/kg)	No. of rats per test	No. of death	Survival	Mortality ratio
50	3	0	3	0/3
100	3	0	3	0/3
800	3	0	3	0/3
1000	3	0	3	0/3
2000	3	0	3	0/3
5000	3	0	3	0/3

3.3 Effect of Hot Water Extract of *P. guajava* on Castor Oil-induced Diarrhea in Rats

From the result, it shows that the group 1 which was given castor oil only has the highest diarrhoea episode which was significantly higher than every other group. There was no significant difference between the group administered 400mg/kg of extract and the group administered the standard drug (Table 3).

Table 3. Effect of Aqueous extract of *P. guajava* on castor oil-induced diarrhoea in rats

Treatment	Mean ± standard deviation
Control (castor oil only)	5.40 ± 1.14^{c}
100mg/kg extract	2.40 ± 1.52^{b}
200mg/kg extract	1.20 ± 0.84^{ab}
400mg/kg extract	0.80 ± 0.45^{a}
2mg/kg loperamide	0.60 ± 0.55^{a}

Values are means ± SD. values with different letter(s) are statistically different (P<0.05).

4. DISCUSSION

Qualitative phytochemical analysis of the crude leaf extract of *P. guajava* revealed the presence of tannins, cardiac glycosides, carbohydrates and saponins. The presence of these secondary metabolites may be responsible for the anti-diarrhoea properties of the sample as was also stated in a previous study [4]. Equally, it has been established that plants which naturally synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils and contain minerals and vitamins, possess medicinal properties [2,14].

The castor oil model for diarrhoea was chosen for these pharmacological studies because it increases the volume of intestinal contents by preventing water reabsorption from the

intraluminal space, stimulates secretion by mucosal glands [15,16]. Its action also stimulates the release of endogenous prostaglandin [17,18], which contributes to the pathophysiological functions in the gastrointestinal tract [19]. This release of prostaglandins is a major cause of rachidonic-induced diarrhea [20,8]. The chemistry of castor oil is centered on its high content of ricinoliec acid, a product of ricinoleate which is reported to be responsible for the diarrhea inducing property of castor oil [16,10].

This present work shows that aqueous extracts of *P. guajava* has the potentials to reverse diarrhea resulting from a wide variety of causes as there was no significant difference ($P>0.05$) between the 400mg/kg extract treated group and the group treated with the standard drug of diarrhea as shown in table 3. It was equally observed that there were significant difference ($P<0.05$) between the group treated with 400mg/kg and the group treated with 100mg/kg of the extract. This result indicates that the effectiveness of the extract is dose dependent. Since it was observed that the extract is not toxic, high does up to 400mg/kg or more can be taken for effectiveness of the plant.

Loperamide is a commonly used opioid anti-diarrhea agent which acts by increasing colonic phasic segmenting activities through inhibition of pre-synaptic cholinergic nerves in the submucosal and myenteric plexuses. These effects result in fecal water absorption thus, reducing the frequency of defecation [21].

CONCLUSION

As there was no significant ($P<0.05$) difference between the drug and the high dose of the extract, it is an indication that the extract possess ant-diarrhea potentials, although the dose was higher more than 100 folds. Therefore, it can be recommended that the extract will be better in treating diarrhea since other nutrients can be gotten from it as well as treatment.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (The specifications of the Bingham University Animal Ethical Research Committee) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee of the University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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