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Original research

Evaluation of *In Vivo* Anti-Diarrheal Activity of Selected Medicinal Plants Traditionally Prescribed for the Management of Diarrhea

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Abstract:

This study explores the anti-diarrheal activities of aqueous-ethanolic extract of selected medicinal plants collected from Rawalakot Azad Kashmir, Pakistan. Different doses of the hydro-ethanolic extract of *these Punica granatum, Aegle marmelos, Myrtus communis, and Helicteres isora* as 250, 500, and 1000 mg/kg body weight (b.w.) were tested for antidiarrheal activity using castor oil-induced diarrhea in standardized animal specimens of albino rats (n = 5 in each group). Loperamide as a standard drug (control group, n = 5) and atropine (control group, n= 5) were utilized to evaluate the frequency of diarrheal feces and to check the intestinal transit by charcoal meal, respectively, while normal saline was used as a negative control group (n= 5). Anti-diarrheal activities of hydro-ethanolic extracts at different doses were compared to standard drug and negative control group. Results revealed that extracts of the selected plants at different doses induced significant ($p< 0.05$) reduction in number of diarrheal feces and this reduction was increased with increasing the dosage. This demonstrated that aqueous ethanolic extracts of the studied plants were able to increase the percentage inhibition of the charcoal meal movement and at high concentration (1000mg/kg b.w.), it significantly decreased the intestinal transit as compared with standard drug (atropine). From these results, it can be concluded that the selected traditional plants can have a significant antidiarrheal effect, but more research is recommended to explore the phytochemical constituents responsible for these anti-diarrheal activities.

Keywords: Medicinal plants, Anti-diarrheal activities, ethanolic extracts, traditional treatments

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1- Introduction

Diarrhea is an intestinal disorder of varied etiology related to loose or watery stools frequently associated with vomiting and fever. In the western world, normal fecal weight is less than 200 gram/day with a firm to hard consistency of the stools (Cummings et al., 1992). In India, the greater dietary fiber content of the diet increases the fecal mass and its water content (Jang et al., 2021). Therefore, for the purpose of this study, diarrhea is defined as the condition in which fecal mass and water content are higher than usual (Sweetser, 2012). Diarrhea is categorized into two major subtypes: infectious and non-infectious.

1.2. Infectious causes of diarrhea

Infectious Diarrhea can be caused by pathogens like bacteria, viruses, and parasites, with infectious agents acting as the most common causes of diarrheal illness globally (Hodges and Gill, 2010; Sisay et al., 2017; Aranda-Michel and Giannella, 1999). Diarrhea affects all age groups but is most commonly seen in children. According to the World Health Organization's 2017 statistics, diarrhea is the second highest cause of death in children (Dadonaite et al., 2018). Transmission of infection mostly occurs through fecal-oral pathway. Viruses like Norwalk virus, cytomegalovirus, viral hepatitis, rotavirus, and even coronavirus disease 2019 are the major infectious viruses that may cause mild to severe diarrhea. Bacterial species like *Salmonella* species, *Shigella species*, *Campylobacter species*, *Clostridium difficile*, and *Yersinia pestis* are other major causes of diarrhea. Toxins of *Staphylococcus aureus, Vibrio cholerae, Escherichia coli* are also held responsible for diarrhea (Hernández-Cortez et al., 2017). There are numerous methods for the investigation of diarrhea which include microscopy and stool cultures (Mokomane et al., 2018). Diarrhea is prevented by hygienic precautions such as hand washing after using the bathroom and before and after meals.

1.3. Non-infectious causes of diarrhea

Non-infectious diarrhea is commonly caused by drugs, toxins, food additives, medications, irritable bowel disorder, malabsorption, and inflammatory bowel syndrome (Ede, 2014). Diarrhea has been reported to occur due to the intake of some drugs such as Digoxin which is usually prescribed for the treatment of chronic heart disorders. Antibiotics are prescribed for the treatment of various ailments including diarrhea, but often these same agents can induce diarrhea (Akram et al., 2020). Diverticulitis, irritable bowel syndrome, and bowel infarction or gangrenous bowel are some common causes of non-infectious diarrhea (Jung et al., 2010).

1.4. Medicinal herbs role in diarrheal illness

Medicinal herbs are well known for their use in a variety of complementary and alternative medicine. In rural areas, people use medicinal plants as self-medication, and they know such plants as medications secondary to their efficacy in chronic disorders. Medicinal plants are being used as traditional medicine for a variety of diseases since ancient times with such precocious knowledge transferred from generation to generation. Herbal pharmacopoeia has been developed by refining and updating this practice. Gonçalves et al., reported several medicinal herbs in Brazil for i*n vitro* anti-rotavirus action beside diarrhea (Gonçalves et al., 2005). The World Health Organization 2019 tried to make a framework (2019-2023) for the use of traditional medicinal plants particularly based on scientifically proven traditional medicine because of their non-well reported side effects on the body (World Health Organization, 2019). Different traditional plants in Pakistan have been used as antidiarrheal drugs such as *P. granatum, A. marmelos*, *M. communis*, and *H. isora* which are the chosen plants for this study.

1.5. *Punica granatum*

Punica granatum is a highly nutritious fruit containing many minerals, vitamins, and antioxidants. In addition to its nutritional benefits, *P. granatum* has been used in the treatment of many parasites for nearly 1500 years (Jurenka, 2008). *P. granatum* acts as anti-diarrheal drugs through its effect on pathogenic bacteria by altering the pH of the intestinal tract. This demonstrates signal interaction between the intestinal tract of the host body and the fruit, raising the immune system to fight these pathogens induced by this anti-inflammatory mechanism (Ramadass, Vaiyapuri, & Tergaonkar, 2020).

1.6. *M. communis* L. (*Myrtaceae*) and *H. isora*

A. marmelos (L.) belongs to the Rutaceae family and its leaves, roots, branches, fruits, and trees are used in the treatment of many diseases in alternative medicine due to its various properties. Scientific studies have demonstrated the effectiveness of *A. marmelos* (L.) leaves as an anti-diarrhea due to its large amount of mucilage (Brijesh et al, 2009). *M. communis* L. (*Myrtaceae*) is the only species found in the Northern Hemisphere. It has many medicinal properties that have been used in alternative medicine in Greece since ancient times. The leaves of *M. communis* L. (*Myrtaceae*) are used to treat abdominal pain and diarrhea in Pakistan and India (Mekonnen et al., 2018). *H. isora* L is a shrub of the Sterculiaceae family, used as an antipyretic, anthelmintic, antispasmodic, and anti-diarrheal (Mekonnen et al., 2018). Various modern medicines are originated from plant sources such as aspirin (Ramadass et al., 2020). This study has shown that multiple plant prescriptions are most commonly used for the control of diarrhea in the diverse system of medicines (Berge et al., 2012).

1.7. Aim and Purpose of the Current Study

The aim of this research work is the provision of scientific evidence for the efficacy of conventionally used herbs native to Rawalakot Azad Kashmir, Pakistan, for the treatment of diarrheal illness. The study was performed to assess the *in vivo* anti-diarrheal activity of local medicinal plants conventionally and/or traditionally used for the treatment of diarrhea. The major research question is, "What antidiarrheal effects do these plants have in living subjects and how do they compare to conventional medical therapies for diarrheal illness?"

2. Materials and Methods

2.1. Collection of plants and preparation of extracts

Selected medicinal plants such as *Punica granatum, Aegle marmelos, Myrtus communis, and Helicteres isora* were procured from the neighboring market of Rawalakot, Azad Kashmir, Pakistan. All these medicinal herbs were authenticated from Botanist, Department of Botany, University of the Poonch, Rawalakot Azad Kashmir, Pakistan. Extraction was carried out using 70% ethanol in water as solvent using the procedure described by Seo *et al*., (2014) and Merhavy *et al.,* (2021). Pulverized herbal substances weighing 100 g were agitated twice using 1000 ml of hydro-ethanol. The material was agitated at room temperature for 48 hours. Filtration was performed using Whatman filter paper No. 1. The resultant semisolid extract was evaporated using a rotary evaporator at a reduced temperature. The resulted extracts were further dried using a water bath at room temperature and solid extracts were packed in airtight glass bottles. This method was chosen, as the water bath was assumed to better provide for uniform drying as compared to simple room temperature drying.

2.2. Experimental Animals

Wistar Albino rats weighing roughly 150-200g were used as an animal models. Animals were placed in standard metal cages and retained in customary housing circumstances. Animals were provided with unlimited contact with food as well as *water ad libitum*. Rats were allowed to familiarize themselves with the environment for one week before initiating the research. Due approval was taken from the institutional ethical committee for conducting the experiments on animal models. All elements of the study were performed following standard animal handling procedures.

2.3.Drugs and Chemicals

All the chemicals utilized were of analytical grade. Atropine sulphate and Loperamide were obtained from Sigma Chemicals USA. The Castor oil, Charcoal meal (activated charcoal 10% in Gum acacia 5%), and Normal saline (0.9% NaCl) utilized were purchased from a local market.

2.5. Anti-diarrheal potential in rats

To evaluate the antidiarrheal impact of selected medicinal plants, Wistar Albino rats were used in current research. Four separate extracts were prepared using *P. granatum, A. marmelos, M. communis, and H. isora* as medicinal plants undergoing testing, thus, animals were divided into major four groups (n=60). These study groups were subdivided into three groups (n=5 in each) based on dosage as 250, 500, and 1000 mg/kg body weight (b.w.). Loperamide a standard drug, (control group, n= 5) and atropine (control group, n= 5) were used to evaluate the frequency of diarrheal feces and to check the intestinal transit by charcoal meal, respectively, while normal saline was used as a negative control group (n= 5). All the studied rats were kept fasting for 16 hours before starting the testing.

After the first hour, all grouped animals were administered castor oil (2 mL/rat) orally using oro-gastric gavages. Separate metabolic cages were used to retain the rats, and, for stool collection, translucent plastic containers were placed underneath the cage. The diarrheal frequency was determined after every hour up to 6 hours. The absolute total of feces expelled out (i.e., both diarrheal and non-diarrheal) was compared with the negative control group and the positive control group. The standard drug control was used as a standard control group and considered as 100% for comparative estimation of percentage inhibition of diarrhea. The percentage of diarrheal inhibition was calculated from the following equation:

$$\% \text{ Inhibition} = \frac{\text{Average number of WFC} - \text{Average number of WFT}}{\text{Average number of WFC}} \text{X } 100$$

Where, WFC = average number of wet feces in the control group
WFT = average number of wet feces in the test group

2.6. Gastrointestinal motility test

Gastrointestinal motility was also evaluated using standard drug atropine sulphate in hundred Wistar rats using activated charcoal according to Sisay et al., (Sisay et al., 2017). Aqueous-Ethanolic extract of plants were given orally to castor oil-induced-diarrhea. Wistar rats as 250 mg/kg, 500 mg/kg, 1000 mg/kg, atropine 5 mg/kg as standard drug (group five rats for each group), to check the intestinal transit by charcoal meal and normal saline was used as negative control group (n= 5). The 100 rats were kept NPO for 16 hours prior to any administration. After 30 minutes charcoal meal (activated charcoal 10% in gum acacia 5%) was supplied orally with

1mL/rat dose to each animal. All the rats were slaughtered after 30 minutes to measure the distance in centimeter (cm) that charcoal meal covered from pylorus to caecum of intestine. The result was shown in percentage of distance covered. Percent was calculated according to the following equation:

$$\text{Peristaltic index} = \frac{\text{Mean distance travelled by charcoal meal}}{\text{Mean length of small intestine}} \text{ X } 100$$

$$\% \text{ of inhibition} = \frac{\text{PIC} - \text{PIT}}{\text{PIC}} \text{ X } 100$$

Where, PIC = Peristaltic index of control
PIT = Peristaltic index of the test group

2.7. Statistical analysis

The obtained data was subjected to statistical analysis for the determination of significance using one way ANOVA followed by Dunnett's test using SPSS 16.0 version (Trial version, USA). Results as means ± standard deviation of mean are presented to compare between positive, negative control, and treatment groups, while statistical significance are expressed by p values less than 0.05.

3. Results and discussion

3.1. Effect of plants extracts on castor oil-induced diarrhea

The results showed that all the selected medicinal plants test doses demonstrated a considerable reduction in the diarrheal stool count in contrast to the negative control group. The groups treated with *P. granatum* extract presented mean number of diarrheal defecation as 0.5±0.8, 0.5±0.8 and 0.33±0.8 at 250, 500, and 1000mg/kg doses, respectively, as compared to the negative control group (1.33±1.2). In *A. marmelos* extract-treated groups was 0.66±0.8, 0.66±0.81, 0.5±0.5 at 250, 500, and 1000mg/kg doses, respectively. In *M. communis* extract-treated groups was 01±0.89, 0.83±0.98, 0.5±0.83 at 250, 500, and 1000mg/kg doses, respectively. In *H. isora* extract-treated groups was 0.16±1.16, 0.60±0.81, 0.5±0.8 at 250, 500, and 1000mg/kg, respectively. Whilst in the group treated with Loperamide it was 0.16±0.40. For percentage inhibition, negative control group (i.e., normal saline (5mL/kg)) was considered as 0% inhibition. Percentage inhibition of wet (diarrheal) feces for *P. granatum were* 62.5%, 62.5%, and 75%, for *A. marmelos* were 50%, 50%, and 62.5%, for *M. communis* were 25%, 37.5%, and 62.5% and for *H. isora* were 12.5%, 50%, and 62.5% at 250, 500, and 1000 mg/kg, respectively. The positive control group treated with Loperamide (5mg/kg) showed 87.5% inhibition of diarrhea. The significant inhibition ($p < 0.05$) was recorded at 1000mg/kg dose concentration for all the four studied plant extracts, out of which *P. granatum* remarked maximum inhibition i.e., 75% followed by the remaining three plants i.e., 62.5%. Effects of studied plants extracts on % inhibition of castor oil-induced diarrhea are as given in Table 1 and Figure 1.

Table 1: Effect of studied plants extract on % inhibition of castor oil-induced diarrhea.

Treated with	Treatment groups\Times in hr.	No of stools (diarrhea)							
		after 1hr	after 2hr	after 3hr	after 4hr	after 5hr	after 6hr	Mean ± SD	% Inhibition
Punica granatum	Normal saline 5mg/kg	3	2	2	1	0	0	1.33±1.2a	-
	Loperamide 5mg/kg+castor oil 2ml	0	0	0	1	0	0	0.16±0.4b	87.5
	PG 250mg/kg+castor oil 2ml	1	2	0	0	0	0	0.5±0.8c	62.5
	PG 500mg/kg+castor oil 2ml	0	1	2	0	0	0	0.5±0.8c	62.5
	PG 1000mg/kg+castor oil 2ml	0	0	2	0	0	0	0.33±0.8b	75
Aegle marmelos	Normal saline 5mg/kg	3	3	1	1	0	0	1.33±1.36a	-
	Loperamide5mg/kg+castor oil 2ml	0	0	0	0	1	0	0.16±0.4b	87.5
	AM 250mg/kg+castor oil 2ml	2	1	1	0	0	0	0.66±0.80c	50
	AM 500mg/kg+castor oil 2ml	1	2	1	0	0	0	0.66±0.81c	50
	AM1000mg/kg+castor oil 2ml	0	1	1	1	0	0	0.5±0.5d	62.5
Myrtus communis	Normal saline 5mg/kg	3	2	2	1	0	0	1.33±1.21a	-
	Loperamide5mg/kg+castor oil 2ml	0	0	0	1	0	0	0.16±0.40b	87.5
	MC 250mg+castor oil 2ml	1	2	2	1	0	0	1.00±0.89a	25
	MC 500mg+castor oil 2ml	1	2	2	0	0	0	0.83±0.98a	37.5
	MC 1000mg+castor oil 2ml	0	0	2	1	0	0	0.5±0.83c	62.5
Helicteres isora	Normal saline 5mg/kg	3	3	1	1	0	0	1.33±1.36a	-
	Loperamide5mg/kg+castor oil 2ml	0	0	0	0	1	0	0.16±0.40b	87.5
	HI 250mg/kg+castor oil 2ml	2	3	1	1	0	0	1.16±0.16a	12.5
	HI 500mg+castor oil 2ml	0	2	1	1	0	0	0.81±0.61a	50
	1000mg/kg+castor oil 2ml	0	0	2	1	0	0	0.5±0.8c	62.5

Note: Result sharing the same alphabets are non-significantly ($p > 0.005$) different from negative control group while different alphabets represent the significant ($p < 0.05$) inhibition of diarrheal condition.

2.1.*In vivo* effect of studied plants extracts on the transit of charcoal meal from intestine of Albino Wistar rats

Castor oil-induced and charcoal meal GI movements were radically reduced at 500 and 1000mg/kg ($p<0.05$) in contrast to the negative control in a dose-dependent manner. At 500mg/kg, the plants extract produced % inhibition at a rate of 60%, 51%, 62%, and 49% for *P. granatum, A. marmelos*, *M. communis*, and *H. isora*, respectively, that is comparable to standard drug Atropine (5mg/kg) i.e., 57%, 54%, 52%, and 56%, respectively. The highest effect was exhibited by dose 1000mg/kg of the extract of *P. granatum* 67%, *A. marmelos* 60%, *M. communis* 68%, and *H. isora* 66% which was more than the effect of the standard drug Atropine. The highest percentage inhibition of charcoal meal transit from the intestine of rats was produced by *M. communis* at 1000 mg/kg (68%, p value < 0.05), 500 mg/kg (62%, p value < 0.05) that has more significant effect than standard Atropine (52%, p value < 0.05) (see Table 2 and Figure 2).

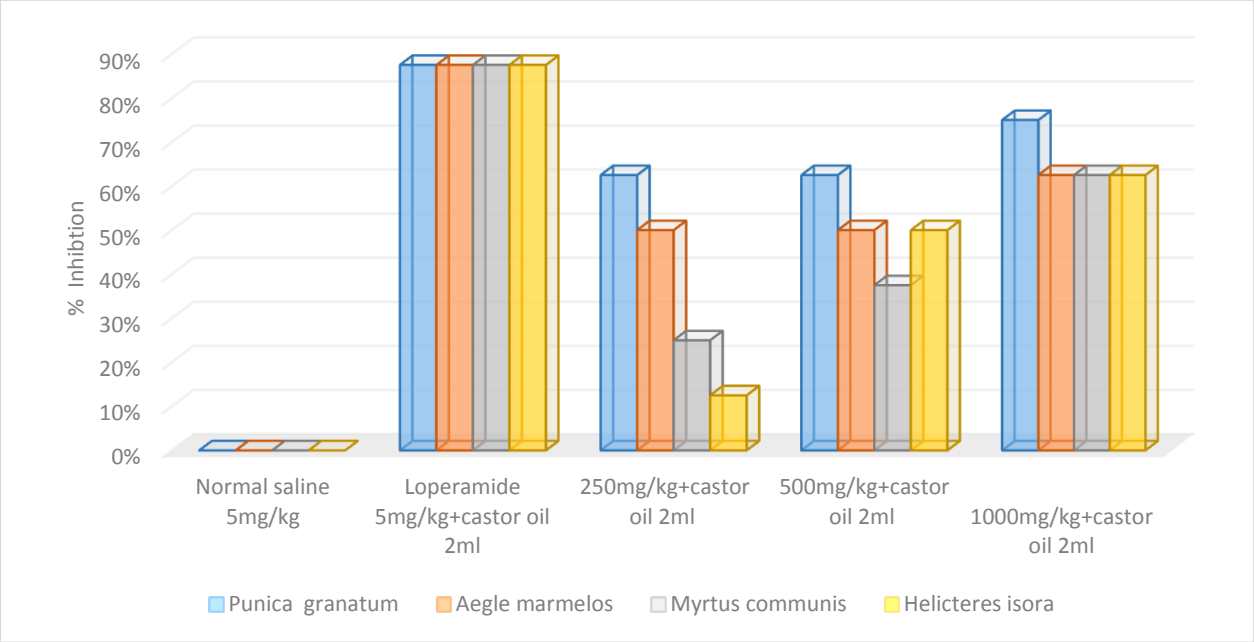

Fig. 1: Effect of studied plants extract on % inhibition of castor oil induced diarrhea.

Table 2: Effect of studied plants extract on charcoal transit from Albino Wistar rat intestine.

Treated with	Treatment groups (Dosages)	Distance (cm) covered by charcoal	% Inhibition
Punica granatum	Normal saline 5mg/kg	70a	-
	Atropine 5mg/kg	30b	57
	PG 250mg/kg	40c	43
	PG 500mg/kg	28d	60
	PG 1000mg/kg	23d	67
Aegle marmelos	Normal saline 5mg/kg	68a	-
	Atropine 5mg/kg	31b	54
	AM 250mg/kg	41c	40
	AM500mg/kg	33d	51
	AM1000mg/kg	27d	60
Myrtus communis	Normal saline 5mg/kg	69a	-
	Atropine 5mg/kg	33b	52
	MC 250 mg/kg	38c	45
	MC 500mg/kg	26d	62
	MC 1000mg/kh	22d	68
Helicteres isora	Normal saline 5mg/kg	73a	-
	Atropine 5mg/kg	32b	56
	HI 250mg/kg	42c	42
	HI 500mg/kg	37b	49
	HI 1000mg/kg	25d	66

Note: Result sharing the same alphabets are non-significantly ($p > 0.005$) different from negative control group while different alphabets represent the significant ($p < 0.05$) inhibition of diarrheal condition.

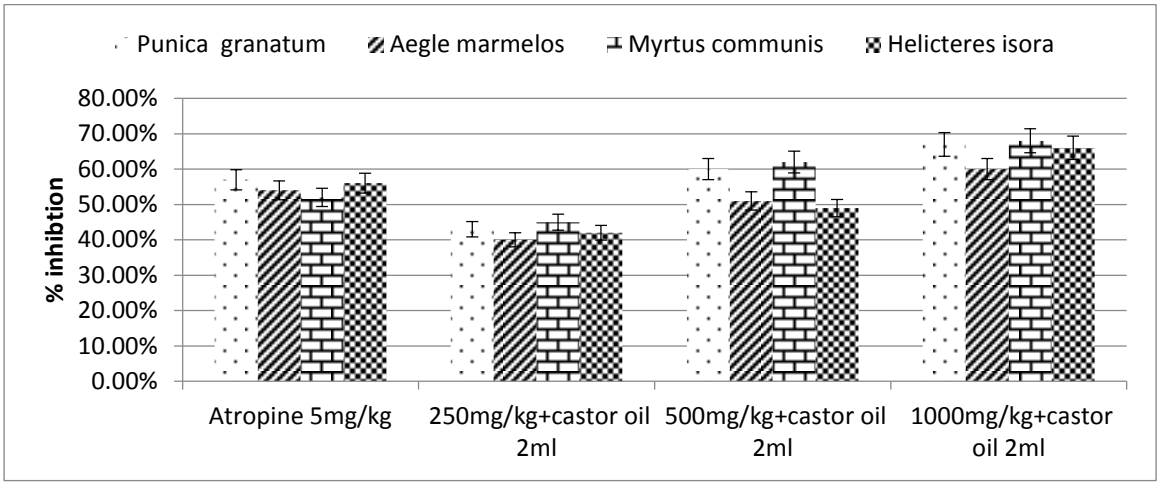

Fig.2. Effect of studied plants extract on charcoal transit from Albino Wistar rat intestine.

3. Discussion

Crude extracts of *P. granatum, A. marmelos, M. communis, and H. isora* were used to investigate and compare possible anti-diarrheal activity and its effect on intestinal motility of Albino Wistar rats. Three doses of hydro-ethanolic extracts 250, 500, and 1000mg/kg were applied, and diarrhea was induced by castor oil. Diarrheal defecation score and charcoal meal test was used for intestinal motility evaluation where standard drugs for antidiarrheal action were Loperamide and for anti-motility test Atropine. Ricinoleic acid, the dynamic component of castor oil, is accountable for the induction of diarrhea. It arouses peristalsis of the small intestine via the release of endogenous prostaglandins while it also alters the permeability of electrolytes in the intestinal mucosa. The present study showed that the aqueous-ethanolic extracts of *P. granatum, A. marmelos, M. communis, and H. isora* at 1000mg/kg show evidence of significant inhibition of diarrhea induced with castor oil in experimental mice.

Studies conducted on the phytochemical screening of *P. granatum* (Pandey and Priyadarshini, 2018), *A. marmelos* (Ariharan and Prasad, 2014), *M. communis* (Bouaoudia-Madi et al., 2017), and *H. isora* (Tambekar *et al.,* 2008), revealed that phenolics, tannins, alkaloids, sterols, saponins, and terpenoids found in these plants might be responsible for anti-diarrheal activity. Further studies are needed to isolate the active substances from *P. granatum, A. marmelos, M. communis, and H. isora* for a clear understanding of the mechanisms of their actions in the inhibition of diarrhea.

The rising concentrations of crude extract of *P. granatum, A. marmelos, M. communis, and H. isora* were given orally in experimental groups animals. The behavior of animals was usual with normal physiological parameters. *A. marmelos* revealed dose-dependent safety on diverse parameters determined during *in vivo* experimentation. *A. marmelos* amplified the onset time of diarrhea and reduced the total number of faeces therefore, intestinal motility was inhibited. More than a few studies stated the occurrence of tannin in *A. marmelos* fruit and is suspected to be liable for the anti-diarrheal effect of medicinal plants (Sarkar, Salauddin, & Chakraborty, 2020).

The presence of tannic acid is assumed to be responsible for the inhibition of diarrhea in experimental mice (Taqvi et al., 2018).

The fruits of *H. isora* have been reported to treat flatulence in children and also in gripping of bowels as it possesses pharmacological actions as astringent as well as a demulcent. Dysentery and diarrhea can be treated by using its bark. It has been shown by toxicity study that the administration via the oral route of aqueous-alcoholic extracts of *P. granatum, A. marmelos, M. communis, and H. isora* up to 1000 mg/kg is safe without any observation of or general weakness in tested animals. Aqueous ethanolic extract of *P. granatum, A. marmelos, M. communis, and H. isora* given at 250, 500, and 1000 mg/kg dose showed significant percentage inhibition for all recommended doses for which negative control i.e., Normal saline 5mg/kg was considered as 100%. Maximum inhibition was shown by *P. granatum* at 1000 mg/kg i.e., 75% followed by *A. marmelos, M. communis and H. isora i.e.,* 62.5% at same dose compared to positive control operamide (5mg/kg) i.e., 87% diarrhea inhibition. This study presented a pronounced reduction in diarrheal incidents where peak effect was shown at 1000 mg/kg in the alcoholic extracts of all plants. Our results are also in agreement with the finding of Qnais et al., (2007) who used *P. granatum* at 100, 200, 300, and 400 mg/kg exhibited 16%, 32%, 53%, and 57% inhibition of diarrhea, respectively explored that increasing the same dose of extract exhibited in increasing of the anti-diarrheal activities as 62.5%, 62.5%, and 75% at 250, 500, and 1000 mg/kg, respectively. The study revealed that the hydro-ethanolic extracts represented marked anti-diarrheal efficacy. In the current study *P. granatum* in contrast to *A. marmelos, M. communis,* and *H. isora,* proved high efficacy as an anti-diarrheal potentiated plant in castor oil-induced diarrhea model. The studied extracts were able to increase the percentage inhibition of the charcoal meal movement in which *M. communis* remained at the top.

Comparable to the current study, Mekonnen et al, 2018, demonstrated in an *in vivo* study that 80ME significantly inhibited the intestinal transit of charcoal meal at all tested doses. The data revealed that the percentage reduction of gastrointestinal transit of charcoal was 33.54% ($p < 0.001$), 46.12% ($p < 0.001$), and 62.31% ($p < 0.001$). Another in vivo study in mice showed a diminution in the diarrheic drops at extract doses 100, 200 (*P value* <0.05), and 400 mg/kg (*P value* <0.01) comparative to the standard drug. The intestinal fluid accumulation and distance travelled by charcoal meal in the intestine resulted in percent inhibition at given doses as 26.83%, 46.34%, 53.66%, and 24.41%, 39.89%, and 51.66%, respectively (Asrie et al., 2016). Moreover, Mekonnen et al., (2017) , performed a similar study i.e. *in vivo* evaluation of *J. schimperiana* leaf methanolic extract (80%) for anti-diarrheal potential, gastrointestinal motility, and enteropooling in mice which demonstrated a considerable decline in total defecation as well as diarrheal drops at 100, 200, and 400mg/kg; wet feces percentage inhibition was 42.58%, 65.07%, and 74.96%, respectively and percent fall in mean weight of intestinal contents was 66.96%, 67.83%, and 76.52%, respectively. The charcoal meal gastrointestinal movement was also significantly reduced at 200 and 400mg/kg doses. Cardiac glycosides, flavonoids, alkaloids, saponins, steroids, tannins, and terpenoids were reported in the methanolic extract of *M. communis* by Mekonnenet al., (2018), might be responsible for therapeutic effects. The above-mentioned facts indicate that these plants are tolerable and safe as well as showed potency in given doses. These facts authorize the effectiveness and safety of these plants in their traditional uses as well.

4. Practical Application

This particular study looked at the efficacy of those medicinal plant which can be utilized as either as adjunctive therapy or instead of those mainstays of antidiarrheal agents such as

loperamide or atropine. These plants have been utilized by traditional medicine for centuries and can be practically implemented in clinical practice which further studies and purifications of those naturally occurring compounds or combination of compounds which provide the antidiarrheal effects. It is the aim of the author team to provide better and more naturally occurring alternative with less side effects to those patients suffering with diarrheal illnesses and those providers who want to avoid harming patients with effective, and yet, problematic agents with large side effect profiles such as atropine and loperamide.

Because of the anti-inflammatory nature of the *Punica granatum* plant as demonstrated by Vucic et al. (2019), Lee et al. (2010), and the current study's demonstration of anti-diarrheal effects, disease states such as inflammatory bowel disease and other disease states in which inflammatory processes cause the diarrheal illness might have increased benefit when administered concurrently with more traditional methods of anti-inflammation. These disease states such as Diverticulitis, irritable bowel syndrome, or infection from *Clostridium difficile,* may benefit from this particular plant's anti-inflammatory effects as the mechanism by which the diarrhea is created is through increased inflammation breaking down the barrier between the gut and the extracellular compartment leading to fluid transposition. Similar thoughts with the anti-inflammatory effects of the other plants of this study might also benefit those patients suffering from inflammatory diarrheal illness either from disease states or infectious causes and help to speed up their healing process by preventing worsening inflammation. Such worsened inflammation increases the overall time for healing to occur (Holzer-Geissler et al., 2022). Therefore, in tamping down this process, these plants might provide for both increased speed of healing, decreased recurrence rates, and better outcomes for the patients as has been seen with other plants from traditional medicine practices (Zhao et al., 2019).

5. Limitations and Future Research Directions

One of the biggest limitations of this current study was the lack of complete extractions and characterization of the specific compound or compounds which produce the antidiarrheal effects in the rat models. Such studies will provide more useful data in locating other plants for potential novel pharmaceutical extractions. One future study could purposefully separate and test the different compounds from the plants to observe which have antidiarrheal effects.

Other limitations include the use of rat models instead of human test subjects. While a comparable gastrointestinal tract, rats are not human subjects. Therefore, the observed changes in the diarrheal symptoms may not be the same in human subjects as they were in the rats. Further studies should look at the effects that these plant extracts have on human subjects suffering from diarrheal illness or drug induced diarrhea.

Lastly, the use of castor oil, while useful in creating diarrhea in the rats does not fully replicate the most common cause of diarrheal symptomatology, Infectious disease. Therefore, further studies in which rat models are purposefully infected with pathogenic causes of diarrhea might provide different data than what was found during our current research project in which non-infectious products were utilized.

6. Conclusion

It can be deducted or inferred from the current study that hydro Ethanolic (70%) extract of *P. granatum, A. marmelos, M. communis and H. isora* have anti-diarrheal potential as proved by a diminution in the GI motility and diarrheal drops. Consequently, this study suggests and/ or recommends the use of these plants in the traditional treatment of diarrhea. Further studies are

needed to isolate the active substances from these plants for a clear understanding of the mechanisms of their actions.

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