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ANTI-ULCER ACTIVITY OF *PSIDIUM GUAJAVA* ON PYLORUS LIGATION INDUCED GASTRIC ULCER IN ALBINO RATS

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ABSTRACT: The present study deals with the phytochemical analysis and evaluation of antibacterial activity of hydroalcohol, acetone, and hexane extracts of the leaves of *Psidium guajava*. The antimicrobial activity was measured by the agar well diffusion method. Gastric volume, pH, total and free acidity and the ulcerative index was also measured in hydroalcoholic extract of *Psidium guajava* on gastric ulcer pyloric ligation in rat. Glycosides, terpenoids, and tannins are only present in the hydro alcohol extract. The extracts are tested against gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) bacterial strains. The zone of inhibition against microorganisms is direct proportional to the concentration of extract. Maximum zone of inhibition (24mm) against *Staphylococcus aureus* except hexane extract was seen. *Psidium guajava* leaves show maximum phytochemicals compounds and inhibition of microorganisms in hydroalcoholic extract. So the hydroalcoholic extraction was used for anti-ulcer activity. The present study was performed in pylorus ligation induced gastric ulcer model in albino rats in which the ability of hydroalcoholic extracts of *Psidium guajava* was tested at a dose level of 400 mg/kg body weight orally and compared with Ranitidine (10 mg/kg) as standard. From the results it is concluded hydro alcohol leaf extracts of *Psidium guajava* 400 mg/kg dose level showed significant anti-ulcer activity when compared to that of standard drug.

INTRODUCTION: Ulcer can be developed inside the inner lining of the stomach (gastric ulcer) or the small intestine (duodenal ulcer). Both the ulcers are also cumulatively referred to as peptic ulcers. It affects nearly 10% of the world population ¹.

There are different types of ulcers, such as pressure ulcers, genital ulcers, mouth ulcers, peptic ulcers, venous ulcers, stress ulcer, esophagus ulcers. A peptic ulcer develops on the inside lining of your stomach, the upper portion of your small intestine.

The two most common types of peptic ulcer are gastric ulcer, duodenal ulcer. They form when the digestive juices damage the walls of your stomach or intestine. Duodenal ulcer develops in the lining of the beginning of the small intestine. Today, research shows that most ulcers (85% of gastric ulcers & 95% of duodenal ulcers) develop as a

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result of infection with a bacterium called *H. pylori* infection, which is generally affecting more than a billion people worldwide. Ulcers also come about as a result of excess secretion of acid than required, and this may take place when due to inequality among the digestive juices utilized by the stomach to break down food and the various factors that protect the lining of the stomach and duodenum².

The major factors that disrupt the equilibrium between the aggressive factors and defensive factors are *Helicobacter pylori*, acid-pepsin hyper secretion, and non-steroidal anti-inflammatory drugs. Where there is a high and uncontrollable production of acid also leads to ulcer formation^{3,4}. The symptoms are burning of abdominal pain, vomiting, nausea, chest pain, unexplained weight loss, heartburn, bloating, inflammation, autoimmune pathologies, and digestive disorders such as gastrointestinal inflammation. Peptic ulcer population based on the study (2001-2014) that evaluated long term mortality in 234 patients who underwent surgery for perforated peptic ulcer, mortality was 15.2% at 30 days, 19.2% at 90 days, and 2.6% at 1 year. When the 30-day mortality data were excluded, 36% of the patient died during a median follow-up of 57 months.

Independent factors associated with an increased risk of long-term mortality rate included age older than 60 years⁵. Synthetic drugs such as proton pump inhibitors, H₂ receptors, cytoprotectants, demulcents, anti-cholinergic, antacids, and prostaglandin analogs are used for the treatment of ulceration, but these drugs are produced several side effect. So, herbal medicines are considered as better alternatives for the treatment of peptic ulcer⁶.

Literature suggests that herbal medicines are considered safe for the treatment of ulcers. *Psidium guajava* L. is commonly known as guava and is an evergreen shrub, aromatic, is found all over the world. Guava leaves, roots, and fruits have been used for the treatment of malaria, gastroenteritis, vomiting, diarrhea, dysentery, ulcers, anti-bacterial activity, and a number of other conditions⁷. This study was investigated in rats to evaluate the anti-ulcer activity by pyloric ligation models using hydroalcoholic extract of *Psidium guajava*. This work was carried out in Sankaralingam Bhuvaneshwari College of Pharmacy, Sivakasi (A

Pioneer Institution in Pharmaceutical Education and Research)

MATERIALS AND METHODS: The collected plant leaves were washed with running tap water and distilled water. Washed leaves were shade dried at room temperature for 3-5 days and ground into a fine powder. After they were kept in air tight container and used for solvents extraction. Powdered leaves were subjected to extraction with hydroalcoholic, acetone, and hexane solvent.

Preparation of Hydroalcohol Extraction: About 25 g of dried leaves powder were mixed with (7:3) 100 ml of ethanol, and distilled water added and incubate for 72 h at 40 °C. Thereafter, it was filtrate through Whatmann no.1 filter paper, and then the filtrate was collected and used for preliminary chemical colour reactions of phytochemical group.

Preparation of Hexane and Acetone Extraction: The extraction procedure for 100% hexane solvent and 25 g of dried leaves powder was extracted using 100 ml of hexane solvent 40 °C for 72 h. The acetone extract was prepared by using 100% of acetone solvent with 25 g of dried powder in 40 °C for 72 h. The extracts were then concentrated by evaporation process under air drying. The samples were stored by the refrigerator. These extracts were used for preliminary different phytochemical screening for the analysis of various phytochemical groups.

Preliminary Qualitative Analysis: Alkaloids, Amino acids, carbohydrates, fixed oil and fat, Glycoside, Phenolic Compounds, Phytosterols, Protein, Saponins, Flavonoids, Tannins, Terpenoids were qualitatively analyzed in all extracts⁸.

Antibacterial Activity Assay (Agar Well Diffusion Method): *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* organisms are maintained in nutrient agar medium at 4 °C. Different volumes of crude plant extracts were dissolved in distilled water (10 mg/ml). The pathogenic microorganism was mature in Nutrient broth for twenty-four h and swapped on the petriplates containing Muller Hinton Agar (MHA). In MHA agar plate, about 6 mm diameter well was made by gel puncture. Diluted extracts with different concentration (50, 75 and 100 µl) were applied into the well, and the plates were incubated

at 37 °C for 24 h. The medicinal drug activity was assayed by measuring the diameter of the inhibition zone shaped around the well.

Anti-ulcer Activity:

Experimental Animals: Albino rats of either sex (130 - 180 gm) were procured from the animal house, Sankaralingam Bhuvaneshwari College of Pharmacy, Sivakasi (A Pioneer Institution in Pharmaceutical Education and Research), Tamil Nadu, India used for the present study. They were maintained under standard conditions (24 – 28 °C) and fed a standard diet for mice and given water.

The care of the animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India. The albino rats were divided into three groups, each with four animals. Group- I received distilled water orally (1 ml/kg, p.o) act as a control. Group- II received Ranitidine (10 mg/kg, p.o) act as a standard. Group- III received hydroalcoholic extract of *Psidium guajava* (400 mg/kg, p.o).

Drugs: Anaesthetic ether, gastric antacid (Dose ten mg/kg, IP; NaOH 0.01N), Topfer's chemical agent, collodion, hydroalcoholic extract of fruit tree 400 mg.

Surgical Procedure: Anaesthetics the overnight fasted rat with anesthetic ether. The rat has been secured on the operating group. The incision of 1 cm long in the abdomen just below the sternum exposed the stomach. Then the thread was passed around the pylori sphincter and applied a tight not. While putting the knot, cared should be taken, so the no blood vessel is tied along the knot.

The abdomen wall was closed by putting the sutures. The skin was cleaned from any blood spots and bleeding. Collodion was applied over the wounds. The rats were kept in a separate cage and allow it to recover. After 30 min period of pyloric ligation, the standard drug Ranitidine (10 mg/kg, ip) and the herbal drug *Psidium guajava* hydroalcoholic extract (400 mg/kg, ip) was injected to rat. After four hours of pyloric ligation, both animals were sacrificed by overdose of ether. The abdomen was opened, and the esophageal end ties the (cardiac end of the stomach). The entire

stomach from the body of the animal was cut and removed, and ulcer scoring was done. Gastric juice was collected, and gastric secretion studies were performed⁹.

Estimation of Gastric Volume, Total and Free Acidity:

Collection of Gastric Juice: The subject is advised not to eat or drink anything during the 12 h preceding the test. Ryle's tube lubricated with a little paraffin in passed to the stomach through oral or nasal passage up to the double mark on the tube. Saliva should not be swallowed afterward. The resting juice is removed with a 50 ml syringe.

The volume is noted, and the contents are kept for further examination. This is called 'zero samples'. The rat is given a pint of oatmeal to drink. The time is noted. About 10 ml of the stomach contents are removed every 15 min for 2½ h into different test tubes marked from 1 to 10. At the end of this period, the stomach is emptied completely, and the volume of the residual contents is measured. The tube is then withdrawn gently. Then gastric juice was then centrifuged, and the clear supernatant was analyzed for total and free acidity.

Acid Titration:

Free Acid: Pipette 1 ml of the filtered gastric content into a small beaker. Add about 2-3 ml of distilled water and then a drop of Topfer's indicator. It will turn pink in the presence of free HCL. Titrate it with N/100 NaOH until the pink color disappears and the color ultimately becomes yellowish orange (pH 4.0). At this pH, all free HCL is titrated. Take the burette reading. The volume of the alkali required for titration represents the free HCL as N/10 acid present in 100 ml of gastric juice.

Total Acid: Now, add a drop of phenolphthalein to the content and continue titration with the alkali until a definite red reappears (pH 8.5). The second reading of the burette was noted. The difference between this reading and the initial reading represents the total acid present in 1 ml of gastric contents.

Measurement of Ulcerative Index: Ulcerative index was measured by the method of briefly, the stomach was opened and washed with running tap

water, then was placed on a flat glass plate and observed under 10X magnification for ulcers¹⁰.

Scoring of the ulcer will be made as follows:

Normal stomach.....	(0)
Red colouration.....	(0.5)
Spot ulcer	(1)
Hemorrhagic streak...	(1.5)
Ulcers.....	(2)

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows

$$\% \text{ Protective} = \frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Determination of Acidity:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times 100}{0.1 \times \text{mEq} / \text{LZ}}$$

Statistical Analysis: The values are represented as mean \pm standard error, and statistical significance between treated and control groups was analyzed using of Graph pad software.

RESULTS AND DISCUSSION:

Phytochemical Analysis: In the present study significant phytochemical analysis is observed by hydroalcohol, acetone and hexane of *Psidium guajava*. Phytochemical screening of *Psidium guajava* leaves extracts were represented in **Table 1**.

TABLE 1: PHYTOCHEMICAL SCREENING OF PSIDIUM GUAJAVA LEAF EXTRACT

Phytochemicals test	Hydroalcohol	Acetone	Hexane
Alkaloids	+	+	+
Flavonoids	+	+	+
Carbohydrate	+	+	+
Glycosides	+	-	-
Tannins	+	-	-
Phenolic compound	+	+	+
Saponins	+	+	+
Fats & Oils	-	-	-
Protein & Amino acid	+	-	-
Phytosterols	+	+	+
Gums & mucillages	-	-	-
Terpenoids	+	-	-

(+) Positive; (-) Negative

The result revealed that the hydroalcoholic extract of guava leaves, the presence of alkaloids, flavonoids, carbohydrates, glycosides, tannins,

phenolic compound, saponins, protein, amino acid, phytosterols, and terpenoids. Fats & oils and gums & mucilages are absent. A similar result was obtained by Narender *et al.*, 2014¹¹. Acetone and hexane extracts show only a few compounds are present when compared to hydroalcoholic extract. In acetone extract of *Psidium guajava* shows the presence of alkaloids, flavonoids, carbohydrates, saponins, phenolic compounds, and phytosterols. Glycosides, tannins, and terpenoids are absent in the acetone extract of our plant, which was similar to the acetone extract of *Psidium guajava*¹².

In hexane extract of *Psidium guajava*, alkaloids, flavonoids, carbohydrates, saponins, phenolic compound, and phytosterols were present. Flavonoids are among the cytoprotective materials for which anti-ulcerogenic efficacy has been extensively confirmed.

It is suggested that these active compounds would be able to stimulate mucus, bicarbonate, and prostaglandin secretion and counteract in gastrointestinal lumen. So, the antiulcer activity of *P. guajava* may be attributed to its flavonoids content¹³.

Phytochemical screening showed that the maximum presence of phytoconstituents in hydroalcoholic extract (40%) more than acetone (30%) and hexane extract (30%). The various phytochemical compounds detected are known to have beneficial importance in medicinal science¹⁴. The phytochemical screening showed that the hydroalcoholic extract has more bioactive metabolites. So, it has higher antibacterial activity.

Anti-bacterial Activity: Various extracts of *Psidium guajava* showed significant antibacterial activity with a variety of pathogens due to the contribution of these phytochemicals. The antibacterial activities exhibited by these extracts are shown in **Table 2**.

TABLE 2: INHIBITION OF MICROORGANISM BY HYDROALCOHOL, ACETONE AND HEXANE EXTRACT OF PSIDIUM GUAJAVA

HYDROALCOHOL EXTRACT				
Organisms	50 μ l	75 μ l	100 μ l	Control
<i>B. subtilis</i>	17 mm	17 mm	18 mm	24 mm
<i>E. coli</i>	14 mm	17 mm	27 mm	21 mm
<i>S. aureus</i>	13 mm	14 mm	24 mm	22 mm

ACETONE EXTRACT

Organism	50 μ l	75 μ l	100 μ l	Control
<i>B. subtilis</i>	13 mm	17 mm	17 mm	22 mm
<i>E. coli</i>	14 mm	15 mm	19 mm	18 mm
<i>S. aureus</i>	12 mm	15 mm	15 mm	19 mm

HEXANE EXTRACT

Organism	50 μ l	75 μ l	100 μ l	Control
<i>B. subtilis</i>	11 mm	14 mm	11 mm	21 mm
<i>E. coli</i>	10 mm	13 mm	11 mm	20 mm
<i>S. aureus</i>	12 mm	13 mm	12 mm	20 mm

An antibacterial activity of leaves of *Psidium guajava* was observed by agar well diffusion method and by measuring the diameter of zone of inhibition (in mm). Three different concentrations of extracts were taken (50 μ l, 75 μ l and 100 μ l) and tested against 3 different bacteria's (*Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*). Ambistryn was used as standard, and their individual solvents were used as control.

In the case of *Escherichia coli*, maximum inhibition (27 mm) was obtained by hydroalcoholic extract, and the second-highest (19 mm) was obtained by acetone extract, and finally, the least inhibition (11 mm) was observed in hexane extract. Maximum inhibition (24 mm) was obtained by hydroalcoholic extract and acetone; hexane extract showed a little inhibition against *Staphylococcus aureus*. A similar result was obtained by Gitika et al., 2016¹⁵ who

found crude hexane extract of leaves exhibit significant antibacterial activity against gram-negative *E. coli* and gram-positive *S. aureus*.

In the case of *Bacillus subtilis* maximum inhibition (18 mm) was obtained by hydroalcoholic extract and acetone; hexane extract showed little inhibition. The difference in the observed activities of the various extracts may be due to varying degrees of solubility of the active constituents in the solvents¹⁶. Biswas et al. has reported that *Psidium guajava* has Gram-negative and Gram-positive antibacterial characteristics¹⁷. We observed the excellent antibacterial activity of hydroalcoholic extract of *Psidium guajava* against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. From the present study, the hydroalcohol extract of *Psidium guajava* leaves show the maximum phytochemicals compound and inhibition of microorganism. So the hydroalcoholic extract can be used for Anti-Ulcer activity.

Anti-ulcer Activity: Plants contain a large variety of natural products with diverse biological activities, including antiulcerogenic actions. In this work, we examined the anti-ulcer activity of hydroalcohol extract of *Psidium guajava* in pyloric ligation induced model is reported in **Table 3** and **Fig. 1**.

TABLE 3: EFFECTS OF HYDROALCOHOL EXTRACT IN *PSIDIUM GUAJAVA* ON GASTRIC ULCER PYLORIC LIGATION IN RAT

S. no.	Group	Volume of gastric juice	pH	Acidity		Ulcer index	% of inhibition of ulcer
				Free	Total		
1	Control	5.42 \pm 1.38	4.2 \pm 1.21	117.25 \pm 1.97	124 \pm 0.31	7.24 \pm 3.23	-
2	Ranitidine 10 mg/kg p.o	4.17 \pm 0.68	5.32 \pm 0.46	53 \pm 2.54	64 \pm 1.46	1.35 \pm 0.40	81.35%
3	Hydroalcohol 400 mg/kg p.o	4.72 \pm 0.75	4.65 \pm 0.75	60 \pm 2.66	74.25 \pm 5.48	2.34 \pm 0.54	68%

Data values are represented by Mean \pm SEM, n=4



Group I
(RANITIDINE (10 mg/kg))

Group II
(HYDROALCOHOL EXTRACT (400 mg/kg))

Group - III

FIG. 1: ANTI-ULCER ACTIVITY OF *PSIDIUM GUAJAVA* (HYDROALCOHOL EXTRACT)

The animals between 130 – 180 gm weight were divided into three groups, *i.e.*, Group – I as control, which is normal, Group – II as a positive control, which is treated with ranitidine (10 mg/kg p.o), Group – III as a sample, which is treated with hydroalcoholic extract (400 mg/kg p.o). In hydroalcoholic extract, the value of ulcer index slightly decreased when compared to the standard (Ranitidine) group, and the value is also reduced compared to the control group. Gastric acidity and the volume of gastric juice are increased in the control group due to pyloric ligation. Hydroalcoholic extract of *Psidium guajava* 400 mg/kg is moderately reduced the gastric acidity and volume of gastric juice when compared to standard (Ranitidine), and the value is also reduced when compared to that of control. **Table 3** indicated the significant anti-ulcer activity of *Psidium guajava* hydroalcoholic extract.

The percentage of inhibition of ulcer in ranitidine is 81.35%, and the hydroalcoholic extract of *Psidium guajava* inhibition of ulcer is 68%. So, it was almost comparable to that of a standard drug. Our results, similar to the antiulcer activity of methanol extract of *B. oleracea* in pylorus ligation model was evident from its significant reduction in gastric volume, total acidity, free acidity, ulcer index, and increase in pH of gastric juice¹⁸. The excess gastric formation of prostaglandin was increased in mucosal resistance as well as a decrease in aggressive factors, mainly acid and pepsin. Gastric acid over secretion is one of the key pathogenic factors for gastric ulcer induction¹⁹.

Similar to our results, the most active subfraction of *Buchanania lanzan* ethyl acetate (BLE) exerted a significant dose-dependent decrease in the ulcerative lesion index produced by APL ulcer model in rats as compared to the standard drugs omeprazole (30 mg/kg, b.w orally) and ranitidine (32 mg/kg, b.w orally), respectively. The reduction in gastric fluid volume, total acidity, and an increase in the pH of the gastric fluid in APL treated rats proved the anti-secretory activity of most active sub-fraction of BLE. The oral administration of most active sub-fraction (F4) of ethyl acetate fraction of methanolic leaf extract of *B. lanzan* Spreng produced significant antiulcer activity²⁰. Several scientific studies revealed that the phytoconstituents like flavonoids, tannins,

terpenoids, and saponins were responsible for gastro protective agents^{21, 22}. Tannins have astringent activities, precipitating proteins of mucosal membranes and skin. Some types of tannins suppress gastric secretion and enhance the mucus layer, and have a local action of protection of the gastric mucosa²³.

CONCLUSION: The anti-gastric ulcer activity *Psidium guajava* leaf extracts in pylorus ligation model is evident from its significant reduction in gastric volume, free acidity, total acidity, ulcer sore, and increase pH when compared to that of standard drug. The anti-gastric ulcer activity 100% Hydro-alcohol extract at 400 mg/kg showed better results. It has also been scientifically proven that these extracts possess energy potential as an anti-ulcerogenic agent. This type of study provides health applications at an affordable cost. Further research needs in the angle whether the phytochemicals could be useful to treat other dreadful diseases. Further studies are being conducted on these plants in order to isolate, identify, characterizes, and elucidate the structure if these bioactive compounds.

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CONFLICTS OF INTEREST: Nil

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