

Proceeding Paper

# Anti-Microbial Activity, Antioxidant and Antidiarrheal Activities of the Leaves of *Carica papaya* L. and *Psidium guajava* L. <sup>†</sup>

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**Abstract: Objective:** The aim of the present study was to investigate the phytochemical and antimicrobial activity as well as the antioxidant and anti-inflammatory properties of *Carica papaya* and *Psidium guajava* leaf extracts. **Methods:** Since phytochemicals are biologically active compounds and a powerful group of plant chemicals believed to stimulate the immune system, the present study focuses on the alcoholic and aqueous extraction of *Carica papaya* and *Psidium guajava* leaf extracts. Antioxidant activity was examined in vitro using DPPH and hydrogen peroxide assay. Gram-negative (*Klebsiella pneumonia* and *Escherichia coli*) and gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) bacteria were obtained as test microorganisms. The results showed that aqueous and alcoholic leaf extracts have antibacterial effects against tested organisms. The antidiarrheal activity of both aqueous and alcoholic leaf extracts, extracts from the leaves of *Carica papaya* and *Psidium guajava*, was studied in rats against castor oil-induced diarrhea at doses of 125 and 250 mg/kg body weight. **Results:** The results of the phytochemical screening showed that the presence of biologically active compounds such as alkaloids, carbohydrates, and amino acids varied in the various solvent extracts, with alcoholic extracts showing the highest levels of phytochemicals and the best anti-microbial, antioxidant, and anti-diarrheal activities. The time it took to induce diarrhoea was greatly increased by the alcoholic extract, and to a lesser extent by the aqueous extract. It also decreased the frequency of diarrheal episodes and increased the movement of charcoal meal through the GI tract. **Conclusions:** In the search for new drugs, the purification and isolation of specific bioactive chemical substances may serve as a natural and promising cure.

**Keywords:** *Psidium guajava* Linn; *Carica papaya* Linn; anti-microbial; anti-inflammatory; phytochemicals; antioxidants

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## 1. Introduction

The amount of plants offered between and among countries has increased significantly in recent years as a consequence of the rising demand for herbal products. Secondary plant metabolites (also known as phytochemicals), which previously lacking known pharmacological properties, have undergone substantial research as a potential source of therapeutic drugs (Krishnaraju et al., 2005). In order to cure bacterial infections, it is envisaged that phytochemicals with sufficient antibacterial efficacy would be utilised (Balandrin et al., 1985). Considering the significant number of hospitalised patients with decreased immunity and the emergence of novel, multi-resistant bacterial strains, this

information should be of concern. As a result, new infections may spread in hospitals, increasing mortality.

The Caricaceae family, which includes the evergreen shrub or tiny plant *C. papaya* Linn, has four different genera and four distinct species in India. According to (Krishna KL et al., 2009, Makkar HP et al., 2009 and Samatha T et al., 2012), this plant was first discovered between Southern Mexico and Costa Rica. Afterward spread as a plantation crop to India, Sri Lanka, the Hawaiian Islands, Australia, and other tropical and subtropical locations. The herbaceous perennial plant known as papaya is also referred to by the names papaya melon tree, pawpaw or papau, kapaya, lapaya, papyas, papye, tapayas, and fan mu gua. The papaya plant as a whole contains a variety of phytonutrients and has potential uses in food, medicine, and industry. A wide range of phytonutrients, antioxidants, antimicrobials, and anti-dengue properties make the papaya plant the best. (Abd Elgadir M et al., 2014)

Some of the important phytochemicals found in *C. papaya* are Benzylisothiocyanate, Benzylglucosinolate, chlorogenic acid, Beta-carotenoid, Lycopene, protocatechuic acid, Quercetin, caffeic acid, etc. (Parle M., et al., 2011 and Ayoola P. B., et al., 2010) phytochemicals that have been known for their ability to help prevent human diseases, including phenols, flavonoids, and tannins. (Jaiswal P., 2010, Meenakshi S. et al., 2011, Wu Y. Y. et al., 2011 and Basalingappa K. M., et al., 2018)

*Psidium guajava*, a plant belonging to the Myrtaceae family, is well-known for its anti-inflammatory, antispasmodic anti-allergic, cardioactive, antimicrobial, cytotoxic, antiplasmodial, antispasmodic properties. (Gill K S, 2016). Guava leaves are used in traditional medicine to treat a variety of human disorders including ulcers, wounds, bronchitis, eye wounds, gastrointestinal diseases, diarrhea, and cholera. The use of the leaves of guava for decoction has been around for a very long time. (Dzotam J K and Kuate V 2017; Begum S et al., 2014) Guava leaves can also be used therapeutically by being chewed. Because herbal remedies are thought to have little negative effects, about 80% of people worldwide rely on them. (Anbuselvi V. and Rebecca J., 2017) The use of guava leaves as a medicine is of great interest to many people. It is thought that guava leaves contain an active ingredient that can be used to treat a number of illnesses. (Biswas B et al., 2013).

Longicyclene, caryophyllene,  $\beta$ -bisabolene, caryophyllene oxide,  $\beta$ -copanene, farnesene, humulene, selinene, cardinene, and curcumene are all present in guava leaves, along with nerolidol,  $\beta$ -sitosterol, and menthol. Triterpenes like oleanolic acid, tannins, eugenol, caryophyllene, flavonone-2, 2'-ene, prenol, dihydrobenzo-phenanthridine, and cryptonine are among the substances that include 3-L-4-4-arabinofuranoside (avicularin) and 3-L-4-yranoside. The essential oils found in the leaves of *Psidium guajava* are abundant in cineol, tannins, triterpenes, flavonoids, resins, eugenol, malic acid, fat, cellulose, chlorophyll, and other compounds. (Gill K S, 2016)

## 2. Materials and Methods

Fresh leaves of *C. papaya* and *Psidium guajava* were collected from the Sahyadri regions of Maharashtra. To prevent surface contamination, the plant parts were cleaned under running water, dried in the shade for about 15 days. Dried leaves were broken up into tiny pieces and ground into a fine powder. The powder was then steeped in ethanol and water before being extracted with a solvent using the Soxhlet device. The extracted material was then kept at 4 °C for a further examination. (Ahmad N., et al., 2011 and Rai et al., 2007) Crude papaya and *Psidium guajava* leaf extract of approx 15 gms and 18 gms were obtained respectively.

### 2.1. Phytochemical Analysis

Alkaloids, carbohydrates, amino acids, glycosides, protein, phenolic compounds, and tannins were all screened for using phytochemical analysis on the corresponding extracts. According to the protocol, the following bioactive chemicals were screened in each

dry extract: alkaloids, flavonoids, terpenoids, phenol and tannins, sugar, quinones, and proteins, saponins. (K. R. Khandelwal, 2002 and Ayoola et al., 2008).

**Alkaloids test:** A yellow colour formed after mixing 1 mL of the extract with 1 mL of Mayer's reagent and a couple of drops of the iodine solution signifies the presence of alkaloids. **Terpenoids test:** In order to detect the presence of terpenoids, 1 mL of the extract was diluted with 1 mL of Conc. H<sub>2</sub>SO<sub>4</sub> and kept in a water bath for 2–4 min.

**Tests for phenols and tannins:** 1 mL of 2% FeCl<sub>3</sub> and 1 mL of the extract were combined. Tannins are present when blue, green, or black colour develops.

**Tests for Sugar:** 1 mL of the extract is combined with 1 mL of Benedict's solution. After that, the sample is incubated for 2–4 min in a water bath. Red, orange, blue, or green hues indicate the presence of sugar.

**Tests for Saponins:** When 1 to 2 mL of distilled water were added to 1 mL of extract, a 1 cm thick layer of foam formed, indicating a presence of saponins.

**Flavonoids test:** Add a few drops of weak NaOH to 1 millilitre of extract, then add a few drops of HCl to neutralise the mixture. The sample will turn discoloured to show that flavonoids are present.

**Tests for Quinones:** In 1 mL of extract, add a few drops of diluted NaOH, and then add a few drops of HCl to neutralise the solution. When flavonoids are present, the sample will get discoloured.

**Tests for Proteins:** When a few drops of concentrated nitric acid were added to 1 millilitre of extract, a yellow hue developed, signifying the presence of proteins.

## 2.2. Evaluation of Anti-Oxidant Activity

### 2.2.1. DPPH Radical Scavenging Activity

The inhibition of DPPH, a stable free radical in nature, is the foundation of the DPPH (1,1-Diphenyl-2-picrylhydrazyl) test method. The highest absorption of the free radical DPPH that has an odd electron occurs at the wavelength of 517 nm (purple color). As antioxidants interact with DPPH, a stable free radical, it pairs off with a hydrogen donor (such as an antioxidant that scavenges free radicals) it is reduced to the DPPH, which results in a decrease in DPPH's absorbance. In methanol, a stock solution of the extracts and the standard component ascorbic acid was produced to a concentration of 1 mg/mL before being diluted to a different concentration. In a test tube, 1 mL each of the of the solutions that were diluted was added along with 1 mL of an ethanolic solution containing 1 mg/mL of DPPH. The absorbance at 517 nm was measured during a 30-min incubation period in complete darkness at room temperature. (Ebrahimzadeh M. A. et al., 2008)

### 2.2.2. Hydrogen Peroxide Scavenging Activity

The approach of (Ruch et al., 1989) was used to evaluate the extracts' capacity to scavenge hydrogen peroxide. Phosphate buffer, which has a pH of 7.4, was used to prepare the solution of a hydrogen peroxide solution (40 mM). A hydrogen peroxide solution (0.6 mL, 40 mM) was mixed with extracts (100 g/mL) in distilled water. Ten minutes later, the hydrogen peroxide absorbance at 230 nm was measured in comparison to a blank solution that contained phosphate buffer but no hydrogen peroxide.

## 2.3. Anti-Microbial Test

The sensitivity of the test organism for the active fractions of ethanolic and aqueous *Psidium guajava* and *Carica papaya* leaf extract showed the broad spectrum of antimicrobial activity against selected gram-negative (*Klebsiella pneumonia* and *Escherichia coli*) and gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) microorganisms were carried out using the disk diffusion technique described by Perez et al., 1990 and Cheesebrough et al., 2000).

The method suggested by Perez et al. (1990) was applied aseptically to a previously established antibiotic control experiment to evaluate the diameter of the zone of inhibition

from the extracts. This is done to promote either the prescription of antibiotics or the use of plant leaves that have antibacterial properties. Sodium ascorbate (25 mg/mL) is used to compare ascorbic acid to extracts.

By spreading the bacterial inoculums to the media, nutrient agar got contaminated with the specified microorganisms. On the agar, 5 mm-diameter wells were drilled. Then, individually, 25  $\mu$ L, 50  $\mu$ L, and 75  $\mu$ L different quantities of plant extract were introduced to the wells. By assessing the diameter of the zone of inhibition, the antibacterial activity of the plates was evaluated after 18 h of incubation at 37 °C. (Nduche M et al., 2019)

## 2.4. Antidiarrheal Activity

### 2.4.1. Animals

Wister rat (150–200 g) of either sex was used. They were provided with a standard laboratory environment, standard food, and unlimited use of water. The standards for animal care were followed in all experiments.

### 2.4.2. Testing of Antidiarrheal Activity

#### Normal Defaecation

Animals were divided into six groups of six animals each, starved for 18 h, placed individually in polythene cage with filter paper at bottom. (0.5 mg/kg) Loperamide was administered as a standard (Arif M et al., 2008) to one group while other two groups were given ethanolic extract at the doses of 125 mg/kg and 250 mg/kg. The fourth and fifth groups were administered petroleum ether extract at the doses of 125 and 250 mg/kg while the sixth group considered as control, was treated with vehicle (5% tween 80). All administrations were by per oral route (p.o.).

#### Castor Oil-Induced Diarrhea in Rat

One hour after the above treatments the animals were challenged with 1 mL of castor oil orally to induce diarrhea. The animals were observed at a time interval of 1 h for the presence of diarrhea upto 4 h with changing the filter paper after every hour. The diarrhea for this purpose was taken as watery (wet), unformed stool. (Mujumdar A M, 1998 and Pazhani G. P., et al., 2001) The total score of diarrheic faeces of control group was considered 100%. The results were expressed as % of inhibition. (Zaval M. A. et. al., 1988)

#### Gastrointestinal Motility in Rat

As a diet indicator in this trial, charcoal meal was used. In, (Boominathan R. et al. 2005) Six groups of six rats each were established and the rats fasted for 18 h before to the experiment. A vehicle (5% Tween 80 in distilled water) was given to the control group. The second group was given atropine sulphate (0.1 mg/kg, i.p.), the conventional medication. Third and fourth groups were administered ethanolic extract at doses of 125 and 250 mg/kg, p.o. respectively. Petroleum ether extract was given to the fifth and sixth groups at dosages of 125 and 250 mg/kg, p.o., respectively. Each animal was given 1 cc of charcoal meal (10% activated charcoal in 5% gum acacia) orally thirty minutes later. Thirty minutes after the charcoal meal administration, each animal was sacrificed. The proportion of the entire distance that had to be travelled from the pylorus to the caecum that the charcoal meal had covered in the intestine was calculated. (Mandal S. C., et al., 1997)

## 2.5. Statistical Analysis

Using computerized GraphPad InStat version 8.1, the data for antidiarrheal action are reported as mean SEM and analysed by a one-way ANOVA following by Dunnett's *t*-test. (Graph Padsoftware, USA).  $p < 0.05$  was considered statistically significant in all the cases.

### 3. Results

When *C. papaya* and *Psidium guajava* leaf extracts are subjected to phytochemical analysis, alkaloids, proteins, glycosides, phenol, tannin, saponin, quinine, oxalate, and anthocyanins are found. Alkaloid, carbohydrate, glycoside, phenols, tannin, and saponins are present, and their existence in alcoholic and aqueous extracts indicates their greater intensity. (Table 1). All of the bioactive substances, with the exception of proteins and anthocyanin, are present in the ethanolic extract, including alkaloids, glycosides, phenol, tannin, saponin, quinine, and oxalate. The total finding validates that ethanol extracts have more bioactive chemicals than other solvents.

**Table 1.** Qualitative assessment of medicinal plant leaf samples.

Phytochemical	Name of Plant			
	<i>Psidium guajava</i>		<i>Carica papaya</i>	
	Alcoholic Extract	Aqueous Extract	Alcoholic Extract	Aqueous Extract
Alkaloids	+	-	+	-
Terpenoids	-	-	-	-
Phenols and Tannins	+	+	+	+
Sugar	+	+	+	+
Saponins	+	+	-	-
Flavonoids	+	+	-	+
Quinones	+	+	-	-
Proteins	+	+	+	+

Values are mean of triplicate determinations on dry weight basis.

Using the agar well diffusion method, the antibacterial effect of the *Carica papaya* and *Psidium guajava* leaf extracts was evaluated by determining the diameter of the growth inhibition zones using 25, 50, 75, and 100  $\mu\text{L}/\text{mL}$  of aqueous and solvent leaf extracts.

The results showed that aqueous and alcoholic leaf extracts possess good antibacterial activity against selected gram negative (*Klebsiella pneumonia* and *Escherichia coli*) and gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) microorganisms. The extracts of *Carica papaya*, and *Psidium guajava* in aqueous and alcoholic (Concentration of 100  $\mu\text{L}/\text{mL}$ ) exhibit relatively higher zone of inhibition compared to Concentration of 75, 50 and 25  $\mu\text{L}/\text{mL}$ . The *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* were resistant to aqueous leaf extracts of *Carica papaya*. In present study, Aqueous extract of *Carica papaya* exhibit lesser zone of inhibition compared to alcoholic extract.

The phytochemical components of alcoholic and aqueous extracts of all the plants, in varying concentrations, inhibited the development of bacteria. In the examined plants, *Carica papaya* and *Psidium guajava* both exhibit comparable to moderate antibacterial activity.

### 4. Discussion

The existence of antibacterial agents in higher plants is a well-established fact (Srinivasan, 2001). Plants have consistently served as a wellspring of inspiration for the discovery of novel drug compounds, with plant-derived medicines making substantial contributions to human health. Phytomedicine, in particular, holds the potential to be employed in the treatment of various diseases, as demonstrated in systems like Unani and Ayurvedic medicine. Furthermore, it can serve as a foundational framework for the development of pharmaceuticals, providing a natural blueprint for drug development.

The evaluation of the antibacterial properties of the ethanolic extract derived from *P. guajava* and *C. Papaya* leaves revealed a concentration-dependent inhibition of growth for both *E. coli* and *Staph. aureus*, as compared to other organisms albeit with a slightly less

significant effect on the latter (as indicated in Tables 4 and 5). One suggested mechanism of action was that lectins found in *P. guajava* bind on *E. coli* thereby preventing adhesion to intestinal walls.

The result of the antioxidant activity of *P. guajava* and *C. papaya* ethanolic leaves extract with by DPPH assay and H<sub>2</sub>O<sub>2</sub> assay shows that the presence of free radicals is more and directly proportional to the concentration of the sample. It means higher the concentration higher will be the percentage of free radicals in the *P. guajava* and *C. papaya* leaves ethanolic extract as compared to aqueous extract (Tables 2 and 3). In conclusion, it is worth noting that oxidative stress has been associated with the development of various health conditions, including heart disease, cancer, and immune deficiencies. The presence of antioxidants, as suggested by multiple research studies, may play a pivotal role in mitigating the risk of such diseases.

The frequency of defecation and the moisture content of fecal droppings, which serves as an indicator of diarrhea, exhibited a notable reduction in the rat experiment induced by castor oil, as evidenced by the data presented in Table 6. Significantly favorable outcomes were observed for the ethanolic extract at both administered doses. Furthermore, the aqueous extract at a dose of 250 mg/kg also demonstrated a noteworthy reduction in these parameters. These findings exhibit a high level of comparability with the performance of the standard drug Loperamide at a dosage of 0.5 mg/kg.

In the gastrointestinal motility test, the ethanolic extract exhibited a significant reduction in intestinal transit at both administered doses, while the petroleum ether extract at 250 mg/kg also displayed statistically significant results when compared to the control group, as illustrated in Table 7. Notably, both extracts induced a substantial decrease in intestinal transit ranging from 8.79% to 27.78% in the charcoal meal test, which is highly comparable to the effects of the standard drug, Atropine Sulfate. Furthermore, for the acute oral toxicity studies, we adhered to OECD guidelines, and the results indicated that both extracts are safe up to a dose of 1000 mg/kg, although specific data is not presented here.

**Table 2.** Effect of leaf extracts of *Carica papaya* and *Psidium guajava* leaf extracts in DPPH antioxidant model.

Sample	Conc. μL/mL	% Inhibition		
		<i>Carica papaya</i>	<i>Psidium guajava</i>	Standard (Ascorbic Acid)
Alcoholic Extract	100	72.54 ± 1.01	69.21 ± 1.01	86.32 ± 1.02
	75	67.33 ± 1.02	65.84 ± 1.02	77.58 ± 1.04
	50	52.78 ± 1.00	49.68 ± 1.01	62.18 ± 1.05
	25	49.37 ± 1.05	44.56 ± 1.06	59.28 ± 1.02
Aqueous Extract	100	68.91 ± 1.11	66.35 ± 1.05	79.58 ± 1.01
	75	58.13 ± 1.01	56.75 ± 1.01	68.35 ± 1.02
	50	51.64 ± 1.04	45.11 ± 1.04	57.47 ± 1.04
	25	45.81 ± 1.01	39.52 ± 1.02	48.74 ± 1.02

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey-Kramer Multiple Comparison test; \*\* *p* < 0.01 vs. standard drug.

**Table 3.** Effect of leaf extracts of *Carica papaya* and *Psidium guajava* leaf extracts in H<sub>2</sub>O<sub>2</sub> radical scavenging assay.

Sample	Conc. μL/mL	% Inhibition		
		<i>Carica papaya</i>	<i>Psidium guajava</i>	Standard (Ascorbic Acid)
Alcoholic Extract	100	78.21 ± 1.01	76.65 ± 1.00	88.72 ± 1.02
	75	68.15 ± 1.06	66.89 ± 1.03	79.10 ± 1.01

	50	56.97 ± 1.02	54.02 ± 1.00	67.69 ± 1.01
	25	51.04 ± 1.00	48.77 ± 1.02	60.31 ± 1.01
<b>Aqueous Extract</b>	100	75.24 ± 1.03	71.36 ± 1.01	83.15 ± 1.00
	75	65.56 ± 1.01	63.74 ± 1.01	75.61 ± 1.01
	50	52.82 ± 1.01	58.64 ± 1.02	62.05 ± 1.02
	25	45.37 ± 1.01	51.45 ± 1.00	54.71 ± 1.04

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey-Kramer Multiple Comparison test; \*\*  $p < 0.01$  vs. standard drug.

**Table 4.** The antimicrobial activity of the plant extracts for *Psidium guajava* extracts.

Sample	Conc. $\mu\text{L}/\text{mL}$	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
<b>Alcoholic Extract</b>	100	4.2	4.1	3.8	4.1
	75	3.8	3.6	3.1	3.8
	50	3.1	3.1	2.4	3.1
	25	2.5	2.1	1.4	2.7
<b>Aqueous Extract</b>	100	3.8	3.9	3.8	4.0
	75	3.4	3.3	3.2	3.3
	50	2.6	2.7	2.3	2.5
	25	1.4	1.7	1.3	1.9

**Table 5.** The antimicrobial activity of the plant extracts for *Carica papaya* extracts.

Sample	Conc. $\mu\text{L}/\text{mL}$	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
<b>Alcoholic Extract</b>	100	3.9	4.2	3.7	4.1
	75	3.5	3.6	3.4	3.7
	50	3.1	3.2	2.8	3.5
	25	2.7	2.8	1.5	3.1
<b>Aqueous Extract</b>	100	4.2	3.9	3.6	3.9
	75	3.6	3.3	3.2	3.3
	50	3.1	2.5	2.7	2.7
	25	2.9	1.5	2.1	1.8

**Table 6.** Effect of alcoholic and aqueous extract of *Carica papaya* and *Psidium guajava* on castor oil-induced diarrhea in rats.

Treatment	Dose (p.o.)	Total No. of Faeces	Total No. of Wet Faeces	Inhibition (%)
Vehicle(5% tween80)	10 mL/kg	32.17 ± 1.138	18.33 ± 0.4944	0.00
Loperamide	0.5 mg/kg	17.00 ± 1.653 ***	9.667 ± 0.3333 ***	47.30
Ethanollic extract	125 mg/kg	26.00 ± 1.366 **	15.33 ± 0.4216 **	16.37
Ethanollic extract	250 mg/kg	20.83 ± 0.8333 ***	11.67 ± 0.5578 ***	36.33
Aqueous extract	125 mg/kg	29.17 ± 1.014	16.33 ± 0.6146	10.91
Aqueous extract	250 mg/kg	25.33 ± 1.453 **	15.00 ± 0.9661 **	18.17

Values are expressed as mean ± S.E.M. (n = 6). \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  when compared with vehicle-control.

**Table 7.** Effect of alcoholic and aqueous extract of *Carica papaya* and *Psidium guajava* on intestinal transit time in rats.

Group	Dose	Distance Travelled by Charcoal Meal as % of Total Length of Stomach	Inhibition (%)
Vehicle	10 mL/kg, p.o.	72.00 ± 1.065	0.00
Atropine sulphate	0.1 mg/kg, i.p.	42.83 ± 1.579 ***	41
Ethanollic extract	125 mg/kg, p.o	59.33 ± 2.629 **	18.06
Ethanollic extract	250 mg/kg, o.p.	52.00 ± 3.183 ***	27.78
Aqueous extract	125 mg/kg, o.p.	65.67 ± 2.246	8.79
Aqueous extract	250 mg/kg, o.p.	62.83 ± 2.786 *	12.74

Values are expressed as mean ± S.E.M. (n = 6). \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  when compared with vehicle-control.

The aforementioned parameters serve as valuable indicators of diarrhea. Castor oil is known to induce diarrhea primarily through the action of its active metabolite, Ricinoleic acid. (Chitme H. R., et al., 2004 and Ammon P. J. et al., 1974) This compound stimulates heightened peristaltic activity within the small intestine, triggering alterations in electrolyte permeability. The liberation of Ricinoleic acid further leads to irritation and inflammation of the intestinal mucosa, resulting in the release of prostaglandins. Additionally, castor oil's mechanism of action includes the stimulation of endogenous prostaglandin release. It has been reported that castor oil induces diarrhea by increasing the volume of intestinal contents and impeding the reabsorption of water within the gastrointestinal tract.

Furthermore, we conducted an assessment of the antimicrobial activity of ethanolic and aqueous extracts at various concentrations, given that various enteropathogenic microorganisms are major contributors to diarrhea. The results unveiled a moderate zone of inhibition, with particularly robust inhibition of *E. coli* growth observed in response to the ethanolic extracts. In line with these findings, prior research has also highlighted the substantial antibacterial activity of ethanolic extracts derived from *Carica papaya* and *Psidium guajava*. (Devmurari V. P., et al., 2010) It is worth noting that this observed activity can likely be attributed to the presence of potent phytoconstituents such as steroids, triterpenoids, glycosides, flavonoids, and tannins, all of which may contribute to the extracts' antimicrobial properties. (Ikram M. and Innamual H., 1980a) Consequently, these findings hold promise for potential advancements in the development of novel antimicrobial drugs for the treatment of diarrhea and other infectious diseases caused by *E. coli*.

## 5. Conclusions

Many plants are showing various pharmacological activities due to the presence of phenolic and flavanoid contents. *Carica papaya* and *Psidium guajava* are the plants having wide range of biological activities. The Preliminary phytochemical evaluation leaf extracts are showing the presence of many constituents like phenolics and flavanoids. From this study it is clear that the both fraction *Carica papaya* and *Psidium guajava* ethanolic and aqueous extract are containing presence of large number of phytoconstituents. So this fraction may show more antioxidant and anibacterial activities. This fraction is to be taken for further study for the isolation of active constituents.

**Conflicts of Interest:** The authors declare no conflict of interest.

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