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Evaluation of Antioxidant, Anti-inflammatory and Antimicrobial Potential of *Aegel marmelos* Fruit Pulp Extracts against Clinical Pathogens

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Abstract: In India, a wide range of medicinal plants are reported. Since ancient times, these medicinal plants have been used by people for the treatment of several diseases. Herbal medicines typically have fewer side effects compared to synthetic medicines, and they are also non-expensive. The aim of this study is to evaluate the antioxidant, in vitro anti-inflammatory activity through HRBC membrane stabilization, as well as the antimicrobial potential of fruit pulp extracts obtained from *Aegle marmelos* (*A. marmelos*). The antioxidant activity of the fruit pulp extracts was assessed using the DPPH assay. Various *A. marmelos* fruit pulp extracts *viz.*, aqueous, chloroform, ethyl acetate, hexane, methanol and L-ascorbic acid were found to have IC_{50} values of 91.168 µg/mL, 153.22 µg/mL, 195.58 µg/mL, 164.741 µg/mL and 39.488 µg/mL, respectively, while for L-ascorbic acid (standard) it was 57.823 µg/mL. The anti-inflammatory activity of the fruit pulp extracts of aqueous, chloroform, ethyl acetate, hexane and methanol are also dependent on the concentrations. The hemp production (IC_{50}) concentration was 99.761 µg/mL, 114.443 µg/mL, 167.423 µg/mL, 118.397 µg/mL and 23.244 µg/mL. Likewise, the antimicrobial activity of *A. marmelos* fruit pulp extracts demonstrated significant effects against clinical pathogens. Comparatively, the methanol fruit pulp extract of *A. marmelos* showed higher antimicrobial activity than that of the other four extracts; methanol fruit pulp extract contributed significantly to the development of antimicrobial properties. The findings of this study, thus, methanol fruit pulp extract, clearly showed that it had the strongest antioxidant, anti-inflammatory and antimicrobial activity when compared to the other four extracts.

Introduction

A medicinal plant is a species of the plant kingdom whose parts (flowers, leaves, roots, stems, fruits, or seeds) are directly used or used in some preparation as a medicine to treat a condition or disease (Acharya et al., 2021, 2022; Rai and Sharma, 2024). They are the subject of current research as stated by Yang et al. (2017), Sarkar et al. (2024), Tyagi et al. (2024), Darro and Khan (2024). Herbal medicines typically have fewer side effects and are less expensive, which are their main benefits (Chothiphirat et al., 2019; Hernández et al., 2022; Mohamad Sitheek et al., 2023). Bhattacharya (2017) has pointed out that confirming the safety and effectiveness of medicinal herbs through research studies is crucial. Medical plants with anti-inflammatory (Bee et al., 2023) qualities have received a lot of interest due to the pressing demand for new treatment agents with improved efficacy and fewer adverse effects as opined by Lourenco et al. (2012).

Antioxidants are largely obtained from medicinal plants (Rice-Evans, 2004; Rami et al., 2023; Bashar et al., 2024; Hijam et al., 2024). Natural antioxidants boost plasma's ability to fight off free radicals and lower the risk of certain diseases like cancer, heart disease, and

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stroke (Prior and Cao, 2000). According to previous reports, plant secondary metabolites including phenolics and flavonoids are powerful free radical scavengers (Chakrabarty et al., 2024). Antioxidants produced synthetically are widely used. However, it is claimed that they have a number of negative effects (Ito et al., 1983; Hassanpour and Doroudi, 2023; Bowen et al., 2024), including the potential for liver damage and the development of cancer in laboratory animals (Osawa and Namiki, 1981; Gao et al., 1999; Williams et al., 1999). Therefore, there is a need for antioxidants that are more efficient, less harmful, and more affordable. These desired comparative benefits seem to be present in medicinal plants, which explains the rising interest in natural antioxidants derived from plants.

One of the most significant medicinal herbs in India, Burma and Ceylon is *Aegle marmelos* (Srivastva et al., 1996). Since Charak (1500 B.C.), the Indian nation has recognized vilvam (*A. marmelos*) as one of its most significant medicinal herbs (Farina et al., 2014). Phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids, and tannins are only a few of the more than 100 phytochemicals that have been isolated from different portions of this plant. On a variety of animal models, the crude extracts of this plant have also been shown to have antioxidant, antiulcer, anti-diabetic, anticancer, anti-hyperlipidaemic, anti-inflammatory, antimicrobial, and anti-spermatogenic effects (Yen et al., 1993; Sur et al., 1999; Kamalakkannan and Prince, 2003; Sabu and Kuttan, 2004; Arul et al., 2005; Jagetia et al., 2005; Kamalakkannan and Prince, 2005; Rajadurai et al., 2005; Saradha Jyothi and Subba Rao, 2010). Every component of the *A. marmelos* plant, including the fruits, stem, bark, and leaves, has medicinal value and is used to treat a variety of eye and skin ailments (Kingston et al., 2009). The leaf is regarded as one of the plant sections with the highest accumulation of bioactive substances, which are produced as secondary metabolites (Cowan, 1999).

Vilva leaves (*A. marmelos*) are helpful in the treatment of wounds and jaundice. Leucorrhoea, conjunctivitis, and hearing can all be effectively treated with leaf extract. Fruits make you feel energized and fresh. Both an astringent and carminative, it is utilized, it works well for thyroid-related disorders, heart stimulant, swollen joints, pregnancy issues, typhoid, and coma are some of the other excellent medicinal uses that have been reported and irritable bowel syndrome is treated using leaf powder that has been dried (Sharma et al., 2007). The edible fruits come in oblong, oval, or spherical shapes. Depending on the type, a thin or firm woody shell that is yellowish when ripe and gray-green when uncooked may envelop the fruit's flesh. The shells provide a distinctive lovely scent. The pulp is delicious, resinous, pale orange, and very fragrant (Manandhar et al., 2018). Its fruit has been found to have the most therapeutic benefit, and the unripe fruit is a fantastic cure for diarrhea, especially chronic diarrheas (Brijesh et al., 2009), anti-pyretic and anti-inflammatory (Kamalakkannan and Prince, 2003; 2005), antidiabetic and anti-obesity (Rajadurai et al., 2005), antimicrobial activity (Kamaraj et al., 2012). The fruit of *A. marmelos* has been added to the British Pharmacopoeia due to its efficacy in treating diarrhea and dysentery (Brijesh et al., 2009). The author also opened that "No drug has been longer and better known nor more appreciated by the inhabitants of India than the Bael fruit." Based on this, the present studies are carried out to check the antioxidant, anti-inflammatory and antimicrobial activity of *Aegle marmelos* fruit pulp against various pathogens.

Materials and Methods

Preparation of Fruit Extracts

Aqueous Extraction

Vilvam fruit was harvested; the fruit's skin was then cut off, and the endocarp was allowed to shade dry. 50 g of dried fruit endocarp were combined with 250 mL of boiling water to make a 5% suspension (w/v), which was then placed in a container and shaken at 200 rpm for 4 h at 37°C. The suspension was stirred, cooled to room temperature, and then run through four layers of ROLEX No. 1 Whatman filter paper. The aqueous suspension from the extraction was dried, and the extract was kept at -20°C for use in other biological experiments (Rajesh et al., 2016).

Chloroform, Ethyl acetate, Hexane and Methanol Extraction

50 g of dried endocarp were extracted in 500 mL of methanol, ethyl acetate, hexane, and chloroform to create the extracts. Dried fruit's endocarp was immersed in methanol, chloroform, ethyl acetate and hexane for 72 h at room temperature (1:10 w/v). The filters were concentrated at 45-55°C under pressure reduction using a vacuum rotary evaporator after the extractors were filtered using a ROLEX Whatman No. 1 paper filter. Extracts were weighted and concentrated, then stored for future research (Rajesh et al., 2016).

Antioxidant Potential of *A. marmelos* Fruit Pulp Extracts

Blois's (1958) method of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) technique was used to examine the antioxidant capability of each of the five extracts of

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A. marmelos. Briefly, to get the necessary concentration, methanol was mixed with L-Ascorbic acid (standard) and each of the five *A. marmelos* pulp fruit extracts. DPPH was dissolved in methanol to produce 0.1 mM of DPPH. *A. marmelos* pulp extracts or L-Ascorbic acid, each at a different concentration (12.5-200 µg/mL), were added to the DPPH solution, which was precisely 150 µl in volume. Methanol was added as a control to the DPPH solution. A micropipette is used to completely mix the reaction mixture, which is then left to sit at room temperature in the dark for 30 minutes. The percentage of inhibition is determined by applying the following formula and an absorbance measurement at 520 nm:

$$Percentage\ Inhibition = \frac{\text{OD of Control} - \text{OD of Experiment}}{\text{OD of Control}}\ X\ 100$$

To determine the effective concentration of the reference standard and sample needed to extract DPPH by as much as 50%, line interpolation analyzes were carried out (IC_{50} value).

Anti-inflammatory Potential of *A. marmelos* Fruit Pulp Extracts

The anti-inflammatory potential of each of the five *A. marmelos* fruit pulp extracts was evaluated using Jayameena et al. (2018) method and a membrane stabilization assay with human red blood cells (HRBC). Briefly, blood sample taken from healthy individuals was combined with an equal amount of the Alsever solution. At 3000 rpm, the mixture was centrifuged for 5 minutes. The packed cells were cleaned using an isosaline solution at 0.85%. The suspension was prepared using 10% volume/volume isosaline. The following ingredients were added to 0.5 mL of HRBC suspension: 1.0 mL of 0.15 M phosphate buffer, 2.0 mL of 0.36% hyposaline, and 1.0 mL each of the five extracts of *A. marmelos* fruit pulp (6.25, 12.5, 25, 50, and 100 µg/mL). Hyposaline was replaced with 2.0 mL of double-distilled water along with the aforementioned reagents in the control sample. All five *A. marmelos* fruit pulp extracts and the control were centrifuged for five minutes at a speed of 3000 rpm after being incubated for 30 minutes at 37°C. At 560 nm, the optical density of the supernatant solution was measured. The percentage of hemolysis was computed under the assumption that any hemolysis caused by the use of distilled water would be 100%. The proportion of HRBC membrane stabilization was determined using the following formula:

$$Percentage\ Inhibition = \frac{OD\ of\ Control - OD\ of\ Experiment}{OD\ of\ Control} \times 100$$

Antimicrobial Potential of *A. marmelos* Fruit Pulp Extracts

Antimicrobial activity (antibacterial and antifungal) of selected clinical pathogens against *A. marmelos* fruit pulp extracts was performed by Bauer et al. (1966) method.

Purchase of Microorganism

Micro-organisms (Bactria and fungi) were purchased from the King Institute of Preventive Medicine and Research, Tamil Nadu, India.

Antibacterial Potential of *A. marmelos* Fruit Pulp Extracts

Microorganism Used in this Study (Bacteria)

Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella typhi. Inoculum's Preparation: The inoculum was standardized at 1x 10^6 CFU/mL compared with turbidity standard (0.5 MacFarland tube). Swabs preparation: Cotton wool swabs on wooden applicator sticks were ready to be used. They were sterilized using dry heat or the autoclave and were placed in tins, culture tubes, or on paper. Agar disc diffusion was used to carry out the assay. Briefly, the petriplate is filled with Muller Hinton Agar (MHA) medium. A sterile brush dampened with the bacterial suspension was used to disseminate the inoculums over the MHA plates after the medium had solidified. In MHA plates, sterile samples (20 µL of standard antibiotic (Ampicillin) disc) and sterile samples (disc form 500, 750, and 1000 µg/mL concentration) were added. For 24 h, the plates were incubated at 37°C. By measuring the diameter of the zone of inhibition, the antibacterial activity was identified.

Antifungal Potential of *A. marmelos* Fruit Pulp Extracts

Candida albicans, and Aspergillus niger fungal strains were used for this study. Briefly, on Sabouraud Dextrose Agar (SDA) slant, stock cultures were kept at 4°C. By transferring the stock cultures into test tubes with SDA broth and incubating them for 48 h at room temperature, active cultures for the experiments were created. Agar disc diffusion was used to carry out the assay. On SDA medium, the disc diffusion method was used to assess the extracts' antifungal activity. An SDA medium solution is added to the petri dish. With a sterile swab dampened with the fungal solution, the inoculums were applied to the solid plates after the medium had been set. The concentrations of the samples were diluted to 1000 µg/mL, 750 µg/mL, and 500 µg/mL, respectively. The positive control used was Amphotericin-B. A positive control of 20 µL of Amphotericin-B was applied to sterile discs with three different sample concentrations, and the discs were then placed in SDA plates. About 48 h of incubation at 37°C

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Table 1. DPPH free radical scavenging activity of various *A. marmelos* fruit pulp extracts.

Concentration (µg/mL)	DPPH activity (% Inhibition)					
	Aqueous	Chloroform	Ethyl acetate	Hexane	Methanol	L-Ascorbic acid
Control (0)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
12.5	82.81 ± 0.10* (-17.189)	88.88 ± 0.14* (-11.125)	92.02 ± 0.13* (-7.976)	92.33 ± 0.12* (-7.673)	71.07 ± 0.16* (-28.933)	70.63 ± 0.25* (-29.373)
25	74.95 ± 0.14* (-25.055)	75.96 ± 0.07* (-24.037)	88.26 ± 0.29* (-11.744)	86.84 ± 0.04* (-13.16)	56.77 ± 0.10* (-43.234)	62.21 ± 0.18* (-37.789)
50	61.59 ± 0.24* (-38.408)	67.53 ± 0.09* (-32.467)	78.23 ± 0.17* (-21.768)	76.43 ± 0.16* (-23.57)	45.09 ± 0.14* (-54.909)	52.60 ± 0.27* (-47.401)
100	47.51 ± 0.15* (-52.489)	56.37 ± 0.15* (-43.633)	64.58 ± 0.16* (-35.424)	60.18 ± 0.29* (-39.824)	32.30 ± 0.13* (-67.698)	35.99 ± 0.18* (-64.013)
200	27.39 ± 0.19* (-72.607)	44.40 ± 0.13* (-55.597)	49.33 ± 0.14* (-50.674)	44.46 ± 0.08* (-55.542)	12.58 ± 0.35* (-87.417)	14.30 ± 0.17* (-85.699)

#Values are mean ± S.E. of six individual observations. #Values in parentheses are per cent change over control. # Denotes per cent decrease over control. #*Values are significant at P<0.001.

was spent on the plates. By measuring the diameter of the zone of inhibition, antifungal activity was then calculated.

Statistical Analysis

The results of at least six tests were mathematically computed and provided as Mean ± SE. GraphPad 5 software was used to do a "Two-Way ANOVA" analysis on the differences between the groups. Values at the P<0.001 level were deemed significant.

Results

Antioxidant Activity of *A. marmelos* Fruit Pulp Extracts

Standard methods were used to test the antioxidant activity (DPPH) of extracts from the fruit pulp of *A. marmelos* in aqueous, chloroform, ethyl acetate, hexane, methanol and L-ascorbic acid. The percentages of DPPH inhibition for the fruit pulp of *A. marmelos* in aqueous, chloroform, ethyl acetate, hexane, and methanol, respectively, were -17.189, -11.125, -7.976, -7.673, and -28.933 in 12.5µg/mL. At 200 µg/mL, the inhibition increased with the dose to -72.607, -55.597, -50.764, -55.542, and -87.417 per cent. The DPPH inhibition was maximum in methanol fruit pulp extract and minimum in ethyl acetate extract. The concentration had a direct relationship with the percentage inhibition of DPPH. Similar to this, when the concentration was increased from 12.5 µg/mL to 200 µg/mL, respectively, the DPPH activity of standard L-Ascorbic acid showed a progressive suppression from -29.373 to -85.699 per cent (Table 1 and Figure 1).

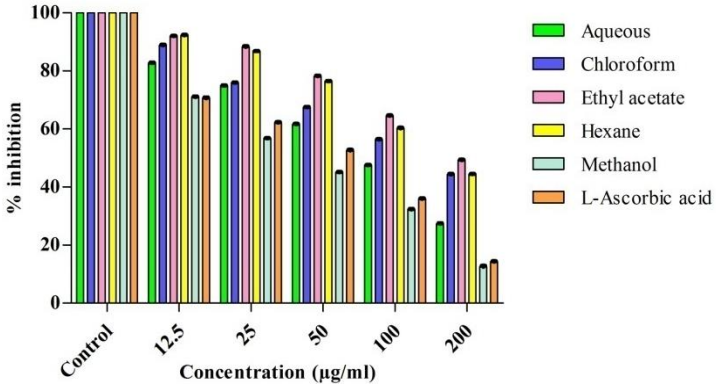

Figure 1. Bar diagram showing DPPH free radical scavenging activity of various *A. marmelos* fruit pulp extracts.

A. marmelos methanol fruit pulp extract had a lower DPPH percent inhibition than standard value (L-ascorbic acid). Two-way ANOVA analysis of the statistical significance revealed that the findings were significant at the P<0.001 level. Various *A. marmelos* fruit pulp extracts *viz.*, aqueous, chloroform, ethyl acetate, hexane and methanol were found to have IC_{50} values of 91.168 µg/mL, 153.22 µg/mL, 195.58 µg/mL, 164.741 µg/mL and 39.488 µg/mL, respectively, while for L-Ascorbic acid it was 57.823 µg/mL (Table 2). The results clearly reveal that *A. marmelos* methanol fruit pulp extract had

Table 2. The exact IC_{50} value of DPPH free radical scavenging activity of various *A. marmelos* fruit pulp extracts

S. No	Extract	Concentration (µg/mL)
1	Aqueous	91.168
2	Chloroform	153.22
3	Ethyl acetate	195.58
4	Hexane	164.741
5	Methanol	**39.488**
6	L - Ascorbic acid	57.823

Table 3. HRBC membrane stabilization of various *A. marmelos* fruit pulp extracts

Concentration (µg/mL)	HRBC Membrane Stabilization (%)				
	Aqueous	Chloroform	Ethyl acetate	Hexane	Methanol
Control (0)	100 ± 0 (0)	100 ± 0 (0)	100 ± 0 (0)	100 ± 0 (0)	100 ± 0 (0)
6.25 g	93.77 ± 0.66* (+6.23)	95.25 ± 0.21* (+4.75)	94.78 ± 0.37* (+5.22)	97.28 ± 0.20* (+2.72)	87.89 ± 0.31* (+12.11)
12.5	85.33 ± 0.46* (+14.67)	73.85 ± 2.42* (+26.15)	86.18 ± 0.21* (+13.82)	91.57 ± 0.25* (+8.43)	64.15 ± 0.20* (+35.85)
25	77.49 ± 0.50* (+22.51)	81.06 ± 0.16* (+18.94)	77.22 ± 0.17* (+22.78)	85.20 ± 0.31* (+14.79)	47.74 ± 0.21* (+52.26)
50	64.62 ± 0.53* (+35.38)	68.74 ± 0.32* (+31.26)	69.14 ± 0.31* (+30.86)	70.56 ± 0.14* (+29.44)	23.47 ± 0.24* (+76.53)
100	49.93 ± 0.32* (+50.07)	54.19 ± 0.11* (+45.80)	60.99 ± 0.24* (+39.01)	55.53 ± 0.22* (+44.47)	11.67 ± 0.07* (+88.34)

Values represented as mean ± S.E. of six individual observations. #Values in parentheses are per cent change over control. # + Denotes per cent increase of hemoproduction over control. # *Values are significant at P<0.001.

the best DPPH-reducing capacity when compared to other extracts.

Anti-inflammatory Activity of *A. marmelos* Fruit Pulp Extracts

Anti-inflammatory activity was evaluated using the HRBC membrane stabilization assay at various concentrations (0, 6.25, 12.5, 25, 50 and 100 µg/mL) of *A. marmelos* fruit pulp extracts. The concentration of extracts was directly linked to per cent hemoprotection. The values increased considerably from 6.23% to 50.07% for the aqueous extract, 4.75% to 45.80% for the chloroform extract, 5.22% to 39.01% for the ethyl acetate extract, 2.72% to 44.47% for the hexane extract, and 12.11% to 88.34% for the methanol extract, respectively. When compared to other extracts, the *A. marmelos* methanol fruit pulp extract showed greater membrane stability. All of the data, when put through a two-way ANOVA, showed that the values are significant at P<0.001 level. It is obvious from the results above that *A. marmelos* methanol fruit pulp extract had anti-inflammatory properties (Table 3 and Figure 2). Aqueous, chloroform, ethyl acetate, hexane, and methanol extracts' respective 50% hemoproduction (IC_{50}) concentration was 99.761 µg/mL, 114.443 µg/mL, 167.423 µg/mL, 118.397 µg/mL and 23.244 µg/mL (Table 4). *A. marmelos* methanol fruit pulp extract clearly showed that it had the strongest anti-inflammatory activity when compared to other four extracts.

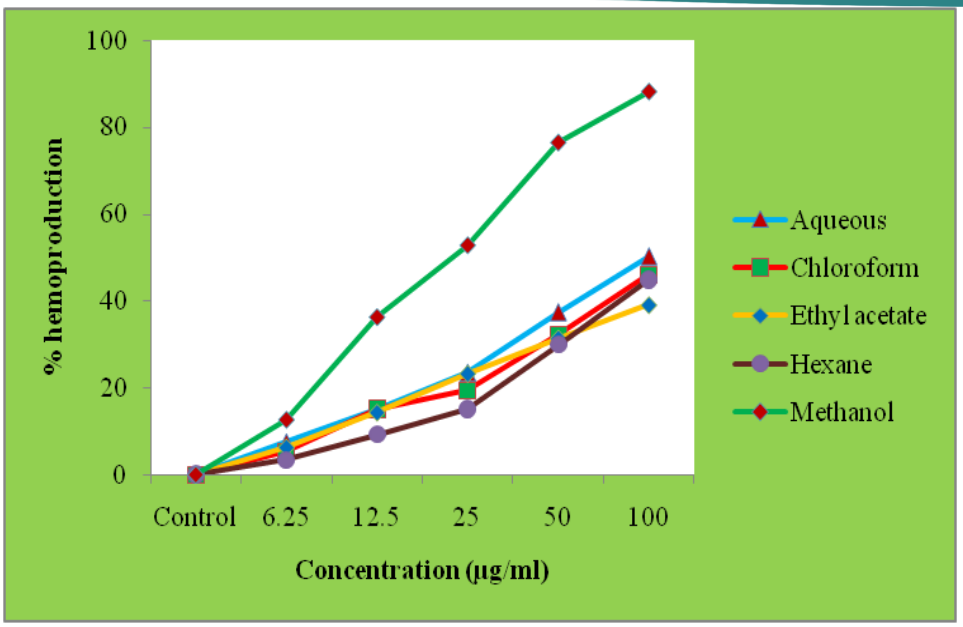

Figure 2. Line diagram showing HRBC membrane stabilization of various *A. marmelos* fruit pulp extracts.

Antibacterial Activity

The antibacterial activity of aqueous, chloroform, ethyl acetate, hexane and methanol fruit pulp extracts from *A. marmelos* were carried out against various bacterial species in the present investigation. Antibacterial activities of different concentrations (500 µg/mL, 750 µg/mL, and 1000 µg/mL) of these extracts were tested against *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Salmonella typhi*. Table 5 and figure 3 revealed that the aqueous fruit pulp extract of *A. marmelos* showed the maximum zone of inhibition in *E. coli* (13 mm), followed by *S. aureus* (10 mm), *B. cereus* (7 mm) and *S. typhi* (6 mm) at 1000 µg/mL concentration, and minimum zone of inhibition occurred in *S. typhi* (3 mm), followed by *B. cereus* (5 mm), *S. aureus* (6 mm) and *E. coli* (9 mm) at 500 µg/mL concentration. All the bacterial species tested were susceptible to Ampicillin (20 µL of Standard disc) and showed the zone of inhibition 14 mm in *S. aureus*, 14 mm in *E. coli*, 11 mm in *B. cereus*, and 10 mm in *S. typhi*.

Likewise, table 6 and figure 4 revealed that the chloroform fruit pulp extract of *A. marmelos* showed a maximum zone of inhibition in *S. aureus* (11 mm), followed by *E. coli* (10 mm), *B. cereus* (8 mm) and *S. typhi* (7 mm) at 1000 µg/mL concentration and minimum zone of inhibition in *S. typhi* (3 mm), followed by *S. aureus* (7 mm), *E. coli* (7 mm), and *B. cereus* (7 mm) at 500 µg/mL concentration. All the bacterial species tested are susceptible to Ampicillin (20 µL of Standard disc) and showed a zone of inhibition of 14 mm in *S. aureus*, 14 mm in *E. coli*, 11 mm in *B. cereus*, and 10 mm in *S. typhi*.

Table 7 and figure 5 revealed that the ethyl acetate fruit pulp extract of *A. marmelos* showed the maximum zone of inhibition occurs in *S. aureus* (11 mm) in 1000 µg/mL concentration, followed by *B. cereus* (9 mm) in 1000 µg/mL concentration, *E. coli* (8 mm) in 1000 µg/mL concentration, and *S. typhi* (7 mm) in 1000 µg/mL concentration, and minimum zone of inhibition occurs in *S. typhi* (2 mm) at 500 µg/mL concentration followed by *B. cereus* (6 mm) in 500 µg/mL concentration, *E. coli* (6 mm) in 500 µg/mL concentration, and *S. aureus* (7 mm) in 500 µg/mL concentration. All the bacterial species tested are susceptible to Ampicillin (20 µL of Standard disc) and showed a zone of inhibition of 14 mm in *S. aureus*, 14 mm in *E. coli*, 11 mm in *B. cereus* and 10 mm in *S. typhi*.

Table 8 and figure 6 revealed that the hexane fruit pulp extract of *A. marmelos* showed the maximum zone of inhibition occurs in *E. coli* (12 mm) in 1000 µg/mL concentration followed by *S. aureus* (10 mm) in 1000 µg/mL concentration, *S. typhi* (10 mm) in 1000 µg/mL concentration, and *B. cereus* (9 mm) in 1000 µg/mL concentration, and minimum zone of inhibition occurs in *S. typhi* (1 mm) at 500 µg/mL concentration followed by *S. aureus* (3 mm) in 500 µg/mL concentration, *B. cereus* (6 mm) in 500 µg/mL concentration, and *E. coli* (9 mm) in 500 µg/mL concentration. All the bacterial species tested are susceptible to Ampicillin (20 µL of Standard disc) showed the zone of inhibition 14 mm in *S. aureus*, 14 mm in *E. coli*, 11 mm in *B. cereus*, and 10 mm in *S. typhi*.

Table 9 and figure 7 depicted that the methanol fruit pulp extract of *A. marmelos* showed the maximum zone of inhibition occurs in *E. coli* (12 mm) in 1000 µg/mL concentration followed by *S. aureus* (12 mm) in 1000

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µg/mL concentration, *S. typhi* (8 mm) in 1000 µg/mL concentration, and *B. cereus* (7 mm) in 1000 µg/mL concentration, and minimum zone of inhibition occurs in *S. typhi* (5 mm) at 500 µg/mL concentration followed by *B. cereus* (5 mm) in 500 µg/mL concentration, *S. aureus* (9 mm) in 500 µg/mL concentration, and *E. coli* (9 mm) in 500 µg/mL concentration. All the bacterial species tested are susceptible to Ampicillin (20 µL of Standard disc) and showed a zone of inhibition of 14 mm in *S. aureus*, 14 mm in *E. coli*, 11 mm in *B. cereus* and 10 mm in *S. typhi*.

Table 4. The Exact IC_{50} value of 50 hemoprotection by HRBC membrane stabilization of various *A. marmelos* fruit pulp extracts

Sl. No.	Extracts	Concentration (µg/mL)
1.	Aqueous	99.761
2.	Chloroform	114.443
3.	Ethyl acetate	167.423
4.	Hexane	118.397
5.	Methanol	**23.244**

Table 5. Antibacterial activity [Zone of Inhibition (mm)] of aqueous fruit pulp extract of *A. marmelos*.

Organisms	Concentration (µg/mL)			
	500	750	1000	Standard 20 µL Ampicillin
Staphylococcus aureus	6	9	10	14
Bacillus cereus	5	6	7	11
Escherichia coli	9	10	13	14
Salmonella typhi	3	5	6	10

Table 6. Antibacterial activity [Zone of Inhibition (mm)] of chloroform fruit pulp extract of *A. marmelos*.

Organisms	Concentrations (µg/mL)			
	500	750	1000	Standard 20 µL Ampicillin
Staphylococcus aureus	7	10	11	14
Bacillus cereus	7	7	8	11
Escherichia coli	7	9	10	14
Salmonella typhi	3	6	7	10

Table 7. Antibacterial activity [Zone of Inhibition (mm)] of ethyl acetate fruit pulp extract of *A. marmelos*.

Organisms	Concentrations (µg/mL)			
	500	750	1000	Standard 20 µL Ampicillin
Staphylococcus aureus	7	10	11	14
Bacillus cereus	6	7	9	11
Escherichia coli	6	6	8	14
Salmonella typhi	2	4	7	10

Table 8. Antibacterial activity (Zone of Inhibition (mm)) of hexane fruit pulp extract of *A. marmelos*.

Organisms	Concentrations (µg/mL)			
	500	750	1000	Standard 20 µL Ampicillin
Staphylococcus aureus	3	6	10	14
Bacillus cereus	6	7	9	11
Escherichia coli	9	10	12	14
Salmonella typhi	1	2	10	10

Table 9. Antibacterial activity [Zone of Inhibition (mm)] of methanol fruit pulp extract of *A. marmelos*.

Organisms	Concentrations (µg/mL)			
	500	750	1000	Standard 20 µL Ampicillin
Staphylococcus aureus	9	10	12	14
Bacillus cereus	5	6	7	11
Escherichia coli	9	11	12	14
Salmonella typhi	5	6	8	10

Figure 3. Antibacterial activity of aqueous fruit pulp extract of *A. marmelos* [A - Standard, 1 - 1000 µg/mL, 2 - 750 µg/mL, 3 - 500 µg/mL].

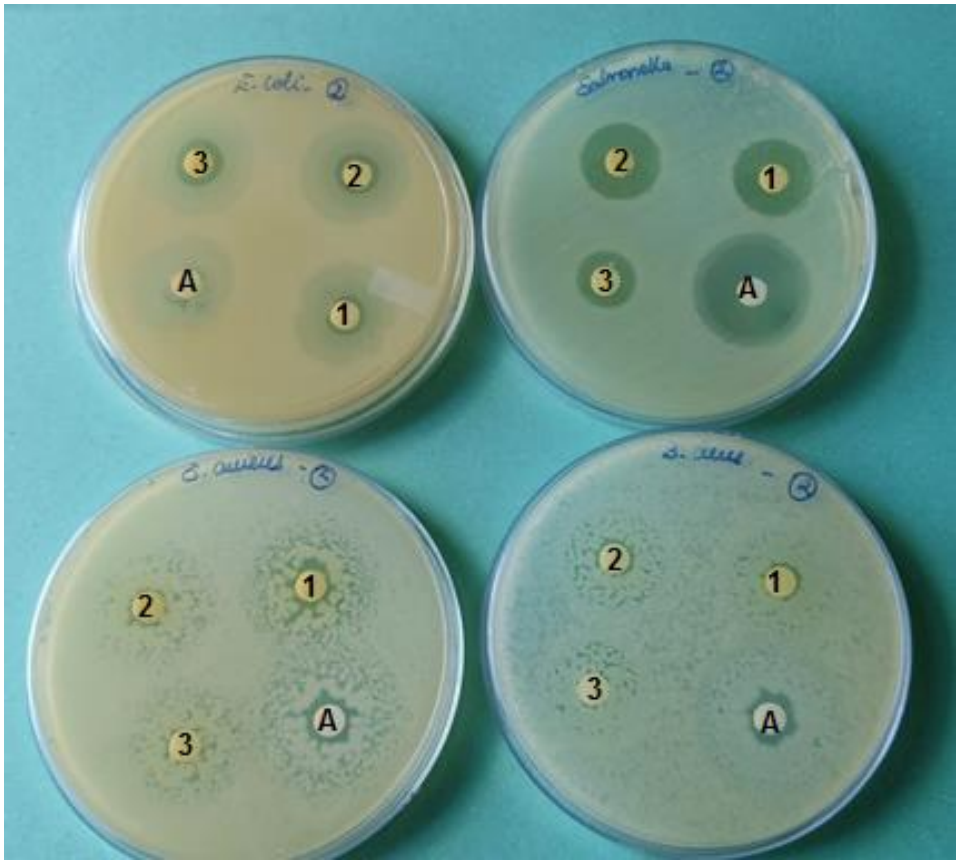

Figure 4. Antibacterial activity of chloroform fruit pulp extract of *A. marmelos* [A - Standard , 1 - 1000 µg/mL, 2 - 750 µg/mL, 3 - 500 µg/mL].

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Figure 5. Antibacterial activity of ethyl acetate fruit pulp extract of *A. marmelos* [A - Standard, 1 - 1000 µg/mL, 2 - 750 µg/mL, 3 - 500 µg/mL].

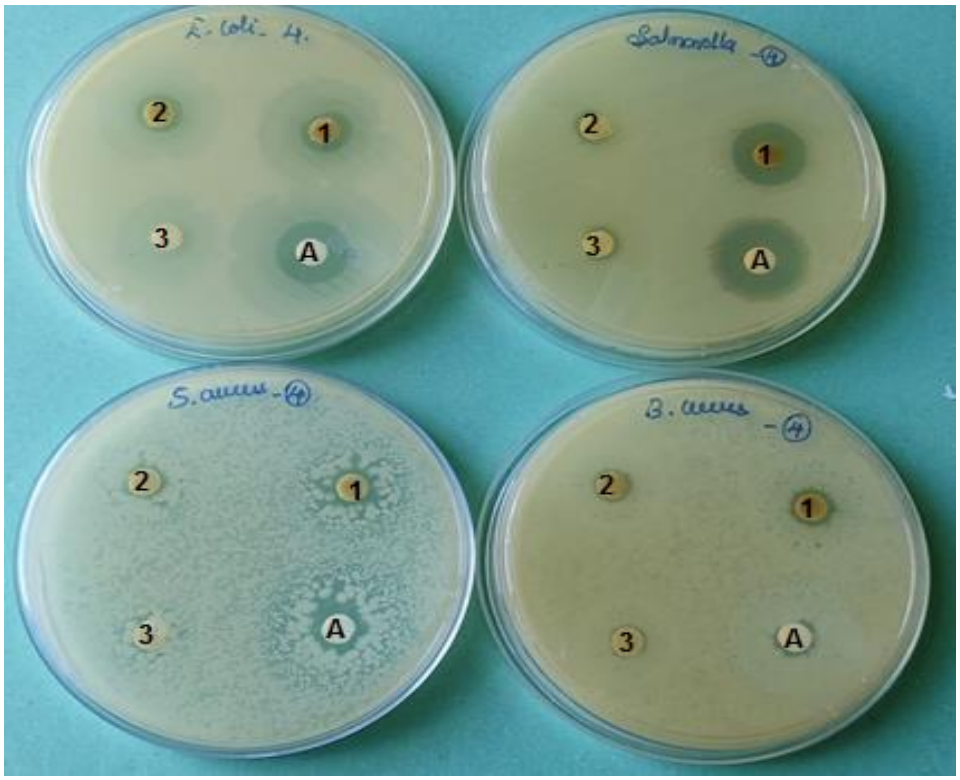

Figure 6. Antibacterial activity of hexane fruit pulp extract of *A. marmelos* [A – Standard, 1 - 1000 µg/mL, 2 - 750 µg/mL, 3 - 500 µg/mL].

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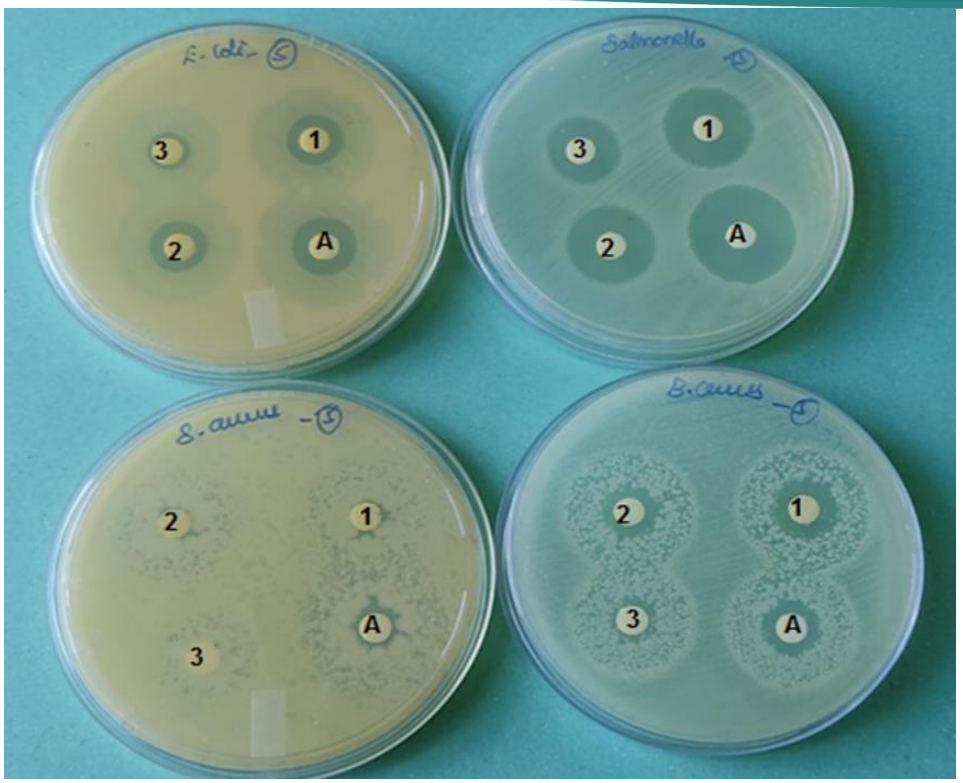

Figure 7. Antibacterial activity of methanol fruit pulp extract of *A. marmelos* [A - Standard , 1 - 1000 µg/mL, 2 - 750 µg/mL, 3 - 500 µg/mL].

Antifungal Activity

The antifungal activity of aqueous, chloroform, ethyl acetate, hexane and methanol fruit pulp extracts from *A. marmelos* were carried out against two fungi in the present investigation. Antifungal activities of different concentrations (500 µg/mL, 750 µg/mL and 1000 µg/mL) of all the extracts were tested against *Candida albicans* and *Aspergillus niger*. The results are given in an approximate table and figures in the text. Antifungal activity of all the five fruit pulp extracts of *A. marmelos* against *Candida albicans* showed the maximum zone of inhibition in methanol fruit pulp extract (15 mm), followed by chloroform and ethyl acetate fruit pulp extracts (14 mm), hexane fruit pulp extract (13 mm), and aqueous fruit pulp extract (10 mm) in 1000 µg/mL concentration, and minimum zone of inhibition showed in ethyl acetate fruit pulp extract (9 mm) followed by aqueous (10 mm), chloroform and ethyl acetate fruit pulp extracts (11 mm), and methanol fruit pulp extract (12 mm) in 500 µg/mL concentration. *C. albicans* tested against Amphotericin-B (20 µL of Standard disc) showed a zone of inhibition of 11 mm in all the five extracts of *A. mamelos* fruit pulp. Likewise, all the five fruit pulp extracts of *A. marmelos* against *Aspergillus niger* showed the maximum zone of inhibition in methanol fruit pulp extract (15 mm), followed by hexane fruit pulp extract (14 mm), chloroform fruit pulp extract (13 mm), ethyl acetate fruit pulp extract (10 mm) and Aqueous (10 mm) in 1000 µg/mL concentration and minimum zone of inhibition showed in ethyl acetate fruit pulp extract (9 mm) followed by aqueous (10 mm), chloroform and ethyl acetate fruit pulp extracts (11 mm), and methanol fruit pulp extract (12 mm) in 500 µg/mL concentration. *A. niger* tested against Amphotericin-B (20 µL of Standard disc) showed the zone of inhibition 11 mm in all the five extracts of *A. mamelos* fruit pulp (Table 10 and figure 8-12).

Table 10. Antifungal activity (Zone of Inhibition (mm)) of all the five fruit pulp extracts of *A. marmelos*.

Sample Name	*Candida albicans*				*Aspergillus niger*			
	Concentration (µg/mL)			Standard 20 µL Amphotericin-B	Concentration (µg/mL)			Standard 20 µL Amphotericin-B
	500	750	1000		500	750	1000	
Aqueous	10	11	12	11	8	8	10	11
Chloroform	11	13	14	11	9	12	13	11
Ethyl acetate	9	10	14	11	10	11	12	11
Hexane	11	13	13	11	13	13	14	11
Methanol	12	14	15	11	09	13	15	11

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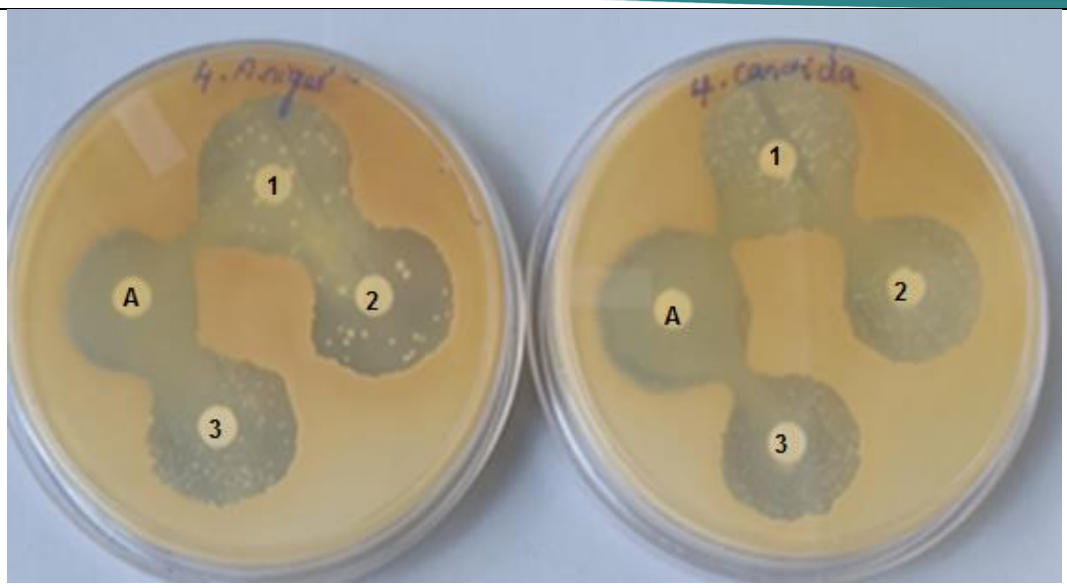

Figure 8. Antifungal activity of aqueous fruit pulp extract of *A. marmelos*.

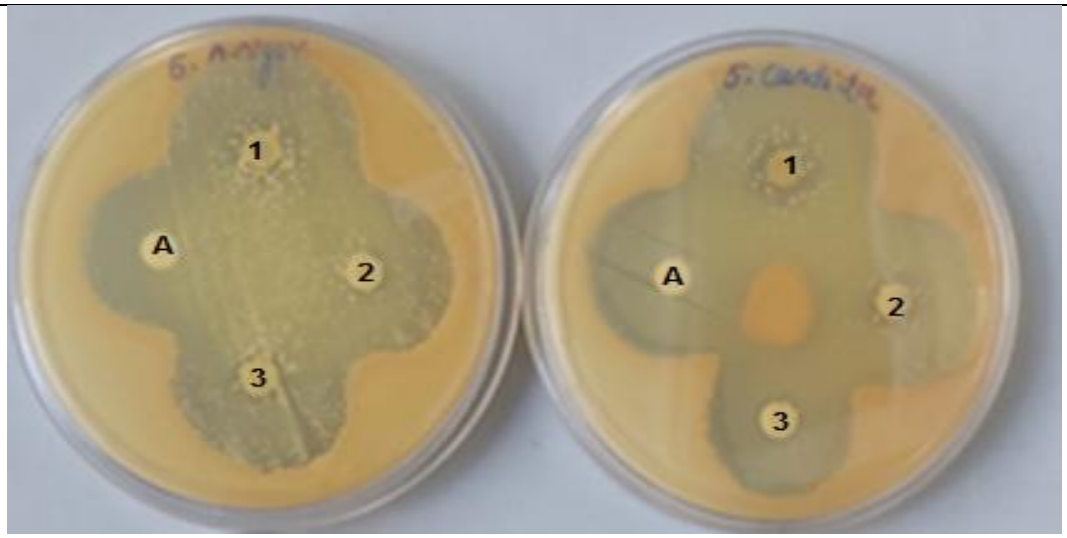

Figure 9. Antifungal activity of chloroform fruit pulp extract of *A. marmelos* [A - Standard, 1 - 1000 µg/mL, 2 - 750 µg/mL, 3 - 500 µg/mL].

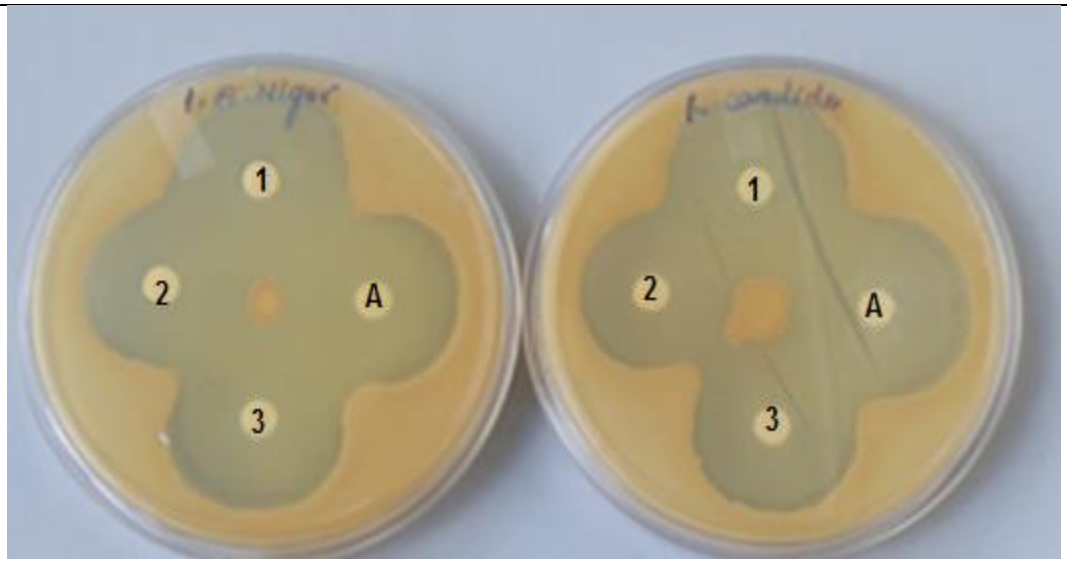

Figure 10. Antifungal activity of ethyl acetate fruit pulp extract of *A. marmelos* [A - Standard, 1 - 1000 µg/mL, 2 - 750 µg/mL, 3 - 500 µg/mL].

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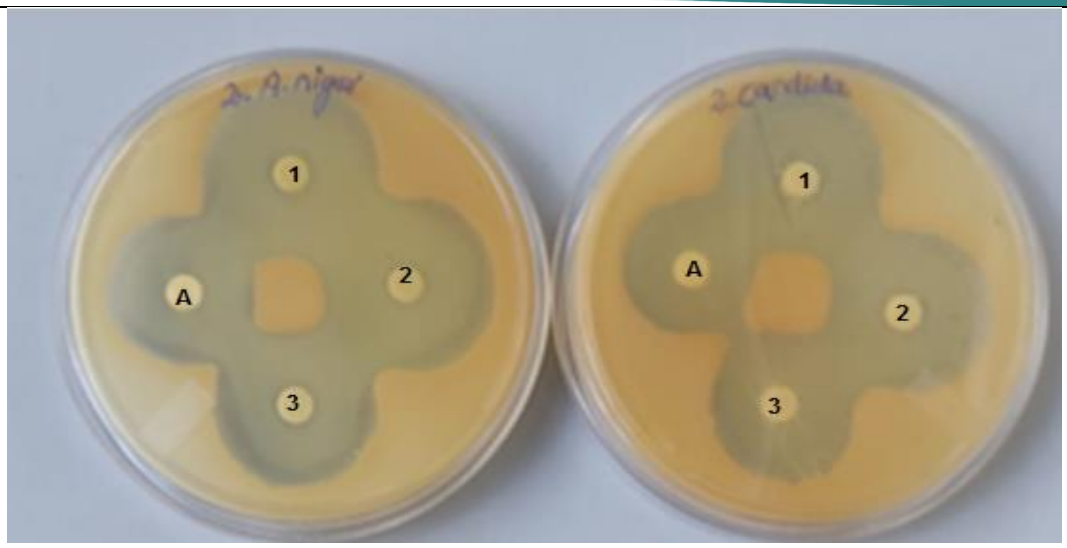

Figure 11. Antifungal activity of hexane fruit pulp extract of *A. marmelos* [A – Standard, 1 - 1000 µg/mL, 2 - 750 µg/mL, 3 - 500 µg/mL].

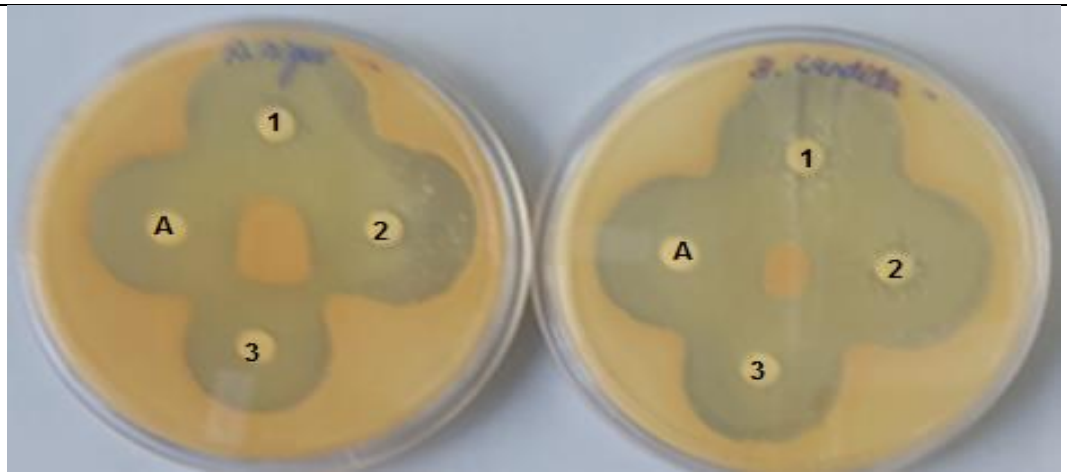

Figure 12. Antifungal activity of methanol fruit pulp extract of *A. marmelos* [A – Standard, 1 - 1000 µg/mL, 2 - 750 µg/mL, 3 - 500 µg/mL].

Discussion

The traditional Indian medical system is aware of the positive therapeutic effects of plants. In India, ethnomedicinal plants have been extensively studied. It has been shown that because plant phytochemical extracts may include antiviral, anticancer and antibacterial properties, they are utilized in allopathic therapy (Nair et al., 2005; Rajesh et al., 2020). Researchers from all around the world have examined the impact of fruit extracts on various biological activity (Padmavathy et al., 2021; Abdul Rahman et al., 2023). In this work, the extracts of *A. marmelos* fruit pulp were obtained by using various ranges of polarity solvents such as aqueous, chloroform, ethyl acetate, hexane and methanol. Extraction yields ranged from 24% for methanol extract to 2% for hexane extract. It could also be seen that the extraction yield of aqueous (20%) is higher than chloroform (4%) and ethyl acetate (3%). The average yields of extraction by various solvents decreased in the following order: methanol > aqueous > chloroform > ethyl acetate > hexane. These results show that the extraction yield increases with increasing polarity of the solvent used in the extraction process of *A. marmelos* fruit pulp. A similar study by Rafi et al. (2020) reported that extraction yields ranged from 18.24% for ethanol to 5.73% for *n*-hexane. It could also be noted in the works of Rafi et al. (2020) that the extraction yield of water (10.94%) was higher than ethyl acetate (9.08%) extracts of *Guazuma ulmifolia* leaves.

Antioxidant findings of the present study, the inhibitory concentration (IC_{50}) of aqueous, chloroform, ethyl acetate, hexane, methanol extracts and L-ascorbic acid was found to be 91.168 µg/mL, 153.22 µg/mL, 195.58 µg/mL, 164.741 µg/mL, 39.488 µg/mL and 57.823 µg/mL, respectively. The results clearly reveal that *A. marmelos* methanol fruit pulp extract has the best DPPH-reducing capacity when compared to stable free radicals. *A. marmelos* extracts' phytoconstituents, which can be used to combat oxidative and inflammatory

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stressors, are closely related to the extracts' antioxidant activities. Similarly, Jayamena et al. (2018) demonstrated the antioxidant activity of the rutin compound, and Rajesh et al. (2020) reported the antioxidant activity of *Cardiospermum halicacabum* extracts. Likewise, the percentage inhibition shown in fruits (apple and mango) was 67.27% and 73.16%, respectively, whereas, for vegetables (bottle gourd and ridge gourd), it was 54.23% and 75.95%, respectively, as reported by Sadef et al. (2022). Our results clearly agree with those results.

Numerous in vitro techniques have been developed to evaluate the effectiveness of isolated chemicals or plant extracts that are natural antioxidants. Two main categories of *in vitro* techniques exist: 1) Reactions involving the transfer of hydrogen atoms, such as the Oxygen Radical Absorbance Capacity (ORAC), Total Radical Trapping Antioxidant Potential (TRAP), and carotene bleaching and 2) Reactions involving the transfer of electrons, such as the trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH) (Huang et al., 2005). These techniques are well-liked because of how quickly and precisely they work. However, because phytochemicals are complex, it is crucial to apply many methods to assess the antioxidant capability of plant materials (Salazar et al., 2008).

Anti-inflammatory drugs have the capacity to significantly stabilize human red blood cell membranes against hypotonicity-induced lysis (Kumari et al., 2015). *A. marmelos* methanol fruit pulp extract demonstrated noteworthy potential in the current investigation for stabilizing human red blood cell membrane by preventing heat-induced hemolysis, which is consistent with its phytochemical and antioxidant potential. The conclusions are consistent with the earlier works of Naz et al. (2017). In the present study, the 50 per cent hemoprotection (IC_{50}) of aqueous, chloroform, ethyl acetate, hexane and methanol fruit pulp extracts of *A. marmelos* was 99.761 µg/mL, 114.443 µg/mL, 167.423 µg/mL, 118.397 µg/mL, and 23.244 µg/mL, respectively. The results obviously depict that A. marmelos methanol fruit pulp extract possesses the highest anti-inflammatory potential compared with that of its other counterparts. Smilarly, *Bacopa monnieri* extracts reduce hemolysis from 100% to 94.80% at 50 µg/ml concentrations. On increasing the concentrations continuously, the hemolysis decreases in the same proportion. It decreases to 90.90% at 100µg/ml concentrations. It decreases hemolysis to 90.90% at 100 µg/ml, 77.92% at 250 µg/ml, 72.72% at 500 µg/ml and 64.93% at 1000 µg/ml. It reduces hemolysis up to 41.5±0.56 % of hemolysis at maximum concentrations, i.e., 2000 µg/ml as demonstrated by Abhishek Kumar (2024).

Hemolysis would decrease with the same ratio as plant extract concentrations. This indicates a rise in hemoproduction. According to earlier research, fruit extracts include secondary metabolites. It's also likely that the anti-inflammatory reaction is caused by the flavonoids found in fruits. Previous research has demonstrated that flavonoids can lower the levels of several pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, IL-8 and monocyte chemoattractant protein-1, in a variety of cell types, including Jukart T-cells, Raw macrophages, and peripheral blood mononuclear cells (Santangelo et al., 2007). Likewise, using the same technique, the anti-inflammatory properties of *Ocimum basilicum* and *Centella asiatica* were observed (Chippada et al., 2011; Abhishek Kumar et al., 2023).

Plants are said to have the ability to reduce inflammation due to the presence of phytochemicals like anthocyanins, stilbenes, phenolic acids, tannins, flavonoids, alkaloids, and steroids (Huang et al., 2009). *In vitro* and in *vivo* investigations have shown that secondary plant metabolites like flavonoids and saponins contribute to the stability of the lysosomal membrane. On the other hand, saponins and tannins have also been found to stabilize biological macromolecules like erythrocyte membranes due to their exceptional capacity to bind cations (Sadique et al., 1989). One of the most crucial enzymes that anti-inflammatory medications target is cycloxygenase. A medication has a significant pharmacological potential to treat inflammatory illnesses with fewer adverse effects when it acts specifically on COX-2, as is known. The research suggests that some plant extracts or their isolated compounds may affect this enzyme and may one day lead to the discovery of novel bioactive substances (Ribeiro et al., 2018). According to the findings of the present study, extracts from the fruit pulp of *A. marmelos* are potentially anti-inflammatory and antioxidant, and methanol fruit pulp exhibits the highest potential compared to other fruit pulp extracts. As a result, methanol extract from the fruit pulp of *A. marmelos* can be rationally considered as a viable medicine to treat oxidative stress-related illnesses and inflammations.

In the present study, methanol fruit pulp extract of *A. marmelos* showed the maximum zone of inhibition occurs in *E. coli* (12 mm) in 1000 µg/mL concentration followed by *S. aureus* (12 mm) in 1000 µg/mL concentration, *S. typhi* (8 mm) in 1000 µg/mL concentration, and *B. cereus* (7 mm) in 1000 µg/mL

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concentration, and minimum zone of inhibition occurs in *S. typhi* (5 mm) at 500 µg/mL concentration followed by *B. cereus* (5 mm) in 500 µg/mL concentration, *S. aureus* (9 mm) in 500 µg/mL concentration and *E. coli* (9 mm) in 500 µg/mL concentration. All the bacterial species tested are susceptible to Ampicillin (20 µL of Standard disc) and showed a zone of inhibition of 14 mm in S. aureus, 14 mm in E. coli, 11 mm in B. cereus, and 10 mm in *S. typhi* compared to other solvents extracts. Antifungal activity of the present study showed all five fruit pulp extracts of *A. marmelos* against *Candida albicans* showed the maximum zone of inhibition in methanol fruit pulp extract (15 mm) at 1000 µg/mL compared with other extracts. Likewise, all the five fruit pulp extracts of *A. marmelos* against *Aspergillus niger* showed the maximum zone of inhibition also in methanol fruit pulp extract (15 mm) at 1000 µg/mL, and both *C. albicans* and *A. niger* tested against Amphotericin-B (20 µL of Standard disc) showed the zone of inhibition 11 mm in all the five extracts of *A. mamelos* fruit pulp.

According to the current findings, the most prevalent pathogens were used to screen the antibacterial activity of all five *A. marmelos* fruit pulp extracts. Fruit pulp extracts from the five different fruit extracts generally seemed to be the best source of an active antibacterial agent. *A. marmelos* fruit pulp extract, on the other hand, showed broad-spectrum antibacterial action against *B. cereus*, *E. coli*, *S. aureus*, *S. typhi*, *C. albicans*, and *A. niger*. As a result, the antibacterial activity is connected to the target bacterium, the extracting solvent, and the plant portion employed for extraction. This result is consistent with Dogruoz et al. (2008) findings that the amount of bacterial inhibition depends on the plant extract, the extraction solvent, and the organism tested. Fruit peel extracts of *Actinidia deliciosa* showed activity against the bacterial (*B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa*) and fungal (*A. fluves*, *S. cerevisiae*, *and C. albicans*) strains (Salama et al., 2018). The nature of biologically active components (alkaloids, flavonoids, essential oils, terpenoids, tannins, etc.,), which may be enhanced in the presence of ethanol, and the stronger extraction capacity of ethanol, which may have yielded a greater number of active constituents responsible for antimicrobial activity (Ghosh et al., 2008). However, the findings of this investigation showed that high levels of antibacterial activities are found in the methanol fruit extract of *A. marmelos*. The findings of this study thus demonstrated the significance of the fruit pulp extracts of *A. marmelos* as an antimicrobial agent and showed that they contributed significantly to the development of antimicrobial properties.

Conclusion

Aegle marmelos in the traditional medicinal system shows good responses as a nootropic drug. Here the result showed *A. marmelos* fruit pulp extracts have significant potential effect for the treatment of antioxidant, anti-inflammation and infectious diseases brought on by pathogenic bacteria like *B. cereus*, *E. coli*, *S. aureus*, *S. typhi*, *C. albicans* and *A. niger*. Based on antioxidant, anti-inflammatory and antimicrobial studies, methanol extract of *A. marmelos* fruit pulp can also able to reduce oxidative stress in *in vitro* conditions. The outcome of this study recommends that methanol extract from A. marmelos fruit pulp should be used as an in vivo model to establish their efficacy. If the methanol extract of *A. marmelos* fruit pulp shows a good response, then clinical trials on a human being can be performed to check the potential of this drug in the human body system.

Conflict of interest

The authors declare that there is no conflict of interest.

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