In-Vitro Antispasmodic Effect of Psidium Guajava L. Leaves Extract on Isolated Chick Ileum

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***Abstract*—Background-An antispasmodic drug is a medication used to relieve or prevent spasms or involuntary muscle contraction in the body. Acetylcholine is a neurotransmitter that can indeed cause muscle contraction or spasms under certain circumstances. Psidium guajava is a commercially available plant that has been used commercially for its antispasmodic properties.**

Aim:

The study aims to access the antispasmodic activity of ethanolic extract in Psidium guajava comparison with Atropine using in vitro experimental models.

Materials and methods:

Ethanolic extracts were prepared by the soxhlation method for the in vitro study, and isolated chick ileum was used. Chick ileum was suspended in an organ bath containing Tyrode solution at 37 0 C with adequate oxygen supply. The effect of Psidium guajava was studied on ileum contraction induced by acetylcholine compared with that of Atropine.

Results:

The mean percentage response was calculated for the ethanolic extract of Psidium guajava and Atropine.

Conclusion:

From this study, it was concluded that Psidium guajava extract has a significant antispasmodic activity compared to Atropine. These findings therefore raise hope for the development of a new antispasmodic drug with few side effects that may be useful in the treatment of conditions like dysenteries, the relief of cramps, abdominal distention, and postprandial lower abdominal discomfort associated with diarrhea which are common symptoms in patients with IBS. Antispasmodic activity can inhibit the muscle spasm with fewer side effects. Adverse effects associated with these agents are anticholinergic in nature.

***Index Terms*—Antispasmodic, spasm, Psidium Guajava, Irritable bowel syndrome (IBS), Neurotransmitter, Plant extract, Diarrhea, Gastrointestinal disorder, Anti-inflammatory.**

I. INTRODUCTION

Gastrointestinal disorders are a prevalent concern among children worldwide, encompassing conditions such as functional abdominal pain, ulcerative colitis, irritable bowel syndrome (IBS), infantile colic, constipation, gastroenteritis, and acute gastrointestinal disorders. These conditions can significantly impact quality of life and are often linked to an increased likelihood of anxiety and depression. Characterized by recurrent or chronic abdominal discomfort, IBS specifically is marked by changes in bowel habits or defecation patterns that may alleviate or worsen symptoms.

Medicinal plants and their herbal products have been integral to traditional healthcare systems for centuries, valued for their diverse nutritional and therapeutic benefits. The guava plant, especially its leaves, is recognized as a powerhouse of bioactive compounds, containing a wide spectrum of phytochemicals that play a pivotal role in its health-promoting effects. These compounds include an array of flavonoids, phenolic acids, tannins, and other secondary metabolites known for their antioxidant, anti-inflammatory, antimicrobial, and cardioprotective properties. Key constituents such as quercetin, kaempferol, myricetin, and catechin are renowned for their ability to neutralize free radicals and reduce oxidative stress, while compounds like gallic acid, chlorogenic acid, and epigallocatechin gallate are celebrated for their contributions to overall health by

modulating inflammatory pathways and supporting immune function. The therapeutic potential of guava leaves is further enhanced by their synergistic interplay of bioactive components, which collectively offer a natural, multifunctional approach to disease prevention and health maintenance. These compounds not only contribute to the plants' antispasmodic, anti-inflammatory, and antimicrobial properties but also enhance their efficacy in treating gastrointestinal (GI) diseases and various other ailments. Their long-standing use across generations underscores their effectiveness and safety, which are further supported by ongoing research and scientific validation. Consequently, these plants continue to play a crucial role in alternative medicine systems, bridging the gap between traditional knowledge and modern therapeutic approaches.

Spasm-relieving compounds are abundantly found in nature, occurring in a wide variety of plants known for their medicinal properties. Antispasmodic therapeutic agents are effective remedies that help alleviate gastrointestinal muscle spasms while also reducing the liquid content in the gastrointestinal tract, thereby preventing diarrhoea. Spasms in the gastrointestinal tract are triggered by the activation of cholinergic/muscarinic, opioid, and histaminic receptors, located in the gastrointestinal system and other parts of the body. The stimulation of cholinergic receptors, specifically M1, M3, and M5 subtypes, initiates intracellular signaling pathways that activate protein kinase A (PKA) and protein kinase C (PKC). These kinases, in turn, promote the opening of calcium channels, leading to an influx of calcium ions into the cells. Additionally, calcium release from the endoplasmic reticulum further elevates intracellular calcium levels. The increased calcium concentration interacts with smooth muscle, causing strong contractions that may also result in diarrhea. This underlying mechanism is fundamental in understanding and conducting antispasmodic studies.

II. MATERIALS AND METHODS

A. Plant materials

Figure 1- Psidium Guajava

Fresh Psidium Guajava leaves were gathered from Ernakulam district, Kerala state.

B. Animals

For the investigation, fresh chicken ileum was collected from the slaughterhouse. It was kept at room temperature with adequate aeration in a freshly prepared Tyrode solution. The research was done at the Chemists College of Pharmaceutical Sciences and Research in Kerala, India, in the Pharmacology laboratory of the Department of Pharmacology.

C. Preparation of plant extract

The Psidium Guajava extract was created using Soxhlet extraction. In this procedure, fresh Psidium Guajava leaves were shade-dried for 7 days before being manually blended into a coarse powder. The Soxhlet extraction chamber was filled with 35g of the powdered substance, and ethanol was used as the extraction solvent. The extraction was performed for 5 days, and 50^0C-60^0C was kept as the extraction temperature. The distillation apparatus evaporated the ethanol at 55^0C after the experiment to produce a crude extract that weighed 6.50g.

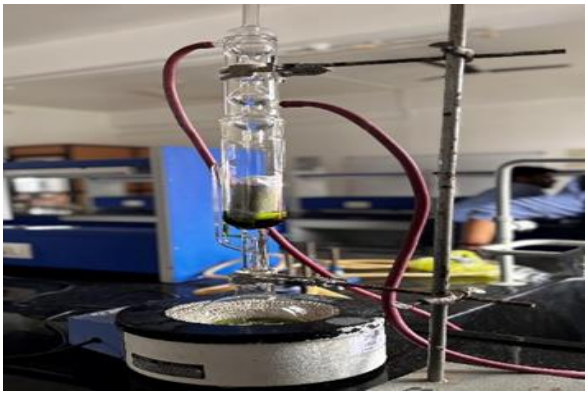


Figure 2- Soxhlation

Figure 3- Plant Extract

D. Drug preparation and dilutions

The concentration of 1 x 10^{-1} g/ml was created by dissolving 100mg of the extract in 100ml of distilled water, from this 20ml was taken and made up to 100ml with distilled water. The conventional medications, 100 µg/ml of Acetylcholine and Atropine, were administered using the same process. Tyrode solution was prepared per liter of water by dissolution of the following substances: NaCl-8g, KCl-0.2g, CaCl2-0.2g, NaHCO3-1g, NaH2PO4-1G, MgCl2-0.1g and Glucose-2g.

In-vitro study of the effect of Psidium Guajava on isolated chick ileum

Figure 4- Dose-response curve

Fresh chicken ileum was collected, placed in a beaker with a Tyrode solution at 37^0C, and then aerated. A portion of the 2-4cm ileum was excised, mounted, and kept at 37^0C in an organ bath containing Tyrode solution with an oxygen supply. The kymograph and its attachments were set up to tension the tissue properly. Before starting the medication infusions, the tissue was given 15 minutes to acclimatize.

Acetylcholine dose responses were established in the following order:

1. Acetylcholine alone.
2. Acetylcholine in the presence of atropine.
3. Acetylcholine in the presence of ethanolic extract of Psidium Guajava leaves.

III. IDENTIFICATION TEST

Psidium guajava is a rich source of bioactive compounds, including flavonoids, phenolic acids, tannins, and various secondary metabolites, which are well-documented for their potent antioxidant, anti-inflammatory, antimicrobial, and cardioprotective effects. Among these, flavonoids are particularly notable for their antispasmodic activity, in addition to their ability to neutralize free radicals and alleviate oxidative stress. Key flavonoid constituents such as quercetin, kaempferol, myricetin, and catechin are instrumental in these effects, while phenolic acids like gallic acid, chlorogenic acid, and epigallocatechin gallate further contribute by regulating inflammatory pathways and enhancing immune responses. To confirm the presence of flavonoids, a standard identification test can be performed as follows:

Tests for Flavanoids

Experiment	Observation	Inference
SHINODA TESTS: 2ml of Extract Solution, Few Magnesium Turnings, 5ml 95% Ethanol, and Few Drops of Conc. HCl was added.	Pink to Red color develops	Presence of Flavanoids
ZN-HCL REDUCTION TEST: To 2ml of the Extract Solution, a mixture of Zn dust and Conc. HCl was added.	Precipitate develops	Presence of Flavanoids

SODIUM HYDROXIDE TEST: Add aqueous sodium hydroxide to the plant extract.	Yellow color develops that gradually disappears on the addition of dilute acid (acetic acid)	Presence of Flavanoids
FERRIC CHLORIDE TEST: To the extract, add a few drops of ferric chloride solution.	A green or blue coloration	Presence of flavonoids

Table 1- Identification test for Flavanoids

IV. RESULTS AND DISCUSSION

Building on the traditional use of *Psidium guajava* in managing hyperactive gut disorders like spasms, its crude extract was evaluated for antispasmodic activity using chick ileum preparations. The crude extract protected spasms, similar to atropine, a standard antispasmodic agent. They relieve spasms or involuntary muscle contraction, which can occur in various body parts, including the digestive tract and the smooth muscles. The Anti-spasmodic activity was evaluated by plotting a dose-response curve. The corresponding percentage response was calculated using the mean heights obtained from DRC. The mean percentage response of Acetylcholine, Psidium Guajava and Atropine was 83.51%, 76.41% and 77.50% respectively. From the observed data, the mean percentage response of Psidium Guajava was found to be less than Atropine.

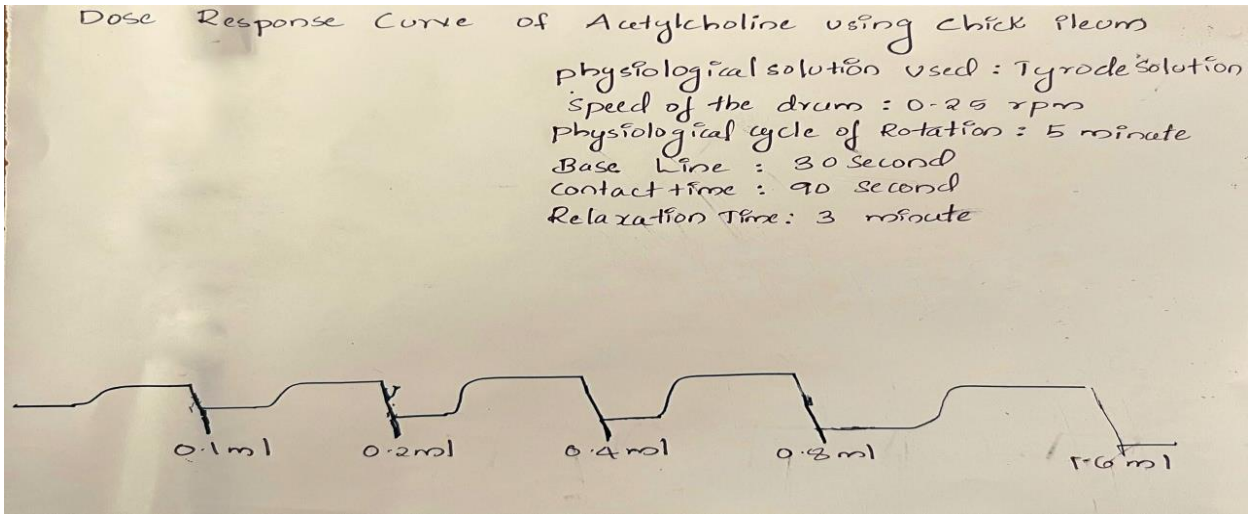


Figure 5- Dose-response curve of Acetylcholine

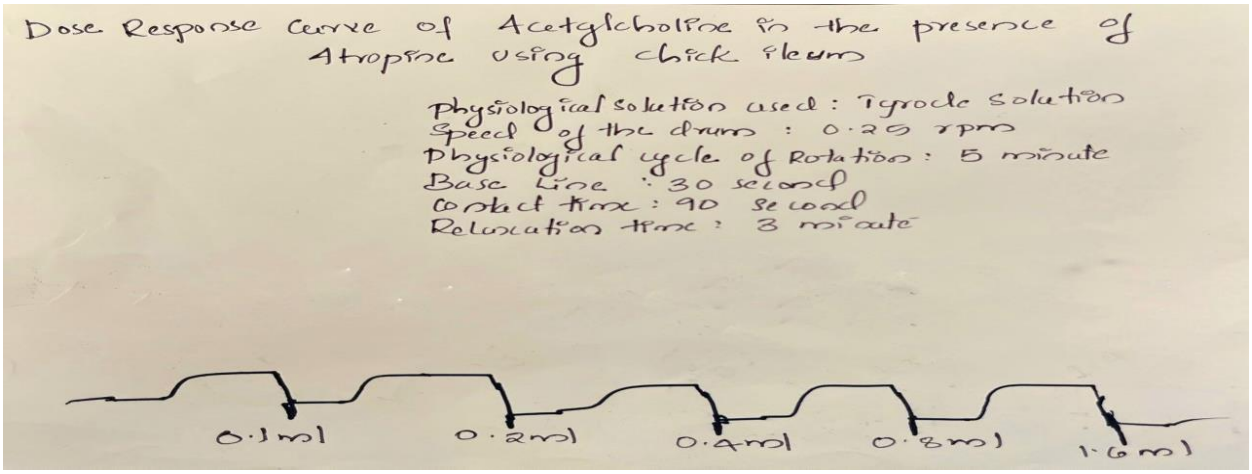


Figure 6- Dose-Response curve of Acetylcholine and Psidium Guajava

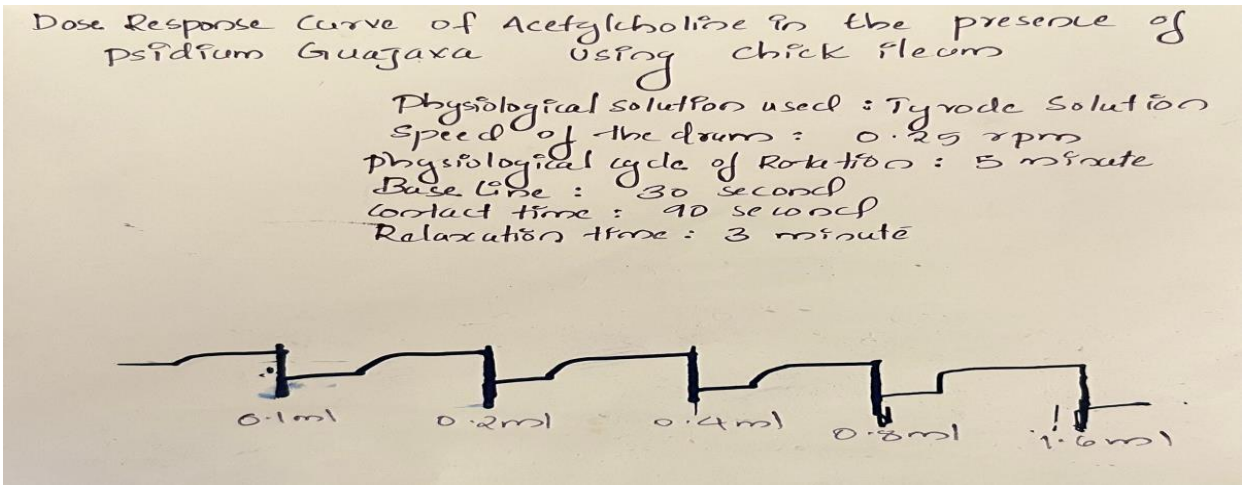
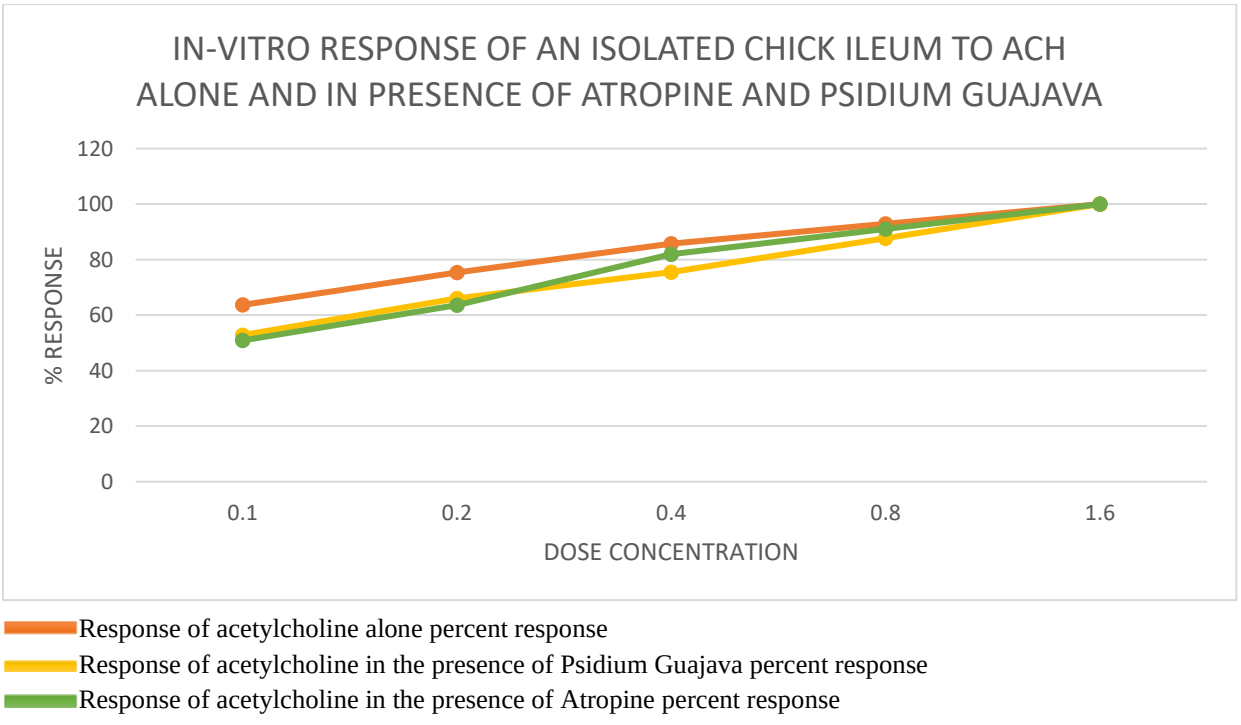


Figure 7- Dose-response curve of Acetylcholine in the presence of Atropine

SI No:	dose of Ach (ml)	Response of Ach alone		Response of Acetylcholine in the presence of Psidium Guajava		Response of Acetylcholine in the presence of Atropine	
		Height in mm±SEM	% Response	Height in mm±SEM	% Response	Height in mm±SEM	% Response
1	0.1	9.3±0.333	63.70	5.6±0.666	52.83	5.6±0.333	50.90
2	0.2	11±0.577	75.34	7±0.577	66.03	7±0.577	63.63
3	0.4	12±0.577	85.71	8±0.577	75.47	9±0.577	82
4	0.8	13.3±0.881	92.85	9.3±0.333	87.73	10±0.577	91
5	1.6	14.6±0.666	100	10.6±0.333	100	11±0.577	100
Mean			83.51		76.41		77.50

Table 2- *In vitro* response of isolated chick ileum to Acetylcholine alone and in the presence of Atropine and Psidium Guajava



Potential Applications:

- Development of plant-based antispasmodic agents.
- Use in managing gastrointestinal disorders like irritable bowel syndrome (IBS).

V. CONCLUSION

The research focused on evaluating the antispasmodic properties of an ethanolic extract derived from the leaves of *Psidium guajava*, comparing its effects to the standard antispasmodic agent, Atropine. Acetylcholine, a neurotransmitter responsible for inducing contractions in smooth muscle, was used to stimulate isolated chick ileum tissue in vitro. The extract's ability to inhibit these contractions and relax the smooth muscle was assessed by recording dose-response curves.

Using Soxhlation, the ethanolic extract was prepared from authenticated, dried, and powdered leaves of *Psidium guajava*. Preliminary phytochemical analysis confirmed the presence of bioactive compounds such as flavonoids, alkaloids, tannins, glycosides, and terpenoids, which are often associated with therapeutic effects, including antispasmodic activity. The chick ileum tissue, rich in various receptors such as muscarinic, histaminic, adrenergic, serotonergic, and GABAergic, served as a model to evaluate the extract's pharmacological effects.

Results demonstrated that Acetylcholine produced dose-dependent contractions in the ileum, while the ethanolic extract effectively inhibited these contractions. The extract’s efficacy was compared to Atropine by analysing the mean percentage inhibition of contractions, showing significant smooth muscle relaxation. These findings suggest that the *Psidium guajava* leaf extract exhibits potent antispasmodic properties, potentially offering an alternative or complementary approach to conventional treatments like Atropine.

Key findings:

Psidium Guajava leaves exhibit a significant antispasmodic effect.

VI. ACKNOWLEDGMENT

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