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PRELIMINARY PHARMACOGNOSTICAL AND ANTI-DIARRHEAL POTENTIAL OF *PSIDIUM GUAJAVA* LEAF

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ABSTRACT:

Millions of people worldwide use alternative health care systems, and traditional medicines are a crucial part of such systems. In contrast to contemporary pharmaceuticals, which are single molecules that have undergone rigorous testing, structural optimisation, and toxicological clearance, traditionally used herbal remedies are multi-constituent medicines, the safety and efficacy of which are dependent on the experiences of the practitioners. More than 80% of contemporary medications are obtained directly from natural sources (plants, microorganisms, cells, etc.) or their molecules/compounds. Plants are becoming recognised as prospective sources for drug development. Diarrhoea and other gastrointestinal diseases are treated using a variety of conventionally used medicinal herbs. The focus of the current study is on testing *P. guajava* ethanolic leaf extract for its potent anti-diarrheal properties. The outcome of the investigation showed that higher concentration of the ethanolic leaf extract of *P.guajava* exhibits potent antidiarrheal activity. The result revealed that % inhibition of the defection of 100, 200 & 400 mg of the leaf extract of *P. guajava* was found to be 39.6,50.2 & 80.9 respectively.

KEYWORDS: *P. guajava,* anti-diarrheal**,** Phytochemical analysis & %TFC.

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INTRODUCTION

An individual with diarrhoea exhibits an elevation in the volume of liquid in their faeces. Diarrhoea is characterised by a water content of 60–90%. Variations in stool consistency and evacuation frequency are seen among individuals. It is one of the leading causes of mortality, particularly in developing countries. In developing countries, millions of persons die each year [1-2]. Children, particularly those under five years of age, are more susceptible to this sickness, which is the leading cause of mortality in this demographic. Symptoms of dehydration often start with irritability and diminished skin turgor; if the situation escalates, it may manifest as decreased urine output, alterations in skin pigmentation, elevated heart rate, and impaired responsiveness [3-4].

Types of diarrhoea

Diarrhoea arises from an imbalance between the secretion and reabsorption of fluids and electrolytes. Based on the frequency and length of the diarrhoeal episodes [5-6].

The types of diarrhoea include:

- Secretary diarrhoea may be induced by microbial toxins, vasoactive intestinal polypeptides, excessive bile acids, laxatives, or unabsorbed fats.
- Mild diarrhoea is often self-limiting and may resolve within 1-2 days.
- Chronic diarrhoea persists for 14 days or more, necessitating a comprehensive diagnosis to ascertain the underlying cause and facilitate the selection of suitable treatment.
- Acute diarrhoea resulting from bacterial infections and other aetiologies can result in considerable fluid and electrolyte depletion, necessitating immediate intervention.

Causes of Diarrhoea [7]

- Food intolerance: especially lactose
- Bacterial infections: *shigella, salmonella, E. coli*
- Viral infections
- Functional bowel disorders
- Reaction to medicines: e.g. antibiotics and magnesium containing antacids
- Parasites : e.g. Entamoeba histolytica
- Intestinal diseases: inflammatory bowel syndrome(IBD), celiac disease
- Nutrient malabsorption due to some diseases and Altered motility
- Secretory tumours of GIT e.g carcinoid, which secrets vasoactive intestinal peptide
- Emotional distress

Psidium guajava L. (guava) is a little tree noted for its delicious fruit, planted extensively in tropical regions worldwide.

Psidium guajava

The fruit is exceptionally abundant in antioxidants, vitamin C, potassium, and dietary fibre [8]. In different parts of the world, this plant holds a special place with respect to fruit and nutritional items. The guava leaves contained numerous chemical constituents such as α-pinene, β-pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, β-bisabolene, caryophyllene oxide, β-copanene, farnesene, humulene, selinene, cardinene and curcumene, mallic acids, nerolidiol, βsitosterol, ursolic, crategolic, and guayavolic acids, cineol, quercetin, 3-L-4-4-arabinofuranoside (avicularin) and its 3-L-4-pyranoside (essential oil), resin, tannin, eugenol, caryophyllene (1a α-, 4a α-, 7 α-, 7a β-, 7b α-)]-decahydro-1H-cycloprop-azulene, Guajavolide (2 α-,3 β-,6 β-,23-tetrahydroxyurs-12-en-28,20 β-olide; 1) and guavenoic acid (2 α-,3 β-,6 β-,23-tetrahydroxyurs12,20(30)-dien-28-oic acid, triterpene oleanolic acid, triterpenoids, flavinone-2 2'-ene, prenol, dihydrobenzophenanthridine and cryptonine [9-10].Guavas contain carotenoids and polyphenols, the major classes of antioxidant pigments giving them relatively high potential antioxidant value among plant foods. The pigment content as polyphenol, carotenoid and pro-vitamin A, retinoid sources than yellow-green ones. Traditionally *P. guajava* is mainly known for its antispasmodic and antimicrobial properties in the treatment of diarrhoea and dysentery. It also been used broadly as a hypoglycaemic agent. Numerous pharmacological studies have demonstrated the ability of this plant to revelation antioxidant, hepatoprotection, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, anti-inflammatory and antinociceptive activities [11].

Experimental works

Procurement and authentication of Crude drugs

The crude drug (leaves) was procured from locally from Bhopal (M.P.) and authenticated.

Evaluation Parameters:

Pharmacognostical examination:

Macroscopic examination:

Color

Untreated samples were examined under diffuse day light. An artificial light source with wavelength similar to those of day light may also be used. The color of sample was recorded.

Surface characteristic, texture and fracture characteristics

Materials were touched to determine if it is soft or hard bend and ruptured it to obtain information on brittleness and the appearance of the fracture plane-whether it is fibrous, smooth, rough, granular etc.

Odor

A small portion of the sample was placed in the palm of the hand and slowly and repeatedly, the air was inhaled over the material.

Taste

A small amount of drug powder was kept over the tounge and the taste was observed.

Extraction: The crude dried powdered drugs 50gm were kept for maceration in 200ml alcohol for 7 days. These drugs were re-macerated and obtained extracts were further used for chemical evaluation.

Evaluation Parameters

Physical evaluation

Various physiochemical evaluation was determined as per standard procedure [12].

Preparation of extracts

The powdered plant material (200gm) was extracted successively with redistilled, analytical grade petroleum ether (40-60^{O}C), chloroform, ethanol, methanol and water.

Qualitative Phytochemical analysis

The extracts obtained were subjected to various qualitative tests to reveal the presence or absence of common phytopharmaceuticals [13].

Determination of Total Flavonoids Content

The total flavonoids content was estimated by $AlCl_3$ colorimetric method. The content of flavonoids was determined as quercetin equivalent. 10 mg/ml of plant extract in respective solvent (stock solution SS) was mixed with 2 ml $AlCl_3$ (2% w/v) in methanol and the solution was made up to 25ml with methanolic solution of acetic acid (0.5% v/v) (Probe solution PS). 1ml of SS was made up to 25ml with methanolic solution of acetic acid (contrast solution CS).The absorbance of PS and CS was measured at 420nm after 30 minutes. The result expressed as % of total Flavonoids content [14].

%TFC = Absorbance at 420 x dilution x 100 / $E^{1\%}_{1\ cm}$ x wt. of extract in gms

Estimation of phenolic content

The phenolic content of the test extract was evaluated using a Folin reagent Ciocateu's microplate assess procedure with minor modifications. The quantity of phenols in the extract was then determined and pronounced in gallic acid equivalent per gram of extract using a standard curve constituted with gallic acid [15].

Effects of the Ethanolic leaf extract of *P.guajava* on castor oil-induced Diarrhoea

The findings of the effect of P. guajava leaf extract on castor oil-induced enteropooling are tabulated as shown below. When compared to the negative control, studies on this model revealed that the test extract considerably reduced the intraluminal volume of fluid accumulation at 200 (P<.01) and 400 mg/kg (P<.01). Conversely, at a dose of 100 mg/kg, the activity of the test extract was not significant statistically. At the highest dose of the test extract, the maximum percentage inhibition of the volume of intestinal content was recorded. Correspondingly, relative to the negative control, the test extract markedly reduced the weight of intestinal contents at 200 (P < .001) and 400 mg/kg (P <.001). At these respective doses, the percentage inhibition in the weight of intestinal content has been shown to be 20% and 25%, respectively. At the extract's ceiling dose (400 mg/kg), a higher degree of reduction of the aforementioned parameter was observed (25%), which is comparable to the existing drug (30%) (Table 4). In contrast, the activity of the extract at 100 mg/kg was minimal relative to the negative control [16].

Result and Discussion

Numerous individuals globally utilise alternative healthcare systems, with traditional medicines being an integral component of these systems. Unlike modern pharmaceuticals, which consist of single molecules that have been extensively tested, structurally optimised, and cleared for toxicity, traditional herbal remedies are multi-constituent medicines whose safety and efficacy rely on practitioners' experiences. Over 80% of modern pharmaceuticals are derived directly from natural sources (such as plants, microbes, and cells) or their molecular components. Plants are increasingly acknowledged as potential sources for pharmaceutical development. Diarrhoea and associated gastrointestinal disorders are addressed using a range of traditionally utilised therapeutic plants. In animal models of diarrhoea, several herbs

have been empirically demonstrated to be beneficial. Nonetheless, only a limited number of them have participated in controlled clinical trials. This work examines the efficacy of P. guajava ethanolic leaf extract as a strong anti-diarrheal agent, as evidenced by in vivo models. activity. Morphological studies of the leaves will enable to identify the crude drug (Table: 1).Various physiochemical parameters like Ash values and extractive values (Table: 2 & fig.1) can be used as consistent aid for detecting adulteration. These straightforward but reliable recommendations will help society use the chemical as a home remedy. Manufacturers might use them to find and choose the raw components needed for the manufacturing of drugs. Since the production of high-quality phytopharmaceuticals is dependent on appropriate raw material selection, standardisation is crucial. The pharmaceutical business places a high priority on the evaluation of crude medicines because official monographs don't include explicit standards. Identifying individuality, purity, and quality are necessary for this. The quality of any crude medication is impacted by several organic and inorganic contaminations that are practically impossible to avoid while collecting crude pharmaceuticals, demanding thorough examination and detection utilising a variety of pharmacognostic and phytochemical indices. Flavonoids are polyphenolic chemicals found in a wide range of plant species. They are mostly used as a source of starting material in the pharmaceutical and food industries, and they exhibit a variety of biological activities of interest, such as antioxidant capacity, anti-inflammatory activity, wound healing properties, antidiarrheal activity and immune system activation.The preliminary phytochemical analysis of various ethanolic leaf extract of *P.guajava* was tabulated in table 3. The result showed presence of flavonoids in methanolic and ethanolic extract of *P.guajava*. Further quantitative analysis of flavonoid was estimated by $AlCl_3$ colorimetric method. The result revealed that % TFC was found to be 42.38 & 51.2 *µg QE/mg extract P.guajava* in methanolic extract and ethanolic leaves extract of *P.guajava* respectively (table.4 & fig. 3).The total phenolic content was determined by Folin reagent Ciocateu's method which revealed that TPC was found to be 48.5 & 41.06 *g GAE/ 100g extract* for ethanolic and methanolic leaves extract of *P.guajava* respectively (table.5 & fig. 4).As per finding of TFC and TPC ethanolic leaf extract showed higher content of flavonoid and plant phenolics. Therefore, ethanolic leaf extract was selected for the *in-vivo* antidiarrheal potential which was determined by castor-oil induced diarrhea on mice. The outcome of the investigation showed that higher concentration of the ethanolic leaf extract of *P.guajava* exhibits potent antidiarrheal activity. The result revealed that % inhibition of the defection of 100, 200 & 400 mg of the leaf extract of *P.guajava* was found to be 39.6,50.2 & 80.9 respectively. The result was tabulated in table 6 and fig.5.

Table: 1 Morphological Parameter

Characteristics	*P. guajava Leaf*
Size	8-12 x 3-5 cm
Shape	Simple, ovate with short petiole
Colour	Dark green (Upper) Pale green (Lower)
Odor	Specific
Taste	Specific

Table 2: Physico-chemical Parameter

S.No.	Parameter	% w/w Content
		P. guajava leaf
1.	Foreign organic matter	1.02
2.	Ethanol soluble extractive	20.92
3.	Pet. ether soluble extractive	2.59
4.	Ethyl acetate soluble extractive	5.62
5.	CCl4 soluble extractive	4.98
6.	Methanol soluble extractive	19.02
7.	Water soluble extractive	13.98
8.	Hydroalcoholic extractive	15.02
9.	Total ash	10.06
10.	Acid-insoluble ash	0.23
10.	Water soluble ash	2.58
11.	Loss on drying	9.6

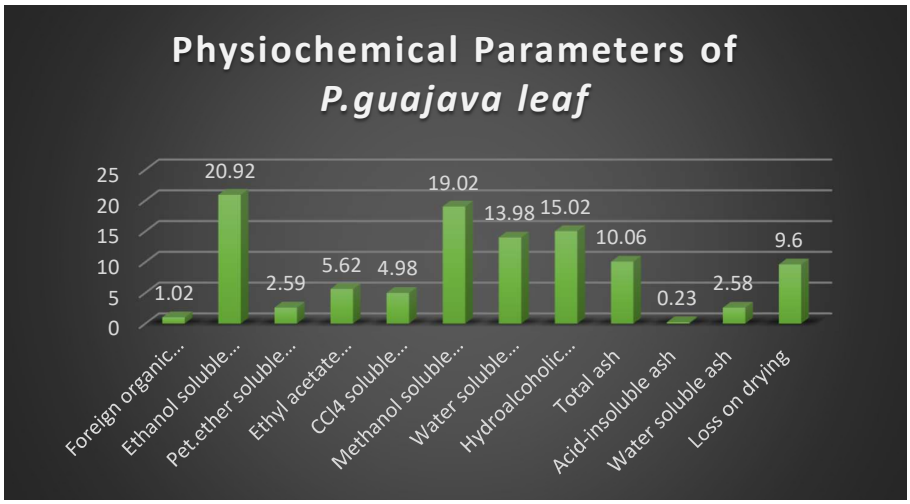

Fig.1 Physiochemical Parameters

Fig.2: Extraction Process

Table 3: Phytochemical Screening of *P.guajava* leaves

Test	Pet ether	Chloroform	Ethanolic	Methanolic	Hydro-alcoholic (70:30)
Carbohydrate					
Molish's	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Benedict	(-)ve	(+)ve	(-)ve	(+)ve	(-)ve
Starch	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
Hexose sugar	(-)ve	(-)ve	(-)ve	(+)ve	(-)ve
Tannin					
$FeCl_3$	(-)ve	(-)ve	(+)ve	(-)ve	(-)ve
Protein					
Biuret	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Xanthoprotein	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Amino acid					
Ninhydrin	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Alkaloids					
Dragnodroff	(+)ve	(+)ve	(+)ve	(-)ve	(+)ve
Mayer	(-)ve	(+)ve	(-)ve	(-)ve	(+)ve
Steroid					
Salkowski	(-)ve	(+)ve	(+)ve	(+)ve	(+)ve
Libermann – Bucher	(-)ve	(+)ve	(+)ve	(+)ve	(+)ve
Flavonoids					
Shinoda	(-)ve	(-)ve	(+)ve	(+)ve	(+)ve
NaOH	(-)ve	(-)ve	(+)ve	(-)ve	(+)ve
Lead acetate	(-)ve	(-)ve	(+)ve	(-)ve	(+)ve
Saponin	(-)ve	(-)ve	(+)ve	(+)ve	(+)ve
Coumarin	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
Glycosides					
Baljet	(-)ve	(+)ve	(-)ve	(-)ve	(+)ve
Legal	(-)ve	(+)ve	(-)ve	(-)ve	(+)ve
Killer-Killani	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve

(+)ve = Present (-)ve Absent

Table 4: Total Flavonoids Content

Sl. No	Sample	µg QE/mg extract
1.	Ethanolic leaf extract of *P. guajava*	51.2
2.	Methanolic leaf extract of *P. guajava*	42.38

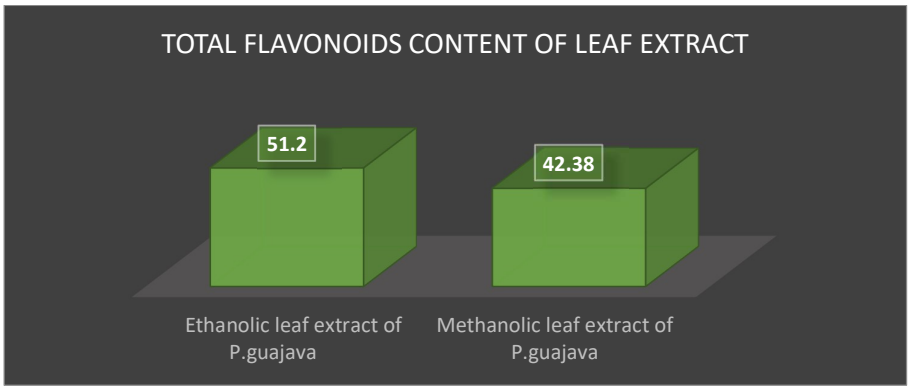

Fig.3: Percentage TFC

Table 5: Total Phenolic Content

S.No	Sample	g GAE/ 100g extract
1.	Ethanolic leaf extract of *P. guajava*	48.5
2.	Methanolic leaf extract of *P. guajava*	41.06

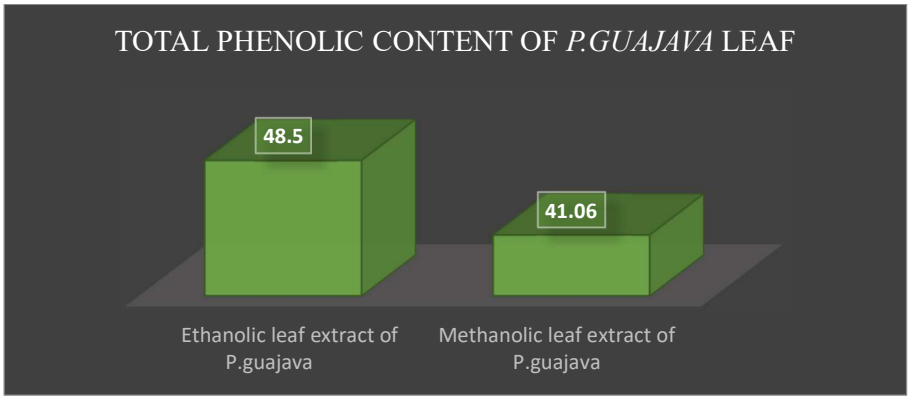

Fig. 4 Total Phenolic content

Table 6: Effect of the ethanolic leaf extract of *P.guajava* on castor oil-induced diarrhea in mice.

Group	Onset of diarrhoea	No. of wet feces	Wt. of feces	Total no. feces	% inhibition of defecation
Control	43.1±2.01	12.8± 1.02	15.2± 0.68	5.2±29	29.6
EtOH leaf 100mg	45.09±2.13	14.2± 1.63	4.6± 0.22	4.1±0.35	39.6
EtOH leaf 200mg	55.6±1.12	9.3± 1.43	4.2±0.39	3.3± 0.20	50.2
EtOH leaf 400mg	63.02±1.52	3.5± 1.08	3.16± 0.26	2.9±0.12	80.9

EtOH = Ethanolic leaf extract of *P.guajava

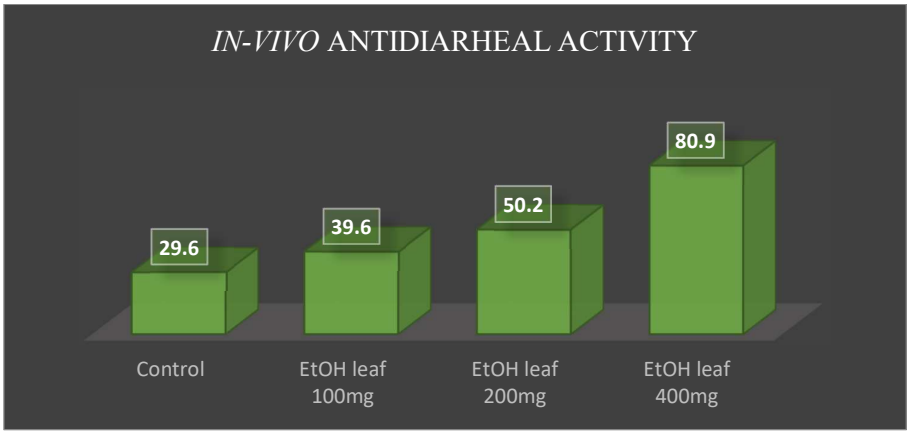

Fig. 5: Percentage inhibition of Defection

CONCLUSION

As per finding of current investigation leaf methanolic and ethanolic extract of *P.guajava* showed presence of flavonoid, phenolic, saponin & phytosterol. The higher phenolic and flavonoid content was found in ethanolic leaf extract of *P.guajava* which further taken in consideration to assessed the anti-diarrheal efficacy *via* castor -oil induced diarrhea on mice as an *in-vivo* model. The findings illustrated that 400mg of ethanolic leaf extract of *P.guajava* showed higher % inhibition of defecation which reflected the potential of *P.guajava* leaf against diarrhea.

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