International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2020): 7.803

In-Vitro Evaluation of Antimicrobial & Antiulcer Activity of Aqueous Extract of *Psidium Guajava*

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Abstract: ***Aim and objective*: The aim of the present study was to develop the In- Vitro Evaluation of Antimicrobial & Antiulcer Activity of Aqueous Extract of Psidium Guajava. It is defined as disruption of the mucosal integrity of the stomach and/or duodenum leading to a local defect or excavation due to active inflammation. *Material and method*: Psidium guajava or commonly known as Guava is a popular fruit tree. It is evergreen, tropical, and small growing only up to 10 m high. Leaves of Psidium guajava were washed with dechlorinated water, dried in shade and powdered with the help of an electric blender. The test materials (1.0 kg) were extracted with different organic solvents viz., acetone, hexane, petroleum ether, chloroform and methanol in a Soxhlet apparatus for 8 h and the extract was concentrated in a rotary vacuum evaporator to yield crude extract, which was used in bioassays. *Result & discussion*: H+/K+ - ATPase Inhibition Activity: The H+/ K+ - ATPase inhibition activity of aqueous extract at a various concentration (20µg, 40µg, 60µg, 80µg, 100 µg) has compared with Omeprazole as standard. The extract significantly showed activity in a dose-dependent manner. Maximum percentage inhibition of 62.18±0.54% has been observed for extract at a concentration of 100µg, and standard Omeprazole showed 69.56±1.72%.***

Keywords: *In- Vitro* Evaluation, Antimicrobial Activity and Antiulcer Activity, TLC Analysis

1. Introduction

It is defined as disruption of the mucosal integrity of the stomach and/or duodenum leading to a local defect or excavation due to active inflammation. Ulcers occur within the stomach or in duodenum and are often chronic in nature. Occasionally it is also described as a break in skin or mucous membrane with loss of surface tissue, disintegration and necrosis of epithelial tissue, and often pus. It is also explained as something that festers and corrupts like an open sore. A peptic ulcer may arise at various locations (Figure 1.1).

- Stomach (called gastric ulcer)
- Duodenum (called duodenal ulcer)
- Esophagus (called esophageal ulcer)

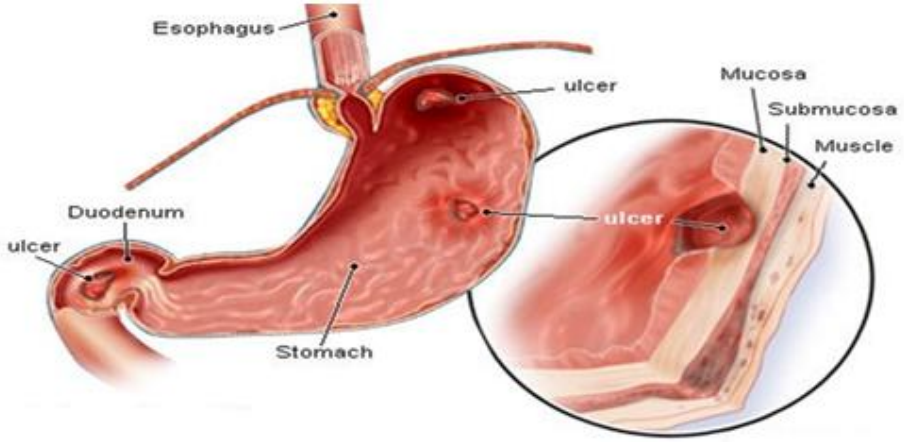

Figure 1.1 (a) Depicting stomach ulcer; (b) duodenal ulcer; and (c) esophageal ulcer

Causes and pathogen city of Ulcers

There are three major causes of peptic ulcers: infection, certain types of medications, and other medical problems that cause the release of too much stomach juices. Research studies have shown that most ulcers are caused by an infection by bacteria called Helicobacter pylori -- also referred to as H. pylori (Figure 1.2).

Figure1.2 A major ulcerogenic Helicobacter pylori

2. Materials and Methods

Plant Materials

Psidium guajava or commonly known as Guava is a popular fruit tree. It is evergreen, tropical, and small growing only up to 10 m high. It is native to the Caribbean, Central America, and South America but now widely cultivated throughout tropical and subtropical regions around the world. It is an excellent pioneer species that can thrive at high temperature and drought conditions. Guava has a wide range of medicinal uses. In particular, it has antibacterial properties, an astringent, anti-inflammatory, and anti-diabetic. It is used against dysentery, diarrhea, hepatitis, gonorrhea, coughs, stomach pain, skin problems, ringworms, wounds, and ulcers. The fruit can be sweet to acidic and is high in vitamin C.

Extraction of Plant Materials

The fresh leaves of Psidium guajava Linn was collected from Local aria of Monad University, Hapur, and U.P. India. A voucher specimen of the plant has been deposited at Monad University, Hapur, and U.P. India. Leaves of Psidium guajava were washed with dechlorinated water, dried in shade and powdered with the help of an electric blender. The test materials (1.0 kg) were extracted with different organic solvents viz., acetone, hexane, petroleum ether, chloroform and methanol in a Soxhlet apparatus for 8 h and the extract was concentrated in a rotary vacuum evaporator to yield crude extract, which was used in bioassays.

Thin layer chromatographic studies

Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using silica gel Gas stationary phase. The mobile phases, results and chromatograms are depicted in Figures 1-3.

Antimicromal Activity

Antimicrobial susceptibility testing was done using the well-diffusion method according to the standard of the National Committee for Clinical Laboratory Standards. The plant extracts were tested on Mueller Hinton II plates to detect the presence of antibacterial activity. Prior to streaking the plates with bacteria, 5mm diameter wells were punched into the medium using a sterile borer. All plates were inoculated with the test bacterium which has been previously adjusted to the 0.5 McFarland standard solution; a sterile cotton swab was dipped into the suspension, rotated several times, and pressed firmly on the inside wall of the tube above the fluid level removing excess inoculums. The surface of the agar plate was streaked over the entire sterile agar surface rotating the plate to ensure an even distribution of inoculums with a final swab around the rim. The plates are allowed 3 to 5min to dry the excess moisture. Fifty µL aliquots of each test extract were dispensed into each well after the inoculation of the plates with bacteria. The wells were also arranged in a triangle formation 2 inches apart. The same extract was used on each plate, with a total of three plates used for each extract for selecting bacterium. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The plates are sealed with par film, labeled, and placed in an incubator set to 37°C. After 24 hours of incubation, each plate was examined for inhibition zones. A ruler was used to measure the inhibition zones in millimeters. Every experiment was carried out in parallel, and the results represented the average of at least three independent experiments. Various extractions of Psidium guajava and Azadirachtolides indica were tested against hazardous bacterial culture of B. subtilis, P. aeruginosa, and aerogenes and S. aureus by disc diffusion method. Strains were swabbed on the surface of the sabered agar plates and discs (Whatman No.1 filter paper with 9 mm diameter) impregnated with the 50 µl of each extract were placed on the surface separately. To compare the anti-bacterial activities, Ampicillins (20 µg/disc) used as standard antibiotic and negative control, a blank disc impregnated with solvent was used. The plates (triplicates) were incubated at 28°C for 72 hrs. The antimicrobial potency of the test samples was measured by determining the diameter of the zones of inhibition in millimeter.

In-vitro Evaluation of Antiulcer Activity:

Acid Neutralizing Capacity: The aqueous extract of acid-neutralizing capacity value is 100mg, 500mg, 1000mg, 1500mg. The aluminum hydroxide and magnesium hydroxide (500mg) have compared for the standard. The total volume was 70ml with the addition of 5ml of a quantity of the mixture and remaining with water to make up the total volume; mix this for one minute. To the standard and test preparation, the 30ml of 1.0 N HCl was added and stirred for 15 minutes after that phenolphthalein was added and mixed. With 0.5N Sodium hydroxide, the excess HCl was immediately titrated until the pink cooler is attained 15. The moles of acid neutralized is calculated by, Moles of acid neutralized = (vol. of HCl × Normality of HCl) - (vol. Of NaOH × Normality of NaOH) Acid neutralizing capacity (ANC) per gram of antacid = moles of HCl neutralized divided by Grams of Antacid/Extract.

H+/K+ - ATPase Inhibition Activity:

Preparation of H+/K+ - ATPase Enzyme

To prepare H+/K+ - ATPase enzyme sample the fresh goat stomach has purchased from the local slaughterhouse, the gastric mucosa of the fundus was cut-off and opened, the inner layer of the stomach has scrapped out for the parietal cell. The parietal cell obtained from the stomach has homogenized in 16mM Tris's buffer with PH of 7.4, which has 10% Triton X-100 and centrifuged at 6000 rpm for 10mins after centrifuged the supernatant solution has used for the H+/K+- ATPase inhibition Protein content are used to find out according to Bradford's method were BSA are used for standard. Assessment of H+/K+ ATPase inhibition: Per-incubated for 60 min at 37 ºC for the reaction mixture of the sample containing 0.1ml of enzyme extract (300µg) and plant extract with different concentration (20µg, 40µg, 60µg, 80µg, 100µg).

The reaction was initiated by adding substrate 2 mM ATP (200µL), in addition to this 2mM MgCl2 (200µL) and 10mM KCl (200µL) has added. After 30 min of incubation at 37 ºC the reaction was stopped by 4.5% ammonium molybdate, and 60% perchloric acid was added and centrifuged at 2000rpm for 10 min, and in spectrophotometrically inorganic phosphate was released

and measured at 660nm by following the Fiske-Subbarow method. Briefly, at 10 min at room temperature, 1ml of supernatant 4ml of Millipore water, 1ml of 2.5% of ammonium molybdate, 0.4ml of ANSA was added. At 660nm inorganic phosphate, absorbance has been measured at various doses of the extract; the enzyme activity has been calculated as micromoles of Pi released per hour. Results were compared with the known anti-ulcer PPA inhibitor Omeprazole and expressed as Mean ± SEM 16 % enzyme inhibition has calculated using the formula:

Percentage of inhibition = [Activity (control) - Activity (test)/Activity (control)] × 100…. **Eqs 1**

3. Result and discussion

3.1 Thin Layer Chromatographic (TLC) Analysis

Among the various methods for separating plant constituents, the thin layer chromatographic procedure is the one of the most commonly used techniques of general application. [6] Thin layer chromatography (TLC) involves the separation of mixtures of organic compounds on thin layers of adsorbents that are usually coated on glass, plastic, or aluminum sheets; and this particular technique is the easiest, cheapest and most widely used method for the characterization of natural products and their preparations. [7] The chloroform extract yielded maximum spots in TLC, followed by methanol and petroleum ether extracts respectively (Figures 5.1-5.3). All of these TLC profiles may serve as characteristic fingerprint of P. guajava leaf. These data would therefore be suitable for monitoring the identity and purity of the plant material and for detecting adulterations and substitutions. [8]

Figure 5.1: TLC profile of the pet. ether extract of P. guajava leaf. Solvent system: benzene: chloroform: ethyl acetate (4: 3: 3). Rf values: 0.10, 0.74, 0.87, 0.93.

Figure 5.2: TLC profile of the chloroform extract of P. guajava leaf. Solvent system: benzene: chloroform: ethyl acetate (3: 4: 3). Rf values: 0.10, 0.15, 0.21, 0.73, 0.84, 0.93.

Figure 5.3: TLC profile of the methanol extract of P. guajava leaf. Solvent system: ethyl acetate: methanol (7: 3). Rf values: 0.34, 0.44, 0.50, 0.68, 0.84.

Table 5.2: TLC characterization of Psidium guajava leaves extracts

S. No	Solvent system	No of spots	*Rf* value
1	Benzene: Chloroform: Ethyl acetate (4: 3: 3)	4	0.10, 0.74, 0.87, 0.93
2	Benzene: Chloroform: Ethyl acetate (4: 3: 3)	6	0.10, 0.15, 0.21, 0.73, 0.84, 0.93.
3	Ethyl acetate: Methanol (7: 3)	5	0.34, 0.44, 0.50, 0.68, 0.84.

3.2 Antimicrobial Activity

The results of the study indicated that only two of the crude solvent extracts prepared from the leaves of Psidium guajava, methanol and ethanol, showed inhibitory activity against bacteria (Table 2). Only Gram-positive bacteria, Bacillus cereus and Staphylococcus aureus, were susceptible to the two extracts, while neither of the Gram-negative bacterium showed any inhibition. At 10 mg/50 µL, the methanol extract had a slightly higher antibacterial activity with mean zones of inhibition 8.27 and 12.3mm than ethanol extract with mean zone of inhibition 6.11 and 11.0mm against B. cereus and S. aureus, respectively. The resistance of the Gram-negative bacteria could be attributed to its cell wall structure. Gram-negative bacteria have an effective permeability barrier, comprised of a thin lip polysaccharide exterior membrane, which could restrict the penetration of the extruding the plant extract. It has been reported earlier those Gram-negative bacteria are usually more resistant to the plant-origin antimicrobials and even show no effect, compared to Gram-positive bacteria [42– 44]. Gram positive bacteria have a mesh-like peptidoglycan layer which is more accessible to permeation by the extracts [22, 28, 42, 43]. Results found in this study were supported and/or opposed in the data reported in literature. Nascimento et al. [45] conducted a study which supports the finding of the present study in which the guava extract was able to have inhibitory effects against Staphylococcus and Bacillus and no effect on the Escherichia and Salmonella, whereas Chanda and Kaneria [46] oppose the findings concerning the Gram-negative bacteria. Mahfuzul Hoque et al. [21] found no antibacterial activity of ethanolic extracts of guava against E. coli and S. enteritis; however, Vieira et al. [26] found guava sprout extracts were effective against inhibiting E. coli. Sanches et al. [24] found that the aqueous extract of guava was effective against Staphylococcus and Bacillus. The methanolic extracts of guava reported by Lin et al. [47] showed significant inhibitory activity against the growth of 2 isolates of Salmonella and enter pathogenic E. coli.

Table 5.5: Antimicrobial activities of Psidium guajava leaves of the screened solvents extracts

Plant extracts	Zone of inhibition* (mm)			
	B. cereus	*S. aureus*	*E. coli*	*S. entertidis*
n-Hexane	—	—	—	—
Ethanol	6.11 ± 0.60	11.0 ± 0.52	—	—
Methanol	8.27 ± 0.44	12.3 ± 0.78	—	—
Water	—	—	—	—

Inhibition zones are the mean including borer (5 mm) diameter ± standard deviation.
—: no inhibitory activity.

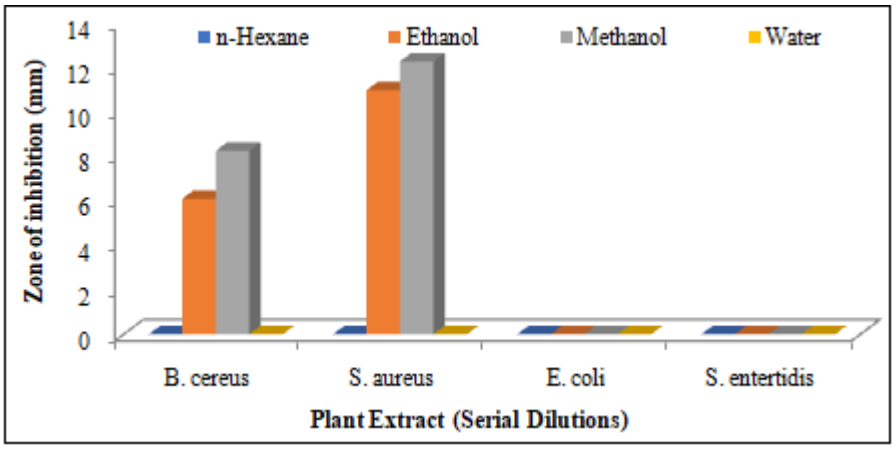

Figure 5.6: Zone of Inhibition of B. cereus, S. aureus, E. coli, S. entertidis

3.3 IN-Vitro Antiulcer Activity

3.3.1 Acid Neutralizing Capacity

The neutralizing effect of the aqueous extract was studied for four concentration (100mg, 500mg, 1000mg, 1500mg) and standard Aluminum Hydroxide + Magnesium Hydroxide [Al (OH)3+Mg (OH)2] (500mg). The results obtained envisage that the extract at concentration 100mg, 500mg, 1000mg, and 1500mg showed a significant reduction in acid-neutralizing capacity (ANC), i.e., 110.5, 35.5, 11.75, and 9.3, respectively, as compared to standard Al (OH)3+Mg (OH)2 (500 mg) which is 15.7. The extract at a concentration of 1500 mg has been found to neutralize acid more significantly as compared to standard. The results have tabulated in Table & Graph 1.

Table 5.3: Effect of Aqueous Extract of on Acid Neutralizing Capacity

S. no.	Concentration (mg)	Volume of NaOH consumed (ml)	mEq of Acid Consumed	ANC per gram of Antacid
1	100	37.9	13.05	110.5
2	500	29.5	17.25	35.5
3	1000	39.5	9.75	11.75
4	1500	42	12	9.33
5	500mg Al (OH)3+Mg (OH)2	45.3	7.85	15.7

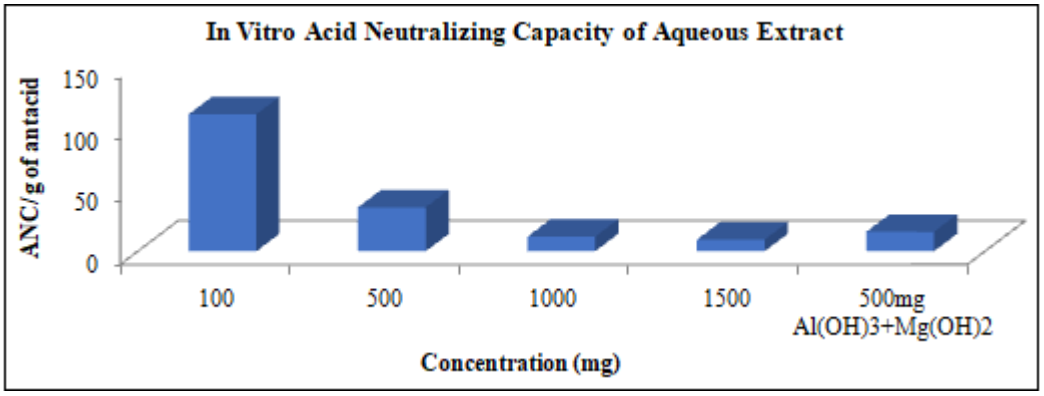

Figure 5.4: Effect of Aqueous Extract of on Acid Neutralizing Capacity

H+/K+ - ATPase Inhibition Activity: The H+/ K+ - ATPase inhibition activity of aqueous extract at a various concentration (20µg, 40µg, 60µg, 80µg, 100 µg) has compared with Omeprazole as standard. The extract significantly showed activity in a dose-dependent manner. Maximum percentage inhibition of 62.18±0.54% has been observed for extract at a concentration of 100µg, and standard Omeprazole showed 69.56±1.72%. The results have been tabulated in Table 2 and Graph 2.

Table 5.4: Effect of Aqueous Extract of on In-Vitro H+/K+ - Atpase Inhibition Activity

S. No.	Concentration (µg)	Percentage Inhibition (%) (Mean ± SEM)	
		Standard Omeprazole	Aqueous extract
1	20	-51.25±0.78	-30.12±0.26
2	40	-56.32±1.24	-18.84±1.86
3	60	36.58±1.58	31.64±0.68
4	80	58.62±0.24	55.36±1.54
5	100	69.56±1.72	62.18±0.54

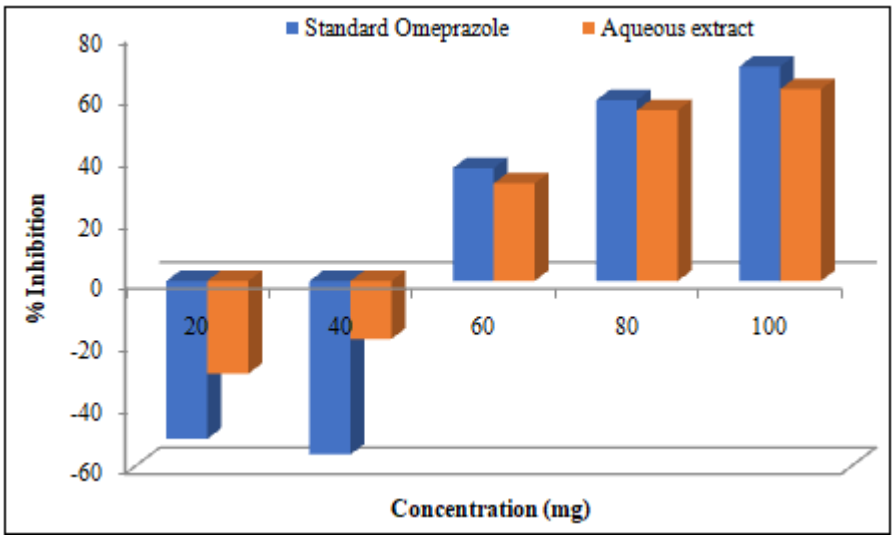

Figure 5.5: Effect of Aqueous Extract of on In Vitro H+/K+ - Atpase Inhibition Activity

4. Summary and Conclusion

Plants are the basic source of knowledge of modern medicine. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant medicines as alternative to synthetic drugs. Nowa- day's herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness and no side effect in clinical experience. Large number of plants belonging to different families has been studied for their therapeutic properties. However, plants such as *Psidium guajava* to Myrtaceous and Meliaceous respectively which have many medicinal properties have not been studied for their antibacterial and pharmacological activities and hence the present study focused on antidiabetic, antioxidant and antibacterial activities of *Psidium guajava* has been investigated in albino rats by using methanolic extracts of both plants. The results were statistically analysed. From the investigations the following observations was made.

- Among the plants, leaf extract of showed potent antimicrobial activity of *Psidium guajava.*
- *Psidium guajava* showed anti-diabetic activity by reducing hypoglycemic effect and by lowering the blood glucose levels in streptozotocin-induced rats.

Antimicrobial, anti-diabetic and antioxidant activities of both plants due to occurrence of polyphenolic compounds such as copaene, caryophyllene, naphthalene, ledol, and phytol from Psidium guajava and à-D-Glucopyranoside, à-D-glucopyranosyl, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl and 3-Heptanol, 3,5-dimethyl, from A indica.

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