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RADICAL SCAVENGING AND DISINFECTION EFFICIENCY OF BAEL FRUIT EXTRACT ON FLOURISHING GREEN VEGETABLES

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ABSTRACT

Background: The public's health is very important; thus, it is crucial for the food business to prevent food contamination brought on by pathogenic microbes throughout production, processing, and packing Consuming fresh produce infected with harmful bacteria is one of the causes of foodborne illness. This article is about prevent population from foodbrone disease by use of bael fruit extract

Ain and Objective: The objective of present research work was to evaluate the Radical scavenging and disinfection efficiency of bael fruit extract on flourishing green vegetables

Materials and Methods: The methodology started by the identification of the plant Bael (*Aegle marmelos*) and then making ethanolic extract. Then estimation of total phenolic content and total Flavonoids content and further their estimation of antimicrobial and antioxidant activity with various assays.

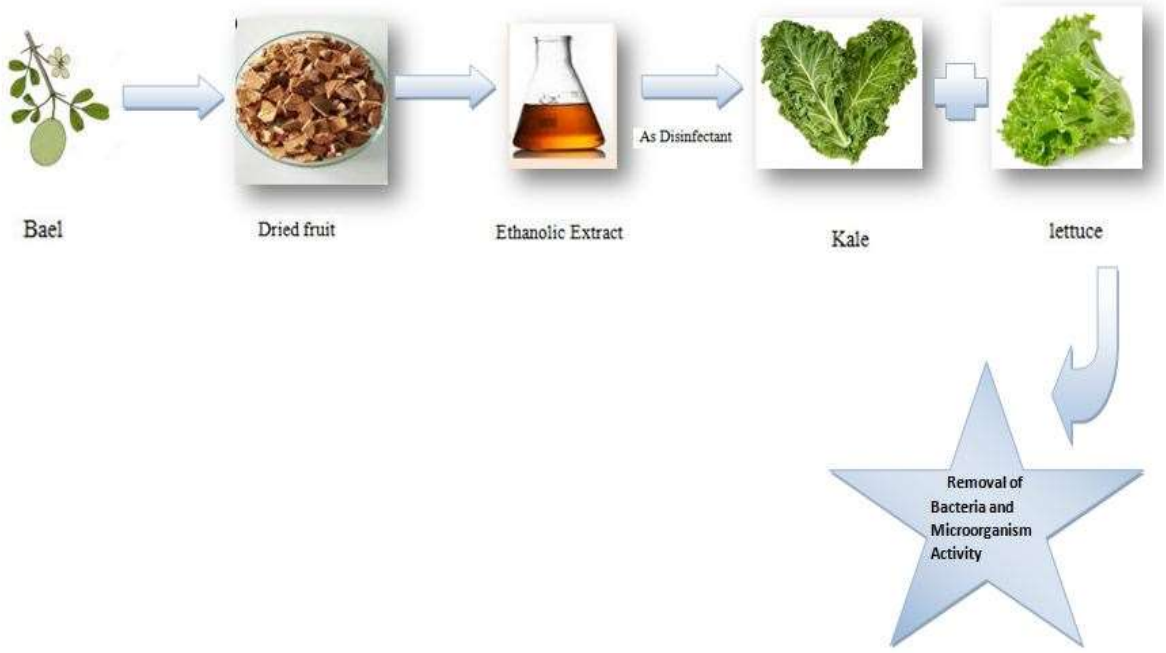
Results: The extract's Total Phenolic content & Total Flavonoid content was found to be 42.65g GAE kg^{-1} and 94.52 g QE kg^{-1}. For DPPH, H_2O_2, FRAC, and PM, the extract showed antioxidant properties of 116.05 and 271.06, 210.20 and 104.52g TE kg^{-1}, respectively. O-(3,3-dimethylallyl) halfordinol, aegeline, marmeline, imperatorin, xanthotoxol, valencic acid, vanillic acid, and rutin were the principal phenolic compounds found. In the MIC range of 17–19 g L^{-1}, the extract reduced

growth *of Salmonella enterica subsp. enterica serovar Typhimurium, Listeria monocytogenes*, and *Escherichia coli*, *Staphylococcus aureus*.

Conclusion: Results from the current study showed that phenolic compounds with antioxidant characteristics and antibacterial action against food originated pathogens are abundant in Bael fruit ethanolic extract. Bael fruit ethanolic extract was also shown to be a useful disinfectant for lowering human pathogenic bacterial populations in fresh green vegetables.

Keywords: Bael Fruit extract, Leafy veggies, natural antimicrobials, Phenolic compunds, antioxidants, food born disease

Pictorial Abstract



1. INTRODUCTION

The public's health is very important; thus, it is crucial for the food business to prevent food contamination brought on by pathogenic microbes throughout production, processing, and packing Consuming fresh produce infected with harmful bacteria is one of the causes of foodborne illness **[1]**.

The leafy vegetables are grown in open filed and contaminated from the soil, sewage, irrigation water and in contact with the wildlife's faces and they often consumed as Raw, there are high possibility of contamination of these vegetables so it's necessary to protect them from contamination **[2]**. In Previous studies it was discovered that

those items which are associated with animal origin having high possibilities of growing pathogenic bacteria such, Salmonella spp., Sthapy loccocus aureus, Escherichia coli and Listeria monocytogenes in fresh fruits, vegetables and especially leafy greens **[3]**. Before distribution to retail markets or during processing for packing, fresh fruit is frequently cleaned with aqueous sanitizers like hydrogen peroxide, trisodium phosphate and chlorine to minimize the microbial burden. However, customers are becoming more knowledgeable of the chemicals used as food preservatives in addition; the use of non-natural disinfectant on sub lethal concentration may induce pathogenic bacteria to exhibit altered phenotypes and activated gene expression, which might result in antibiotic resistance **[4]**. These findings emphasize the need of searching for new effective antibacterial substances without side effects or the development of antibiotic resistance. In this situation, using natural antimicrobials derived from plants as a substitute for synthetic sanitizers has been suggested **[5]**.

According to the previous research by Ravichandran and colleagues and Sa'nchez-Maldonado, The most prevalent and extensively dispersed faction of plant secondary metabolites, phenolic compounds, exert antibacterial effects on a series of pathogenic microorganisms, including *Salmonella species, L. monocytogenes, E. coli*, and *Bacillus subtilis, S. aureus* **[6]**. Phenolic compounds have a variety of biological effects that have been linked to their antioxidant properties, such as anti-allergic, anti-inflammatory, hepatoprotective, antiviral, anticarcinogenic, antithrombotic and vasodilatory activity **[7]**. Due to the possibility of using them as organic antibacterial agents, these qualities have caught the interest of the food industry **[8]**.

Native to India, *Aegle marmelos* sometimes referred to as "bael," is a wild plant that may be found growing all across the central and southern deciduous forests of that nation. Because it is commonly used in the worship of numerous deities, especially Lord Siva, it is exceedingly religious. It is a sparse, small- to medium-sized tree with trifoliate leaves that alternate, fragrant white blossoms, and fruit that resembles berries (amphisaraca) **[9, 10]**. One such herbal source that is rich in bioactive compounds with oxygen scavenging activity and useful for treating a range of ailments, including diabetes **[11]**, diarrhoea, cancer, ulcers, and more is this tree, which includes its root, leaf, trunk, fruit, and seed.

Numerous pharmacological activities of Bael are well-known, including anti-inflammatory,

analgesic and antipyretic, wound-healing, antineoplastic, radioprotective, chemoprotective, and chemopreventive actions **[12, 13]**, antidiabetic, chemoprotective **[14]** and radio-protective **[15]**. In this study, ethanolic extracts of the Bael fruit were examined for their high antioxidant activity in vitro, total phenolic content, and flavonoid content to find possible sources for emerging novel antioxidants in food and medicinal formulations. The current study also sought to: determine the in vitro antimicrobial activity of Bael Fruit Extract in opposition to pathogenic bacteria; and as a model for fresh green vegetables, lettuce and kale, to evaluate the efficacy of their disinfection characteristics.

2. MATERIALS AND METHODS

2.1 Plant sample collection

Fruit (*Aegle marmelos*) was obtained from a local market in Delhi, India. The fruit was recognised and verified as real by the branch of forest products at the Dr. YS Parmar University of Horticulture and Forestry in Nauni Solan, Himachal Pradesh, India, ref.no UHF-Harbarium no. 13957 on 12/4/2022. In our lab, the fruit was manually dried. Prior to extraction, fruit (*Aegle marmelos*) was cut into small pieces and kept at -20 °C.

2.2 Bael fruit ethanol extract Preparation:

Bael *(Aegle marmelos)* fruit was broken up and dried outside in the sun. The fully dried fruits were pulverised, and 15 g of the powdered fruit was used in a Soxhlet device for extraction over the course of four hours. By evaporating the solvent over a water bath, the extract was concentrated into a syrupy liquid and kept at 40°C.

2.3 Characterization of Fruit (*Aegle marmelos*) extract's phenolic content

2.4 *Total Phenolic content*

The folin-Ciocalteu technique was used to find out the total phenolic content. In methanol, a stock solution of the extract containing 5mg/ml was created. 500 mL of the stock solution and 2.5 mL of the folin-Ciocalteu reagent (1:10, v/v) were combined while the vial containing the combination was continuously shaken. After combining, 2.5 mL of a 7.5% sodium bicarbonate solution that had already been prepared was added from the tube's side wall. The tube was then left to stand for 30 minutes in a dark area while being periodically shaken. A calibration curve of gallic acid (10–1000 g/mL) was plotted against absorbance to determine the quantity of phenols. In order to determine the absorbance, a spectrophotometer was used at 765 nm. Gallic acid and total phenol concentration (measured in mg/g of extract) were comparable. All the measurement were

taken in triplicate and the obtained data was expressed statistically as Mean ±SD (n=3).

2.5 *Total flavonoid content*

The aluminium chloride technique was used to measure the samples' total flavonoid content. In methanol, a stock solution of the extract containing 5mg/ml was created. With the appropriate shaking, 500 mL of stock solution was combined with 1.5 mL of methanol. 0.1 mL of sodium acetate (1M), 0.1 mL of aluminium chloride (10%), and 2.8 mL of water were added after waiting for five minutes. After the incubation period, absorbance at 415 nm was calculated via spectrophotometer. A concentration curve of rutin (10-1000 g-mL) was plotted against absorbance to determine the flavonoid content. The amount of flavonoids present in an extract is measured in mg of rutin equivalents per gramme of extract. All the measurements were taken in triplicate and obtained data was expressed statistically as Mean ±SD (n=3)

2.6. Column Chromatographic analysis of phenolic compounds

Column chromatography was performed for the separation of phytoconstituents from the complex matrix of *Aegle marmelos* Fruit Ethanolic Extract and their further sepration for pure constituents using different gradients of the solvents system. 5 g of extract were briefly loaded or absorbed. The sample was loaded onto 15 g of 60–120 mesh size silica gel, while 230–400 mesh size silica gel was used for column packing. The sample was then subjected to the column (column length x diameter (40x3 cm)) for the gradient separation of phytoconstituents using chloroform and ethanol as the mobile phase. Each portion that was gathered had a 10 ml volume. During CC, the eluted solvent flow rate was set to 31 ml/min.

2.7 Free radical scavenging capacity

2.7.1DPPH radical scavenging activity

The outlined technique was used to assess the antioxidant activity of the Bael ethanolic extract **[16]**. 20 mL of material from each created concentration, ranging from 1000 to 7.81 g/mL, was blended with 180 mL of a DPPH (0.1 mM) solution that had already been made. The resultant mixes were kept at room temperature and incubated for 30 min. in a comparatively dark area. At a wavelength of 517 nm, the absorbance was measured using a spectrophotometer. IC 50 values (g/mL) were used to express the results. To assess the fruit ethanolic extract's anti-oxidant potential in relation to standard medication, ascorbic acid (vitamin C) was utilised as a positive control. All the measurement were taken in triplicate and the obtained data was expressed statistically as Mean ±SD (n=3).

This effect was determined by using the equation

% scavenging activity = ($A_{Control} - A_{sample} - A_{control}$) x 100

2.7.2. Ferrous reducing antioxidant capacity assay

By using Oyaizu's approach **[17]**, the ferric reducing antioxidant capacity (FRAC) of the samples was assessed. The production of Perl's Prussian blue is monitored at 700 nm, the Fe2+ is observed. The test tubes were filled with 0.25 mL samples/standard solutions with various concentrations (12.5-150 g/mL),), 0.625 mL of 1% potassium ferricyanide and 0.625 mL potassium buffer (0.2 M [K3Fe (CN)6] solution. All the mixtures were incubated for 20 min. at 50 °C for completion the reaction. Then test tubes were filled with 0.625 mL of a 10% TCA (trichloroacetic acid) solution. After centrifuging the entire mixture at 3000 rpm for 10 minutes, 1.8 mL of the supernatant was removed from the test tubes and combined with 1.8 mL of distilled water and 0.36 mL of a 0.1% solution of ferric chloride (FeCl3). A spectrophotometer was used to test the solution's absorbance at 700 nm in comparison to a blank.

2.7.3. Hydroxyl radical scavenging activity

The extractives' ability to scavenge hydroxyl radicals was assessed using the Halliwell *et al*. Technique **[18]**. The Fe3+-ascorbate-EDTA-H2O2 system (Fenton reaction) produced a hydroxyl radical. The test is based on the measurement of the 2-deoxy-D-ribose breakdown product, which when heated with TBA at a low pH produces a pink chromogen. The reaction mixture contains 0.2 mL of extractives/standard at various concentrations (12.5-150 g/mL), 0.8 mL of phosphate buffer solution (50 mmol L-1, pH 7.4), 0.2 mL of EDTA (1.04 mmol L-1), 0.2 mL of FeCl3 (1 mmol L-1), and 0.2 mL of 2-deoxy-D-ribose (28 mmol L-1). The mixture was heated for 15 minutes at 100 °C before being cooled with water. Using a spectrophotometer, the solution's absorbance at 532 nm was determined. The quantity of 2-deoxy-D-ribose oxidation that was prevented was used to gauge the capacity to scavenge hydroxyl radicals. The formula used to determine the percentage of hydroxyl radical scavenging activity is as follows:

% hydroxyl radical scavenging activity=$[A_0-(A_1-A_2]\times100/A_0$% hydroxyl radical scavenging activity=$[A_0-(A_1-A_2]\times100/A_0$

where A_0 represents the control's absorbance in the absence of a sample. The absorbance at the sample and 2-deoxy-D-ribose addition is A_1. The sample's absorbance without 2-deoxy-D-ribose is A_2. Then the concentration

was plotted against the percentage of inhibition, and the IC50 was determined from the graph. Three times at each concentration, the experiment was repeated.

2.7.4. Phosphomolybdenum (PM) assay,

According to the steps outlined by Prieto P *et al*. **[19],** the phosphomolybdenum technique was used to assess the methanol extract's overall antioxidant capability. 2 ml of the reagent solution 0.6 M sulfuric acid, 28 mmol sodium phosphate, and 4 mmol ammonium molybdate—were mixed with 0.2 ml of the extract. After incubating the reaction mixture at 95°C for 90 minutes and cooling it to room temperature, the absorbance of the mixture was measured at 695 nm using a spectrophotometer against a reagent blank. The extract was replaced with reagent (2 ml), which served as the blank. The amount of ascorbic acid equivalent in mg/gm units is used to express the antioxidant activity.

2.8. Antimicrobial assays

2.8.1. Minimum bactericidal and inhibitive concentration

Escherichia coli, L. monocytogenes, S. aureus, and Salmonella enterica subsp. enterica serovar Typhimurium were tested for the extract's antibacterial effects. A broth microdilution method was used to calculate the minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) and of the extract.

2.9. Effect of fruit extract (*Aegle marmelos*) in cleaning up leafy vegetables

Lettuce and kale were put inside sodium hypochlorite at a 250 ppm concentration to trim down the local bacteria. Before being dried for 30 minutes in an Esco II Airstream biosafety cabinet, samples (10 g) were soaked for 2 min in several bacterial solutions (each containing 1x106 CFU mL-1 of S. aureus, E. coli, or S. enterica ser. Typhimurium L. monocytogenes, respectively). After each contaminated vegetable had dried, it was immersed for 2 minutes in 200 mL of Fruit extract (*Aegle marmelos*) solution (25 g L^{-1}). The sample were dried for 30 minutes in the air. Then, each injected bacterium's initial load was noted. The bacterial load on aerobic Plate Count Agar was compared before and after the treatments **[20]**.

2.10. Statistic evaluation

All studies were conducted using a purely random design. Three independent, first-objective experiments were carried out, and the results were reported as mean Standard Error of the Mean. In Antimicrobial tests the effect of extract (0, 0.5 9 x MIC and MIC) on the kinetic parameters of bacterial growth in *S. aureus, E. coli, L. monocytogenes and S. enterica ser. Typhimurium* (lag phase, growth

rate, and Ymax) was evaluated. The impact of the extract was assessed on the log decrease of pathogenic germs introduced in leafy vegetables (Kale and lettuce). The entire set of data was put through an ANOVA and a multiple range test (Tukey's test) using NCSS 2007 (p B 0.05).

3. RESULTS AND DISCUSSION

Fruit extract from (*Aegle marmelos*) was studied for its primary phenolic components, antioxidant activity, and identification and quantification.

The fruit extract's total yield, phenolic and flavonoid compositions, and antioxidant activity are all listed in **Table 1**. The powdered Fruit extract has phenolic content 42.65±1.32 g GAE kg^{-1} dw and a total flavonoid content of 94.52±1.62 g QE kg^{-1} dw. Moreover, the extract showed radical scavenger activity against the free stable radicals DPPH (116.05 ±1.54), H_2O_2 (271.06 ±2.34), FRAC (210.20 ±1.32), and PM (104.52 ±1.23 g TE kg^{-1}, respectively). Our results are consistent with information from studies that have already been published and which identify fruit extracts from (*Aegle marmelos*) as a significant source of phenolic compounds with anti-inflammatory properties.

Fruit extract's In vitro antibacterial effects against pathogenic microorganisms

Long established as a substantial source of phenolic compounds with antioxidant action, fruit extracts (*Aegle marmelos*) The antibacterial activity of fruit extracts (*Aegle marmelos*) was examined using a broth microdilution test on two Gramme positive (*L. monocytogenes and S. aureus*) and two Gramme negative (*E. coli and S. enterica ser. Typhimurium)* bacteria. For each bacteria, **Table 2** gives the minimal bactericidal concentrations and minimal inhibitory (MBC and MIC).

Pathogen-removing abilities of *Aegle marmelos* fruit extract on green leafy vegetables

On the leaves of kale and lettuce treated with harmful bacteria (*E. coli or S. enterica ser. Typhimurium or L. monocytogenes, S. aureus*), the antibacterial action of Bael (*Aegle marmelos*) Fruit extract (25 g L^{-1}) is shown in **Table 3**. The control treatment was distilled water. Initial pathogenic bacterial populations in Kale varied from 4.796–4.421 log CFU g^{-1}, whereas those in lettuce leaves ranged from 4.792–4.012 log CFU g^{-1}. Water was used to obtain reduction of 0.011-0.747 log CFU g^{-1} in inoculated Kale and 0.889-1.232 log CFU g^{-1} in inoculation lettuce

Table 1: Shows the Bael fruit extract from yield, its total phenol and flavonoid concentration, and its antioxidant activity

Percentage yield	Content of Flavonoid (g QE kg^{-1} dw)	Phenolic content (g AM kg^{-1} dw)	PM (g TE kg^{-1})	FRAC (g TE kg^{-1})	H_2O_2 (g TE kg^{-1})	DPPH (gTE kg^{-1})
39.2	94.52± 1.62	42.65±1.32	104.52 ±1.23	210.20 ±1.32	271.06 ±2.34	116.05 ±1.54

Table 2: Fruit extract's minimum bactericidal and inhibitory concentrations (MIC and MBC, respectively) against pathogenic microorganisms

Microorganism	MIC(g L^{-1})	MBC(gL^{-1})
S. aureus	17	>21
E. coli	19	>23
S. enterica ser. Typhimurium	17	>23
L. monocytogen*es*	19	>23

Table 3: Fresh lettuce and kale leaves with lower harmful bacterial loads (log CFU g-1) after 25 mg mL-1 of *Aegle marmelos* Fruit extract

Treatment	Kale		Lettuce	
	Log CFU g^{-1}	Log reduction	Log CFU g^{-}	Log reduction
L. monocytogenes				
Initial	4.421	-	4.012	-
Water	3.674	0.747	3.123	0.889
Extract	3.359	1.062	3.098	0.914
S. aureus				
Initial	4.796	-	4.792	-
Water	4.173	0.623	3.802	0.99
Extract	4.136	0.66	3.014	1.778
E. coli				
Initial	4.698	-	4.649	-
Water	4.472	0.226	3.624	1.025
Extract	4.463	0.235	2.520	2.129
S. enterica ser. *Typhimurium*				
Initial	4.608	-	4.328	-
Water	4.597	0.011	3.096	1.232
Extract	2.621	1.987	2.529	1.799

Correspondingly 1.778 and 0.914 log decreases. The most significant reduction in Kale was shown in *S. enterica ser. Typhimurium* (1.987 log), and others such as *L. monocytogenes, E. coli, and S. aureus* (1.062, 0.66, and 0.235 log CFU g1, respectively). According to these findings, Ethanolic Fruit extract may be utilised to successfully lower the amount of human pathogenic bacteria in fresh green vegetables. With the help of chlorine sanitizer, which is frequently used to clean vegetables, similar reductions might be made. Despite this, it is necessary to research the efficacy of other decontamination approaches due to the worry over the creation of potentially carcinogenic chemicals obtained from chlorine-based sanitizer in wash water **[24]**. Based on their antibacterial properties, Bael fruit extract may

be used as a natural substitute disinfectant to clean up leafy vegetables.

According to our findings, there were no significant differences in plant Ethanolic extract antibacterial effectiveness against Gramme positive and Gramme negative bacteria. **[21]** These findings highlight the significance of testing the antibacterial efficacy of natural extracts utilising various pathogenic microorganisms.

Fruit extract from *Aegle marmelos* has an antibacterial effect due to the existence of phenolic compound. The flavonoid rutin, which is included in fruit extract, has been found to preferentially boost topoisomerase IV, an enzyme that is essential for *E. coli* survival **[22]**. The physical elimination of disease cells from vegetable surface was the main cause of this incident **[23]**. However, following treatment with Fruit extract at a dosage of $25gL^{-1,}$ the levels of *Staphylococcus aureus*, *Listeria monocytogenes*, and *S. enterica ser. Typhimurium*, *Escherichia coli* in leaves of kale and lettuce were significantly reduced. The log reduction of fruit extract were greater (p[0.05] than water for *E. coli, S. aureus, S. enterica ser. Typhimurium*, and every strain of S. aureus in lettuce leaves and kale leaves. The most effective bacteria against *S. aureus*, *L. monocytogenes* and *S. enterica ser. Typhimurium* in lettuce were *E. coli* (2.129 log drop).

According to these findings, Ethanolic Fruit extract may be utilised to successfully lower the amount of human pathogenic bacteria in fresh green vegetables.

With the help of chlorine sanitizer, which is frequently used to clean vegetables, similar reductions might be made. Despite this, it is necessary to research the efficacy of other decontamination approaches due to the worry over the creation of potentially carcinogenic chemicals obtained from chlorine-based sanitizer in wash water **[24]**. Based on their antibacterial properties, Bael fruit extract may be used as a natural substitute disinfectant to clean up leafy vegetables.

5. CONCLUSION

Results from the current study showed that phenol compound with antioxidant characteristics and antibacterial action against foodborne pathogens are abundant in Bael fruit extracts. Bael fruit extract was also shown to be a useful disinfectant for lowering human pathogenic bacterial populations in fresh green vegetables. The usefulness of Fruit Extract as a disinfectant in fresh green vegetables has not been studied, as was previously noted; nevertheless, several research have been conducted using natural plant extracts for this purpose. According to

these findings, plant extracts can effectively lower the pathogenic burden in fresh vegetables. Our findings in the current study showed that Bael Fruit extract is efficient in reducing *S. aureus, L. monocytogenes, S. enterica ser. Typhimurium* and *E. coli*, from the leaves of lettuce and Kale.

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DECLARATION:

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Ethical Clearance: Nil

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