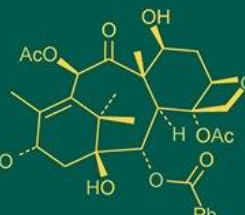


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Exploring the role of different plant extracts and bioactive phytochemicals in combating foodborne pathogens

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Abstract

This study investigated the comparative biological activities of four medicinal plants leaf Red Mulberry (*Morusrubra*), Gooseberry (*Phyllanthusemblica*), Guava (*Psidium guajava* L.), and Moringa (*Moringa oleifera*), focusing on *Phyllanthusemblica* (Gooseberry) and *Psidium guajava* (Guava) due to their higher extract yields and promising bioactivities. Ethanolic and aqueous extracts were evaluated for antibacterial, anti-diarrheal, anti-inflammatory, biofilm inhibition, and antioxidant properties. Guava yielded the highest extract residues (1.21 g aqueous; 1.02 g ethanolic). Antibacterial assays revealed strong inhibitory effects of Gooseberry and Guava against *E. coli* and *C. jejuni*, with ethanolic extracts generally outperforming aqueous ones. Phytochemical screening showed solvent-dependent variations in bioactive compounds. Both extracts exhibited dose-dependent anti-inflammatory and biofilm inhibition activities, with Gooseberry showing higher anti-inflammatory potential and Guava stronger anti-biofilm effects. Antioxidant assays confirmed significant free radical scavenging activity, with Guava ethanolic extract achieving the highest (80.75%). These findings scientifically validate the traditional uses of Gooseberry and Guava, highlighting their potential for developing natural antimicrobial and antioxidant therapeutics.

Keywords: Foodborne disease, plant extracts, herbal drugs, MIC, antibacterial and antifungal activity, phytochemicals, multidrug resistance, phytocompounds.

Introduction

Food poisoning is regarded as one of the leading causes of disease and mortality in underdeveloped nations [1-3]. The majority of reports of food poisoning are linked to bacterial contamination, particularly those caused by Gram-negative bacteria such as *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa* [4,5]. Other Gram-positive bacteria, such as *Bacillus cereus* and *Staphylococcus aureus*, have also been found to be the cause of food borne illness and food spoiling [6]. Conventionally, chemical preservatives are used to prevent food rotting and the etioloical agent that causes it [7, 8]. Although these chemical preservatives have demonstrated effectiveness in preventing and controlling outbreaks of foodborne illnesses, their repeated use has led to the buildup of chemical residues in the food and feed supply, the development of microbial resistance to the chemicals used and negative side effects on human health [9,10]. As a result of this issue, efforts have been concentrated on developing healthier, safer and more natural food preservation methods [11, 12]. In recent times, there has been growing emphasis on creating natural medicines and products. Studies have shown that several fruits, fruit extracts, arrowroot tea extracts and caffeine exhibit antimicrobial effects against *E.coli* O157:H7 [13, 14]. This indicate that plants with strong antimicrobial properties may contain compounds capable of inhibiting the growth of foodborne pathogens. When exposed to plant extracts, bacterial cells may be destroyed through the breakdown of their cell walls and membranes, as well as the disruption of their internal structures [11]. Many parts of the guava (*Psidium guajava*) have been used in traditional medicine to treat a variety of ailments, including malaria,

gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothaches, coughs, sore throats, inflamed gums and many more. The guava is a phytotherapeutic plant that is thought to contain active ingredients that help treat and manage various diseases [15-17]. This herb has also been utilized to treat life-altering diseases like obesity, diabetes and high blood pressure [18]. Guava trees are tiny, evergreen trees. The leaves have a dull-green appearance, are stiff yet coriaceous, have noticeable veins and are 2 to 6 inches long by 1 to 2 inches wide. When crushed, they release a pleasant smell [19]. The guava, mulberry, moringa and gooseberry leaf contains bioactive substances that can control blood sugar, combat infections and even help people to lose weight. Cineol, tannins, triterpenes, flavonoids, resin, eugenol, malic acid, fat, cellulose, chlorophyll, mineral salts and several other fixed compounds are all abundant in the essential oil found in all the leaves [20-23]. The standard techniques for plant extraction include maceration, infusion, percolation, digestion, decoction, soxhlet extraction, aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction, supercritical fluid extraction and phytonic extraction. Maceration extraction is a form of crude extraction in which solvents dissolve molecules with comparable polarity by diffusing into solid plant material [24].

The second plant is Mulberry, since ancient times, mulberries have been used as herbal remedies; in recent years, they have emerged as the most widely used herbal remedy. Chlorogenic acid, caffeic acid, vanillic acid, hydroxybenzoic acid, p-coumaric acid, sinapic acid, protocatechuic acid and ferulic acid are the main phenolic acids found in mulberry leaves [25]. Due to certain compounds known as kuwanon C, mulberrofurin G, mourin, and albanol B, mulberries have demonstrated potent antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Streptococcus faecalis*, *Mycobacterium smegmatis*, *Streptococcus mutants*, *Streptococcus sanguis*, *Porphyromonas gingivalis*, *Aspergillus tamari*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium oxalicum* and some mold species [26-29]. Another plant leaf name *Moringa oleifera*, is a drought-resistant, fast growing tree in the moringaceae family. It is sometimes referred to as the 'drumstick tree' because of its long, slender, triangular seed pods and the 'horseradish tree' because of its horseradish-like root flavour. It is an important medical herb that has long been used as a vegetable. This plant is indigenous to South America, Africa, Arabia, Southern Asia and also in India. *Moringa oleifera* has been a part of diets since ancient times because of its many biological qualities as an antibacterial, anti-inflammatory and anti-diabetic agent [30, 31]. In addition, *Moringa oleifera* is a significant source of minerals, tocopherol, beta carotene, vitamin C, and important amino acid. *Moringa oleifera* offers twice as much vitamin A in carrots, seven times as much vitamin C in oranges, four times the potassium in bananas, nine times the iron in spinach and fourteen times the calcium in milk. Its nutritive value is complemented by a pleasant flavour and aroma. Moreover, the presence of phytochemicals, such as flavonoids and other phenolics in their leaf extract, can prolong the shelf life of food and prevent the formation of harmful germs [32]. Due to the long-standing reputation of *Moringa oleifera* as a "miracle tree" and its traditional use

in treating various illness, along with the limited number of published studies on its effectiveness as a natural food preservative with antimicrobial properties-particularly against foodborne pathogens.

The last plant is *Emblica*. *Emblica officinalis* (commonly known as amla or aonla) is a well-known medicinal plant, with the fruits therapeutic benefits widely recognized. However, the medicinal potential of amla leaves has been less explored. Here, in this study, the antimicrobial activity of petroleum ether extract of amla leaves was evaluated using disc diffusion method. The minimum inhibitory concentration (MIC), tested in a range from 1000 to 62.5, was compared to standard antibiotics-streptomycin (10mg) and amphotericin B (20mg). The results indicated that the leaf extract exhibits significant antibacterial activity, demonstrating broad-spectrum antimicrobial properties and suggesting its potential as a source for developing new antibiotics for treating infectious disease [33,34]. The aqueous crude extract of amla was tested for antibacterial activity using the agar well diffusion method against five human bacterial pathogens: *Bacillus* spp., *Lactobacillus* spp., *Pseudomonas* spp., *Proteus* spp., and *Streptococcus* spp., at concentrations of 30, 60 and 90 microliter. The results clearly indicated that the extract exhibited strong inhibitory effects against all tested bacteria [35]. Additionally, *in vitro* antibacterial activity of aqueous, ethanolic and acetone extracts of amla fruit was assessed against both gram-positive and gram-negative bacteria represented by *Staphylococcus aureus* and *Escherichia coli* respectively. All extracts showed significant antibacterial activity, with stronger effects against *S. aureus* than *E. coli* [36].

These medicinal plant extracts used in Nigerian traditional medicine shown strong antibacterial activity against a few food-borne diseases. Once some pathogens or microbes are eaten with any food items they get colonised to the internal organs like stomach, intestine and any other parts of the body and causes several infection that can occasionally be fatal. Although the symptoms vary depending on the amount of the microbe consumed, some symptoms are common to all of these organisms, but they mostly tend to change the gut microbiota, resulting in nausea, diarrhoea, stomach discomfort and other symptoms [37].

Even though food technology is constantly evolving, food-borne illness morbidity is still widespread, particularly in developing nations. This trend has a negative impact on the economic and health sectors of nations all over the world [38]. A systematic examination of phytochemical content in medicinal plants known for their antimicrobial effects could help discover new antimicrobial compounds with distinct way of working. These new compounds might either directly kill bacteria, target essential cellular functions needed for growth and reproduction, or work together with existing antibiotics by blocking resistance mechanisms in drug-resistant bacteria. In certain case, the aim is to restore the effectiveness of antimicrobial treatments and make resistant bacteria more responsive to them.

Numerous studies have consistently highlighted the antimicrobial properties of traditional medicines. Medicinal plants present a valuable resource, as they contain a variety of bioactive compounds (phytochemicals) that can function as antimicrobial agents. Natural products have also been shown to enhance the effectiveness of modern drugs against multidrug-resistant (MDR) pathogens. As a result, exploring these natural antimicrobials offers promising potential for

controlling foodborne illness. Traditional remedies have been used for a long time to treat diseases caused by foodborne pathogens and research has confirmed their effectiveness. Recently, there has been growing interest in natural preservatives due to concerns over the side effects of synthetic ones used in food. Crude plant materials, such as extracts, essential oils and isolated compounds have been widely studied for their ability to prevent the growth of food-spoiling pathogens and may help reduce the spread of foodborne infections [39-41].

Plant-based products can inhibit or alter bacterial growth through various mechanisms [42]. These include preventing pathogens from attaching to host cells, disrupting the microbes ability to regulate water balance and maintain its electrochemical gradient across membranes, increasing nitric oxide (NO) production which can be lethal to the

bacteria, and interfering with the synthesis of the pathogens cell wall, proteins and genetic material [43]. This research highlights the therapeutic potential of medicinal plants and natural compounds in combating pathogens responsible for foodborne infections [44].

Materials and Methods

In this study plant leaf of four plant leaf Red Mulberry (*Morusrubra*), Gooseberry (*Phyllanthusemblica*, Guava (*Psidium guajava* L.), and Moringa (*Moringa oleifera*), were collected from the locality of Dehradun, Uttarakhand. The plant leaves were firstly washed with running tap water after that leaves were disinfected and washed with distilled water to remove all the dust particles and then kept into the blotting paper in a shade for drying.



Fig 1: Sample collection:(A) Red Mulberry (*Morusrubra*), (B)Gooseberry (*Phyllanthusemblica*), (C) Guava (*Psidium guajava* L.), (D)Moringa (*Moringa oleifera*)

A fine powder of the dried leaves were made by the mixture grinder so that it could pass through a 100 mm sieve. 10 g each plant extracts powder were soaked into 100 ml of distilled water and ethanol in the ratio 1:10 and kept for stirring for about 48-72 hrs in a shaker. After that the soaked extracts were filtered through double layer muslin cloths and then centrifuged at 9000 rpm for about 10-15 minutes and at last the centrifuged extract were again filtered through whatman filter paper No. (41) to get the clear extracts. The volume of the filtrate was measured and evaporated and dried at 40-50 degree Celsius in using hot water bath and hot air oven. The yield of the extract were measured. 10% DMSO solution was prepared and mixed with the dried plant extract and stored in the refrigerator at 4 degree Celsius. The yield percentage of the dried extract were calculated by using the following formula: Extract yield% = $R/S \times 100$. (Where R; weight of extracted plant residues and S; weight of raw plant sample).

Antimicrobial activity of the plant extract

The antimicrobial potency of the above mentioned 4 plant extract was evaluated by using 2 bacterial strains that causes the food poisoning or food borne illness like stomach pain by inflammation, diarrhea and dysentery. The bacteria were isolated from spoiled and contaminated food samples by performing serial dilution. 1ml of each sample were spread into the nutrient agar plate. The plates were incubated for 24 hrs at 37 degree Celsius. Suspected colonies were picked up and re-streaked onto new solidified nutrient agar plate till obtaining pure separate colonies [45]. The purified culture were identified and confirmed by investigating morphological characters and biochemical tests according to Bergey's Manual [46, 47]. Each bacterial strain was sub-cultured overnight at 37 degree in a nutrient broth media.

Preparation of Muller Hilton Agar Media for Antimicrobial Activity

A sterile conical flask was used and add 3.9 g of MHA media and 100 ml of distilled water, add 2% agar-agar powder and mixed properly until it get dissolved. The medium were kept into autoclave for 15-20 mints at about 121 degree Celsius or 15 psi for sterilization. After sterilization, the media was taken out and kept into bio-safety cabinet and poured into the sterile petri-plates and allowed for solidifying.

Swabbing

A sterile cotton swab was used to make a lone culture on the solidified agar plate from freshly cultured bacteria.

Antimicrobial activity of plant extract using agar well diffusion method

On the inoculated plate a proper well was prepared by using 1 ml pipette tips and different concentration (20 micro litter, 50 micro litter and 100 micro litter) of plant extract was added for the activity and incubated for 24 hrs at 37 degree Celsius. The inhibition zones were measured by vernier calliper and recorded against the concentrations of effective plant extracts.

Phytochemical test for the plant extract

To examine the different phytoconstituents found in the 2 plants, an aqueous and ethanol extract of each plant was made. Specific tests for particular phytochemicals were conducted using various chemical reagents. The tests were qualitative in nature so they term as phytochemical screenings. Fisher Scientific in india was the supplier of all chemicals and solvents. The tests were done by following standard procedures based on journal articles [48-50].

Test for Tannin/polyphenol ^[50]: Three to four drops of 10% FeCl₃ were added to the diluted extract; the presence of catechol tannin caused the solution to turn green, whereas gallic tannins showed a blue hue.

Test for saponins ^[48]: 2 ml of extract was mixed with 2 ml of distilled water was shaken in a test tube. The appearance of frothing indicates the presence of saponins.

Test for terpinoids ^[48]: 1 ml of extract mixed with 2 ml of chloroform. 2 ml of concentrated sulphuric acid (H₂SO₄). The formation of reddish brown colour confirms the presence of terpinoids.

Test for flavanoids: 1 ml of extract mixed with 4 drops of 10% NaOH solution and heated in waterbath for 10 minutes. Intense yellow colour formed which becomes colourless upon the addition of 5 drops of 1% hydrochloric acid (HCL) confirms flavanoids.

Test for alkaloids: 2 ml of plant extract was mixed with few drops of Mayer's reagent (potassium mercuric iodide), reddish brown colour precipitate indicates the presence of alkaloids.

Test for steroids: 1 ml of plant extract was mixed with 2 ml of acetic anhydride, add 2 ml of H₂SO₄. Colour changes from violet to blue or green in the extract indicates the presence of steroids.

Test for coumarins: 2 ml of 10% sodium hydroxide was mixed with 2 ml of plant extract, an appearance of yellow colour depicts the presence of coumarins.

Test for protein: 1 ml of conc. H₂SO₄ added into 2 ml of extract was boiled for 10 minutes into hot waterbath, white to yellow after addition of Ammonium hydroxide indicates the presence of protein in the sample.

Test for reducing sugar ^[49]: 1 ml of water, five to eight drops of fehling's solution, and 0.5 ml of plant extract were heated. Brick-red precipitation appeared, indicating the presence of reducing sugar.

Test for quinine ^[49]: In a test tube 1 ml of extract, 1 ml of FeSO₄ solution and ammonium thiocyanate were added then concentrated H₂SO₄ was added drop by drop. The presence of quinine was detected by the rich red colour.

Test for glycosides ^[48]: 1 ml of plant extract mixed with 2 ml of glacial acetic acid containing two drops of 2% FeCl₃. The mixture was poured into another test tube containing 2 ml of H₂SO₄. Formation of brown ring at the interphase indicates the presence of glycosides.

Test for amino acid: A Ninhydrin solution (2 ml of 0.2% in acetone) was added to the 2 ml of plant extract and boiled for 10 minutes in hot waterbath. A violet, bluish or purple colour indicates the presence of amino acid.

Test for quinones: 1 ml of diluted NaOH was added to the 1 ml of plant extract. A blue green or red colour indicates the presence of quinones.

Anti- Inflammatory Potential of Leaf extract

Anti- Inflammatory test was used to evaluate the effect of leaf extract on growth and stress activity of Protein (egg albumin or egg white). This test was used to simulate a potential mechanism of anti-inflammatory activity through protein modulation. Egg white react to stressors like inflammatory inducers like diclofenac sodium (which is also used as a standard) or HCL (Hydrochloric acid) and plant extracts can modulate these responses. To perform anti-inflammatory test, take a sterile test tube, add 2 ml of desired plant extract, 2.8 ml of phosphate-buffer saline (PBS) and 2 ml of egg albumin (Fresh chicken egg), the mixture was incubated for 15-20 minutes at 37 degree Celsius. After incubation, the mixture was heated in a water bath for 10 minutes at 70 degree Celsius for denaturation. After cooling the sample, absorbance was measured at 660 nm.

Percent inhibition of protein denaturation was determined using the following formula: % inhibition = (Ac - As)/Ac * 100; Where, As = Absorbance of sample; Ac = Absorbance of control.

Anti-biofilm assay using plant extract

^[51]

According to Sandasiet *al.* and Mohsenipour and Hassanshahian's modified procedure, the plant's aqueous (cold) extract's ability to suppress the formation of biofilms was evaluated by the following procedure: Culture the biofilm producing bacteria into the nutrient broth medium. The freshly prepared bacterial culture was inoculated in the ratio 1:100 into the desired wells of the microtiter plate. Add 100 micro-litter of LB broth to the desired wells of the plate then add 100 micro-litter of plant extract to the wells. Incubate the plate for 24 hrs at 37 degree Celsius.

After incubation carefully discard the liquid from wells. Gently wash the wells 3 times with PBS (Phosphate buffer saline), 200 micro-litter each wash to remove non-adherent cells.

Crystal violet staining Assay 200 micro-liter of 0.1% crystal violet solution was added into each well. Rinse the plate 3-4 times with distilled water. 200 micro-liter of 95% ethanol (30% acetic acid) were added to each well to dissolve the bound crystal violet. Incubate for 10-15 minutes at room temperature, Observe the OD at 630 nm.

Antioxidant Activity using DPPH Assay

The DPPH (1,1-Diphenyl 2-picryl hydrazyl) Radical Scavenging Assay was carried out, with minor adjustments, in accordance with the experimental protocol provided in standard references. 11.83 mg of the DPPH reagent were dissolved in 100 milliliters of ethanol to create 0.3 milliliters of the solution. In each test tube, 2.5 ml of an extract at a different concentration was added. After that, aluminium foil was placed over each test tube because DPPH is a light-photosensitive reagent. One millilitre of 0.3 mM alcoholic solution of the DPPH reagent was applied to each test tube that was covered with aluminium foil and contained varying concentration of extract. The same process was used to prepare standard ascorbic acid in various concentration by substituting extract with standard ascorbic acid in varying quantities. The addition of 1 milliliter of 0.3 mM alcoholic DPPH solution was then added. Similarly, a control solution was made by adding 1 milliliter of 0.3 mM alcoholic solution of DPPH and substituting ethanol for the extract. 3 milliliter of 95% ethanol were used to create a blank

solution. Now each test tube with varying quantities of extract, control, blank and standard ascorbic acid was kept for dark incubation for about 30 min at room temperature. Each test tubes content's were placed into quartz cuvette in an amount of 2 ml, and the absorbance at 517 was measured by using UV-visible spectrophotometer. The % of DPPH Scavenging activity was calculated by the following formulae:

$$\% SA = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100$$

Where, % SA= Percentage of Scavenging activity.

The percentage of inhibition is then plotted against the concentration in µg/ml to create curves. With the use of this

curve's equation, the IC₅₀ for the sample concentration that decreased the original DPPH absorbance by 50% could be determined.

Result and Discussion

Plant extract yield

The ethanol and aqueous data of the employed plants and their extracts percentage yield are illustrated in Table 1. The extract of 10 g of dried plant materials with aqueous yielded plant extract residues ranged from 0.97 to 1.21 g and ethanol yielded plant extract residues ranged from 0.86 to 1.01 g. The highest yield of plant extract was obtained from *Psidium guajava* (1.21 g & 1.02 g), while *Morus rubra* give the lowest extract yield (0.97 g & 0.86 g) respectively.

Table 1: Weight of leaf extract dissolved in aqueous & ethanol solvent

Plantspecies	Family	Local name	Commonname	Partused	Yield %	
					Aq	Eth
<i>Moringaoleifera</i>	Moringaceae	Sahjan	Drumstick	Leaf	1.03	0.95
<i>Psidium guajava</i>	Myrtaceae	Amrood	Guava	Leaf	1.21	1.01
<i>Morusrubra</i>	Moraceae	Shahtoot	Redmulberry	Leaf	0.97	0.86
<i>Phyllanthusemblica</i>	Phyllanthaceae	Amla	Amla	Leaf	1.02	0.92

Antibacterial properties of plants extracts

Four plant species were investigated to evaluate their antibacterial against food poisoning bacteria including two stains of gram negative bacteria (*E.coli* and *C. jejuni*) using well diffusion method. Evaluation of antibacterial activity of these plant leaf extracts was recorded in Table 2 & 3 and illustrated in Fig 2 & 3. This result revealed that among the four plant leaf extract only two plant leaf namely *Psidium guajava* (Guava) and *Phyllanthusemblica* (Gooseberry) were potentially effective in suppressing microbial growth of food poisoning bacteria with the variable potency. The ethanolic extract of *Phyllanthusemblica* was the most effective at suppressing the microbial growth on *E.coli* at

concentration of 100 mg/ml while ethanolic extract of *Psidium guajava* was effective against *C. jejuni* at concentration of 100 mg/ml. Results of antimicrobial activity of these plant extracts can suggest that *E.coli* and *C.jejuni* were the most resistant strain to the Mulberry and Moringa leaf but are susceptible against the extract of Gooseberry and Guava in ethanol and aqueous solvents. Moreover, *Phyllanthusemblica* and *Psidium guajava* extracts were the most effective extract and showed a strong antibacterial activity against food poisoning bacteria. Hence, more experiments were conducted to determine their anti-diarrheal, anti-inflammatory, biofilm inhibition assay and anti-oxidant test.

Table 2: Antimicrobial activity of leave extract against Gram negative bacteria:

Plant Leaves	<i>E.coli</i> (Zone of inhibition in mm in ethanol)	<i>C. jejuni</i> (Zone of inhibition in mm in ethanol)
Moringa	NA	NA
Mulberry	NA	NA
Gooseberry	19±0.3 mm	21±0.4 mm
Guava	21±0.2mm	19±0.3 mm

Table 3: Antimicrobial activity of leave extract against two gram negative bacteria.

Plant Leaves	<i>E. coli</i> (Zone of inhibition in mm in ethanol)	<i>C. jejuni</i> (Zone of inhibition in mm in ethanol)
Moringa	NA	NA
Mulberry	NA	NA
Gooseberry	28±0.32 mm	18±0.28 mm
Guava	20±0.41 mm	21±0.51 mm

Result values are expressed as Means±standard deviation.

NA = There is zone of inhibition. All experiments were conducted in a triplicate.

Minimum Inhibitory Concentration of the most active Plant leaf: The Minimum Inhibitory Concentration (MIC)

of *Phyllanthusemblica* (Gooseberry) and (Guava) leaf extracts was evaluated at varying concentrations (20 mg/ml, 50 mg/ml, and 100 mg/ml), along with positive and negative controls, as detailed in Tables 4-7 and Figures 4-7. The results showed that the ethanolic extract of Gooseberry leaves effectively inhibited *E. coli* by forming a clear zone,

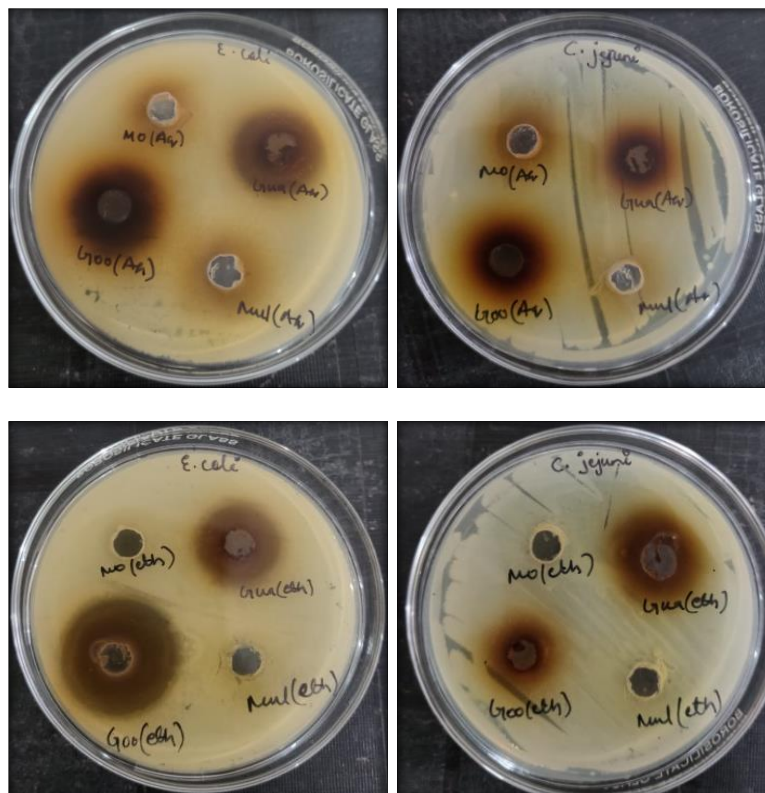


Fig 2: Zone inhibition of food poisoning bacterial against different plant extracts leaf

While the aqueous extract produced a slightly smaller zone at 100 mg/ml. Similarly, for *C. jejuni*, the *Psidium guajava* ethanolic extract of Guava leaves demonstrated a 22 mm zone of inhibition, whereas the aqueous extract showed a 20 mm zone at the same concentration. These findings indicate that *E. coli* and *C. jejuni* were more resistant to Mulberry and Moringa extracts but highly susceptible to Gooseberry and Guava extracts in both ethanol and aqueous forms.

Overall, the MI Canal analysis high lights that Gooseberry and Guava extracts possess strong antibacterial potential against foodborne pathogens. This aligns with previous studies suggesting that organic solvent extracts exhibit superior antibacterial activity compared to aqueous extracts due to their enhanced ability to dissolve bioactive compounds [52].

Table 4: MIC of gooseberry leave on aqueous extract against *E. coli* and *C. jejuni*

Name of Bacteria	Different concentration of Gooseberry leaf extract in aqueous solution				
	GTM (+Ve)	100 mg/ml	50 mg/ml	20 mg/ml	DMSO (-ve)
<i>E.coli</i> (zone of inhibiton in mm)	24±0.35mm	23±0.23mm	18±0.23mm	11±0.35mm	NA
<i>C. jejuni</i> (zone of inhibiton in mm)	22±0.33mm	22±0.21mm	16±0.42mm	12±0.32mm	NA

Table 5: MIC of gooseberry leave on ethanol extract against *E. coli* and *C. jejuni*

Name of Bacteria	Different concentration of Gooseberry leaf extract in ethanol solution				
	GTM (+ve)	100 mg/ml	50 mg/ml	20 mg/ml	DMSO (-ve)
<i>E.coli</i> (zone of inhibiton in mm)	24±0.21mm	19±0.35 mm	16±0.54mm	11±0.69mm	NA
<i>C. jejuni</i> (zone of inhibiton in mm)	21±0.31mm	19±0.35 mm	16±0.22mm	11±0.68mm	NA

Table 6: MIC of guava leave extract on aqueous extract against *E. coli* and *C. jejuni*

Name of Bacteria	Different concentration of Guava leaf extract in aqueous solution				
	GTM (+ve)	100mg/ml	50mg/ml	20mg/ml	DMSO(-ve)
<i>E.coli</i> (zone of inhibiton in mm)	23±0.31mm	20±0.33mm	17±0.45mm	11±0.52 mm	NA
<i>C. jejuni</i> (zone of inhibiton in mm)	22±0.2mm	19±0.3mm	17±0.44mm	11± 0.5mm	NA

Table 7: MIC of guava leave extract on ethanol extract against *E. coli* and *C. jejuni*

Name of Bacteria	Different concentration of Guava leaf extract in ethanol solution				
	GTM (+ve)	100mg/ml	50mg/ml	20mg/ml	DMSO(-ve)
<i>E.coli</i> (zone of inhibiton in mm)	22±0.2 mm	18±0.3mm	16±0.4mm	10±0.53mm	NA
<i>C. jejuni</i> (zone of inhibiton in mm)	21±0.31mm	19±0.29mm	15±0.43mm	12±0.54mm	NA

*Result values are expressed as Means± standard deviation.

NA = There is zone of inhibition. All experiments were conducted in a triplicate. GTM: Gentamicin zone of inhibition as +ve control

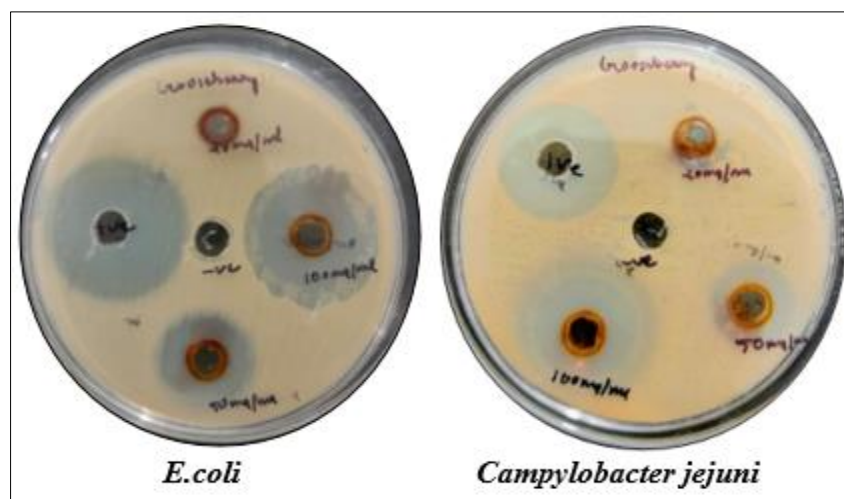


Fig 4: MIC of gooseberry leave on aqueous extract against *E. coli* and *C. jejuni* at different concentration

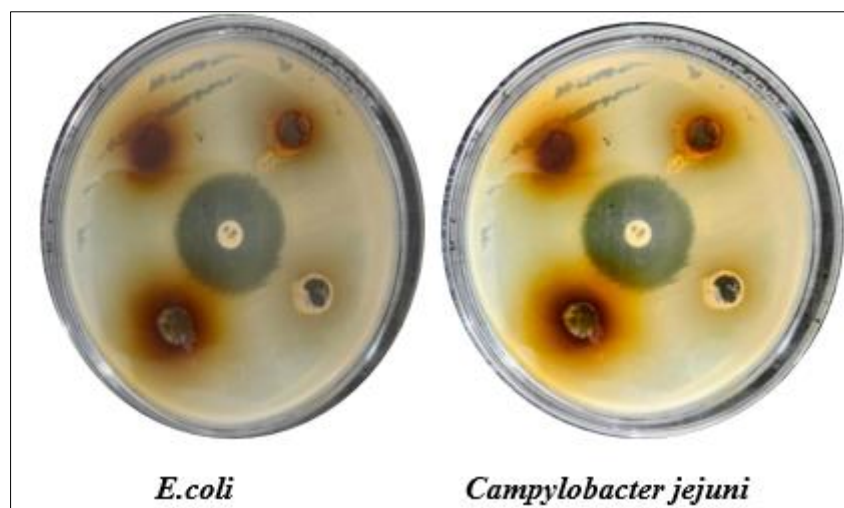


Fig 5: MIC of gooseberry leave on ethanol extract against *E. coli* and *C. jejuni* at different concentration

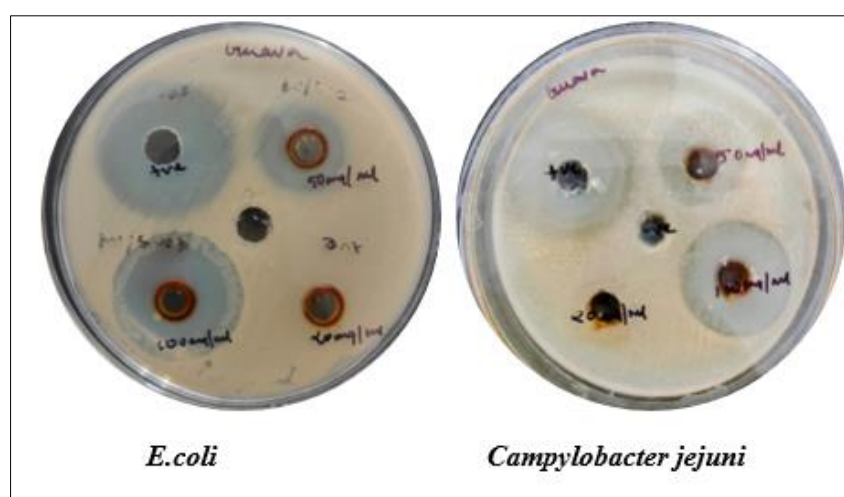


Fig 6: MIC of guava leave on aqueous extract against *E. coli* and *C. jejuni* at different concentration



Fig 7: MIC of guava leave on ethanol extract against *E. coli* and *C. jejuni* at different concentration

Phytochemical test of plant leaf

The phytochemical screening of *Phyllanthusemblica* (Gooseberry) and *Psidium guajava* (Guava) leaf extracts revealed noticeable variations in the presence of bioactive compounds depending on the solvent used. In Gooseberry leaf extracts, the aqueous extract tested positive for saponins, phenols, tannins, glycosides, alkaloids, quinine, proteins, and quinones, while terpenoids, steroids, and reducing sugars were not detected; flavonoids, volatile oils, coumarins, and amino acids were absent. Conversely, the ethanolic extract contained phenols, tannins, glycosides, alkaloids, volatile oils, and quinones but lacked saponins, flavonoids, quinine, coumarins, proteins, and amino acids, with terpenoids, steroids, and reducing sugars remaining undetected. Similarly, the aqueous extract of Guava leaves showed the presence of phenols, tannins, glycosides, alkaloids, coumarins, proteins, quinones, and amino acids, but not terpenoids, steroids, or reducing sugars, while saponins, flavonoids, volatile oils, and quinine were absent. The ethanolic Guava extract contained phenols, tannins, glycosides, alkaloids, volatile oils, and quinones but lacked several other phytochemicals including saponins, flavonoids, quinine, coumarins, proteins, and amino acids. These findings suggest that solvent type significantly influences the extraction of specific phytoconstituents, a trend consistent with prior studies on medicinal plants [53].

Table 8: Phytochemical analysis of Gooseberry leaf

Sl.No.	Test name	Aqueous extract	Ethanol extract
1	Saponin	+	-
2	Phenol	+	+
3	Tannin	+	+
4	Terpenoids	*	*
5	Flavonoids	-	-
6	Glycosides	+	+
7	Alkaloids	+	+
8	Steroids	*	*
9	Reducing sugar	*	*
10	Quinine	+	-
11	Volatile oils	-	+
12	Coumarins	-	-
13	Protein	+	-
14	Amino acid	-	-
15	Quinones	+	+

Table 9: Phytochemical analysis of Guava leaf

Sl.No.	Test name	Aqueous extract	Ethanol extract
1	Saponin	-	-
2	Phenol	+	+
3	Tannin	+	+
4	Terpenoids	*	*
5	Flavonoids	-	-
6	Glycosides	+	+
7	Alkaloids	+	+
8	Steroids	*	*
9	Reducing sugar	*	*
10	Quinine	-	-
11	Volatile oils	-	+
12	Coumarins	+	-
13	Protein	+	-
14	Amino acid	+	-
15	Quinones	+	+

Note: + (positive); - (negative); * (not detected)

3.5 The anti-inflammatory activity of Plant extract

The anti-inflammatory activity of *Phyllanthusemblica* (Gooseberry) and *Psidium guajava* (Guava) extracts were assessed at concentrations of 20 mg/ml, 50 mg/ml and 100 mg/ml by using absorbance based analysis. Evaluation of anti-inflammatory activity of these plant leaf extracts was illustrated in Fig 8 & 9.

The result demonstrate a clear dose-dependent anti-inflammatory activity of Gooseberry and Guava leaf extracts. At 20 mg/ml gooseberry extract showed 50% of inhibition, Guava showed 42.30% inhibition, at 50 mg/ml the inhibition increased to 58.5% and 53.8%, while at 100 mg/ml both the extract achieved a maximum inhibition. Gooseberry extract showed 63.98% inhibition and Guava extract showed 61.2% inhibition. This result showed that Gooseberry leaf extract has stronger anti-inflammatory effect as compared to Guava leaf at 100 mg/ml. These results aids the traditional use of Gooseberry in treating inflammatory conditions and managing inflammation by reducing oxidative stress and can be used in the development of natural anti-inflammatory therapies especially in mild to moderate inflammatory conditions or for the patients seeking plant-based therapies [51].

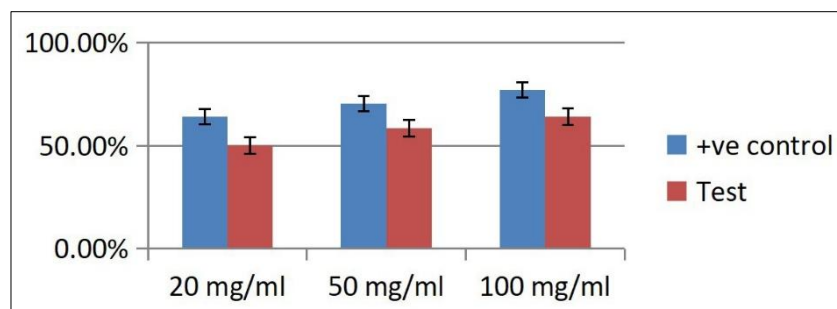


Fig 8: Anti-inflammatory activity of Gooseberry leaf extract and Diclofenac sodium

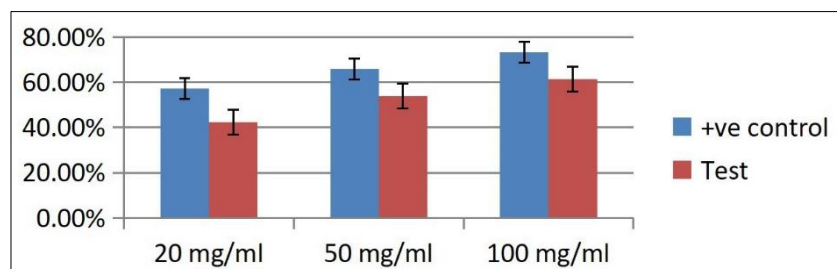


Fig 9: Anti-inflammatory activity of Guava leaf extract and Diclofenac sodium

Biofilm inhibition activity of Plant extracts

The biofilm inhibition activity of *Phyllanthusemblica* (Gooseberry) and *Psidium guajava* (Guava) leaf extracts was evaluated against two significant pathogenic bacteria, *Escherichia coli* and *Campylobacter jejuni*, at varying concentrations of 20 mg/ml, 50 mg/ml, and 100 mg/ml. As detailed in Figures 10-13, reveal a clear concentration-dependent reduction in biofilm formation across both bacterial strains. At the lowest tested concentration (20 mg/ml), Gooseberry leaf extract showed biofilm inhibition ranging from 8.7% to 12.6%, while Guava leaf extract demonstrated slightly higher inhibition between 14% and 20.2%. With increasing concentrations, the inhibitory effects improved, exceeding 50% at 100 mg/ml for both extracts. Specifically, *E. coli*, known for causing urinary

tract and gastrointestinal infections where biofilm formation is a key virulence factor, showed maximum inhibition of 52.9% with Gooseberry extract and 58% with Guava extract at the highest concentration. Similarly, *jejuni*, a common cause of foodborne gastrointestinal illness, exhibited inhibition levels of 51.6% and 56.2% with Gooseberry and Guava extracts, respectively, at 100 mg/ml. These results clearly suggest that Guava leaf extract possesses strong anti-biofilm potential in a dose-dependent manner, supporting its traditional use as an antimicrobial agent and highlighting its promise as a natural, broad-spectrum anti-biofilm compound. These observations are consistent with previous reports confirming the biofilm-inhibitory properties of various medicinal plants ^[54].

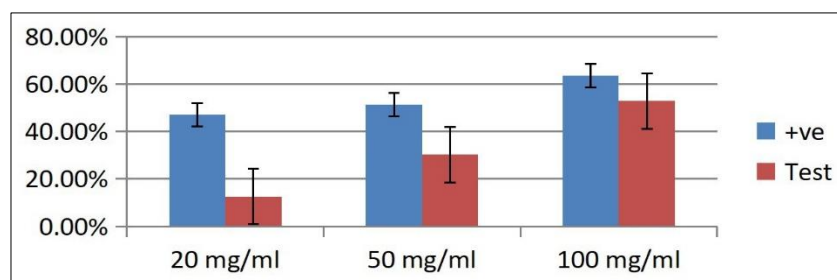


Fig 10: Percentage of bio-film inhibition of *E.coli* by Gooseberry leaf extract

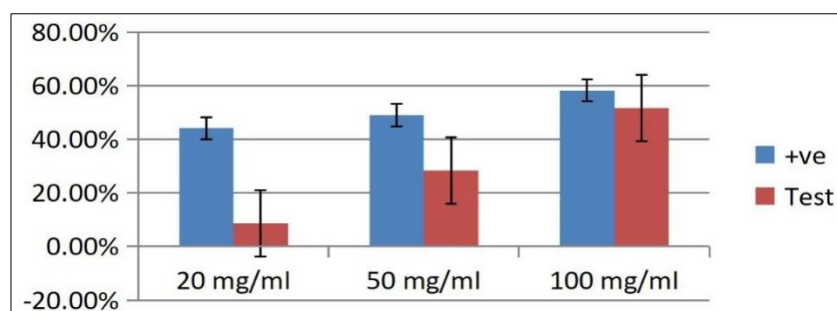


Fig 11: Percentage of bio-film inhibition of *C. jejuni* by Gooseberry leaf extract

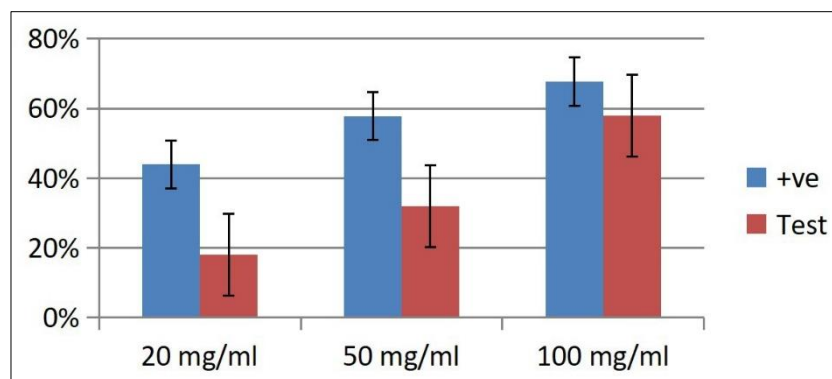


Fig 12: Percentage of bio-film inhibition of *E. coli* by Guava leaf extract

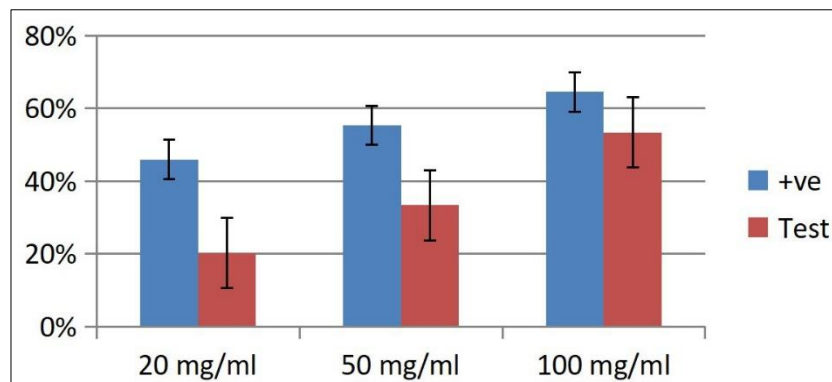


Fig 13: Percentage of bio-film inhibition of *C. jejuni* by Guava leaf extract

Antioxidant activity

The antioxidant activity of *Phyllanthusemblica* (Gooseberry) and *Psidium guajava* (Guava) leaf extracts, prepared using both aqueous and ethanolic solvents, was evaluated using the DPPH free radical scavenging assay, with ascorbic acid serving as the reference standard. Results, as presented in Tables 10-13 and Figures 14-17, demonstrated that increasing the concentration of both extracts led to a corresponding rise in free radical scavenging activity, evidenced by decreasing absorbance values. The aqueous extracts of Gooseberry and Guava

leaves exhibited scavenging activities ranging from 33% to 77.71% and 37.2% to 79.9%, respectively. Ethanolic extracts showed slightly higher activity, with Gooseberry ranging from 41.33% to 78.18% and Guava from 43.13% to 80.75%, the latter displaying the strongest antioxidant effect among all samples tested. Additionally, the IC₅₀ values indicated greater potency of ethanolic extracts, with Guava ethanolic extract showing the lowest IC₅₀ (21.1 mg/mL), suggesting superior radical scavenging efficiency.

Table 10: Antioxidant activity of Gooseberry leaf on Aqueous solvent

Concentration mg/ml	Absorbance 517nm (Gooseberry)	Scavenging Activity (%)	IC ₅₀ value
0	0.709	0%	The IC ₅₀ value for the following is 33.4 mg/ml
10	0.475	33%	
20	0.403	43.15%	
40	0.331	53.31%	
60	0.268	62.20%	
80	0.202	71.50%	
100	0.158	77.71%	

Table 11: Antioxidant activity of Guava leaf on Aqueous solvent

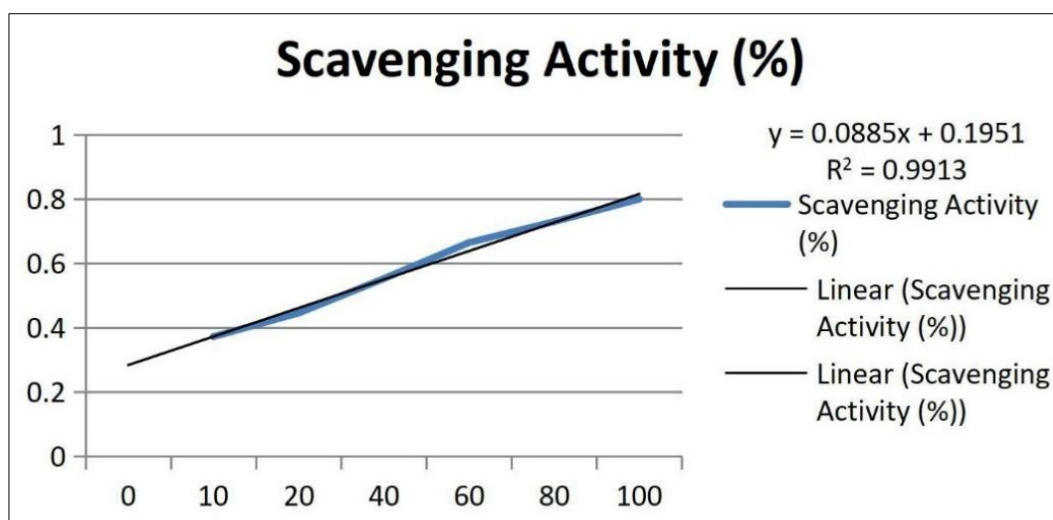
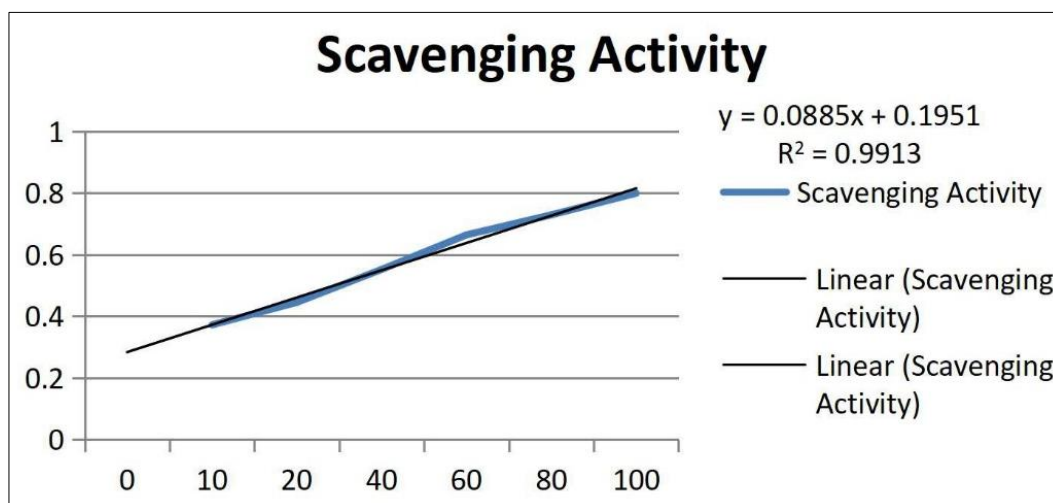
Concentration mg/ml	Absorbance 517nm (Guava)	Scavenging Activity	IC ₅₀ value
0	0.709	0%	The IC ₅₀ value for the following is 30.2 mg/ml
10	0.445	37.2%	
20	0.393	44.5%	
40	0.317	55.2%	
60	0.238	66.4%	
80	0.192	72.9%	
100	0.142	79.9%	

Table 12: Antioxidant activity of Gooseberry leaf on Ethanol solvent

Concentration mg/ml	Absorbance 517nm (Gooseberry)	Scavenging Activity	IC50 value
0	0.816	0%	The IC50 value for the following is 22.24 mg/ml
10	0.478	41.43%	
20	0.417	48.89%	
40	0.351	56.98%	
60	0.259	68.25%	
80	0.201	75.36%	
100	0.178	79.18%	

Table 13: Antioxidant activity of Guava leaf on Ethanol solvent

Concentration mg/ml	Absorbance 517nm (Guava)	Scavenging Activity	IC50 value
0	0.816	0%	The IC50 value for the following is 21.21 mg/ml
10	0.464	43.13%	
20	0.412	49.50%	
40	0.339	58.45%	
60	0.246	69.85%	
80	0.197	75.85%	
100	0.157	80.75%	

**Fig 14:** Calibration of Gooseberry leaf on aqueous extract.**Fig 15:** Calibration of Gooseberry leaf on aqueous extract.

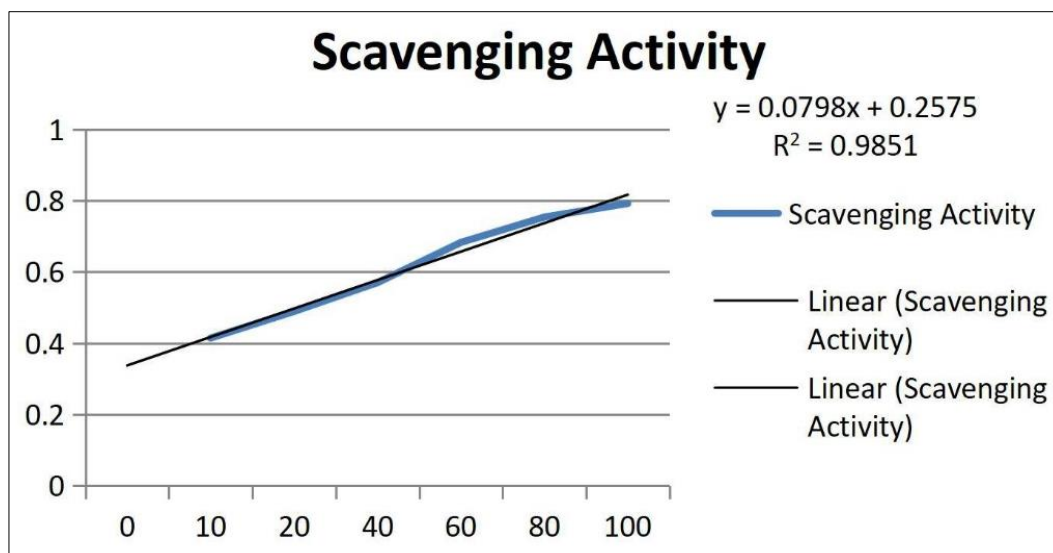


Fig 16: Calibration of Gooseberry leaf on ethanol extract.

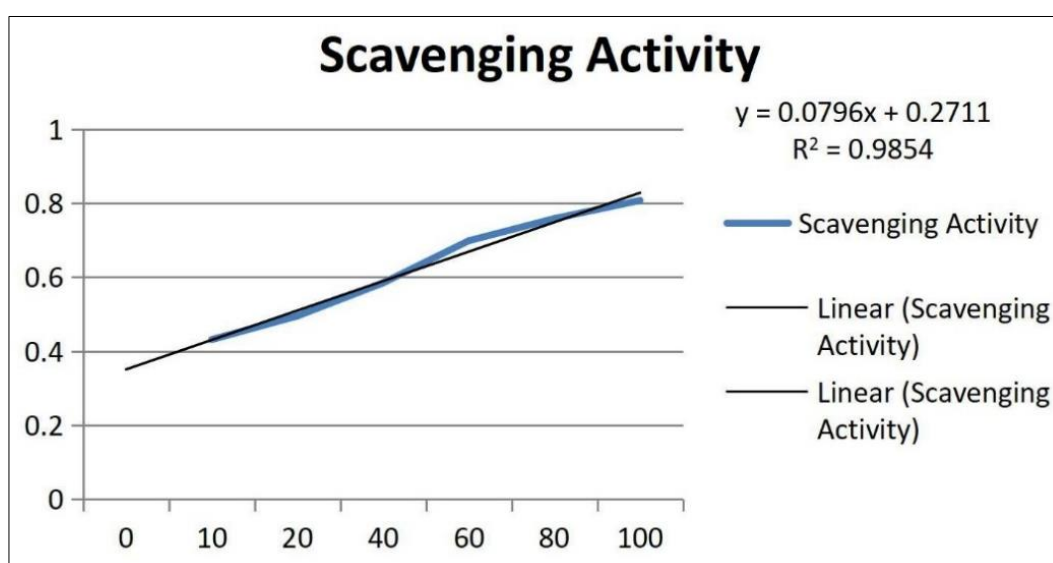


Fig 17: Calibration of Guava leaf on ethanol extract.

Note: IC₅₀ (μg/mL): 10-20 (μg/mL) -Very strong Antioxidant Activity; 20-50(μg/mL) Strong Antioxidant Activity; 50-100 (μg/mL) -Intermediate Antioxidant Activity; > 100 (μg/mL) -Weak Antioxidant Activity (According to Phongpaichit, S., *et al.*, 2007)

Conclusion

This study comparatively evaluated the biological activities of four medicinal plants, highlighting *Phyllanthusemblica* (Gooseberry) and *Psidium guajava* (Guava) for their superior extract yields and promising bioactivities. Both ethanolic and aqueous extracts exhibited concentration-dependent antibacterial, anti-diarrheal, anti-inflammatory, biofilm inhibition, and antioxidant effects. Gooseberry showed stronger antibacterial and anti-inflammatory potential, while Guava demonstrated higher antioxidant and biofilm inhibition activities, especially against *C. jejuni*. Overall, the findings scientifically support the traditional uses of these plants and emphasize the influence of extraction solvents on bioactive compound availability, underscoring their potential in managing foodborne and inflammatory disorders.

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