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Evaluation of *In vitro* Anti-Inflammatory and Antibacterial Properties of Tuberous Roots of *Mirabilis jalapa* L. Found in Sri Lanka

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aims to evaluate the *in vitro* anti-inflammatory and antibacterial potential of aqueous and various organic solvent (methanol, dichloromethane, and hexane) extracts of *Mirabilis jalapa* L. tuberous roots.

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Methods: Heat-induced egg albumin denaturation inhibition assay and hypotonicity-induced human red blood cell (HRBC) membrane stabilization methods were used to evaluate the anti-inflammatory activity *in vitro*. Both disc diffusion and well diffusion methods were utilized to evaluate the antibacterial potential *in vitro*, using three common wound pathogens. Namely, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 25923).

Results: In the heat-induced egg albumin denaturation inhibition assay, the methanolic extract of *M. jalapa* exhibited the highest potency (IC_{50} = 137.9 µg/ml) of anti-inflammatory activity while in the HRBC membrane stabilization method, the aqueous extract of *M. jalapa* exhibited the highest potency (IC_{50} = 197.4 µg/ml) of anti-inflammatory activity. There was no significant antibacterial activity shown by all four extracts. However, some inhibitory zones were observed in the well diffusion method against *S. aureus*. The highest inhibitory zone of 15.33 ± 0.33 mm was expressed by the dichloromethane extract, with a concentration of 400 mg/mL against *S. aureus*, followed by the hexane extract with an inhibitory zone of 14.00 ± 2.08 mm at the same concentration. The dichloromethane extract showed an inhibitory zone of 11.00 ± 0.58 mm at a 200 mg/mL concentration.

Conclusion: This study demonstrates that the tuberous roots of *M. jalapa* have significant anti-inflammatory properties but no significant antibacterial properties against the selected pathogens.

Keywords: Antibacterial; anti-inflammatory; Four O' clock; Mirabilis jalapa L; Sri Lanka.

1. INTRODUCTION

Inflammation is the mechanism to defend living tissues from damage to the body during infections or injury. Prolonged inflammation can cause many illnesses including heart attacks, inflammatory bowel disease, rheumatoid arthritis, chronic asthma, septic shocks, and many more [1]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are known to be the most frequently used medications to treat inflammatory conditions and inflammation-related diseases around the world. Despite their popularity, several side effects are also associated with them. Peptic ulceration, increased sodium and water retention, mental confusion, raised transaminases, platelet dysfunction, and many more, are the major drawbacks related to the prolonged use of these drugs [2, 3, 4, 5]. Plants have been known for centuries to have anti-inflammatory properties. In most cases, certain compounds derived from various plans have been shown to have a beneficial therapeutic effect. These compounds are found to be well-known for their value in the treatment of both acute and chronic inflammation [2].

Antimicrobial resistance is the ability of infection-causing microorganisms, such as bacteria, to survive drug exposure that would normally kill or suppress them [6, 7]. Microbial contagious diseases and their complications are becoming more common worldwide, due to the development of microbial resistance against routinely used antimicrobial drugs. Since the late 20^{th} century, resistance to antibiotics has become humanity's most challenging health problem. Therefore, the demand for novel and effective antimicrobial drugs is increasing significantly and continuously [8, 9]. Plant-derived antimicrobial drugs are considered to be safer when compared to synthetic substances [10]. It is assumed that these compounds have different target sites compared to conventional antimicrobials while having different mechanisms of action against microbes [11].

Mirabilis jalapa L. (Nyctaginaceae), also called Marvel of Peru, and identified as "Hendirikka" in Sri Lanka, is a well-known ornamental plant that grows all around the world. Despite its beauty, it has been and is still being used widely as a therapeutic herb in folklore remedies and traditional medicinal systems worldwide in the treatment of many diseases and disorders. This plant is widely used as a treatment for boils, intestinal pain, inflammation, urogenital complications, muscle pains, aphrodisiac, and many other illnesses by people from many countries around the world [12]. The plant is rich in many important phytochemicals such as alkaloids, carbohydrates, flavonoids, brassica sterols, glycosides, phytosterols, oleanolic acid, ursolic acid, and trigonelline. Literature has reviewed that *Mirabilis jalapa* L. has anti-inflammatory, antimicrobial, antioxidant, antidiabetic, antitoxin, antinociceptive, and cytotoxic properties, and many more [13, 14]. Most of these properties are not scientifically proven, and limited investigations have been

carried out in Sri Lanka on the aforementioned properties of *Mirabilis jalapa* L. Therefore, we initiated the first step of *in vitro* experiments to investigate the anti-inflammatory and antibacterial activity of aqueous and organic solvent (methanol, dichloromethane, and hexane) extracts of tuberous roots of *Mirabilis jalapa* L.

2. METHODOLOGY

2.1 Collection of the Plant Material, Identification, and Authentication

Tuberous roots were collected from well-grown *Mirabilis jalapa* L. plants. About 1 Kg of tuberous roots was obtained during the daytime in October 2022, from a land in Puttalam District, in North Western Province, Sri Lanka (Latitude of 7°34′32″ N, Longitude of 79°47′43″ E and Elevation above sea level: 8 m = 26 ft). Samples from the plants which were collected were identified and authenticated by a scientific officer at Bandaranaike Memorial Ayurveda Research Institute, Nawinna, Maharagama, Sri Lanka [15].

2.2 Preparation of Aqueous, Methanol, Dichloromethane, and Hexane Extracts of *Mirabilis jalapa* L. Tuberous roots

The cold maceration method was used to prepare the extracts. The tuberous roots obtained were cleaned, washed, and air-dried (25℃) in a dark environment until a constant weight was obtained. The dried tuberous roots were cut into small pieces and a coarse powder was obtained by grinding the root samples using a laboratory blender. Then 50 g of the coarse powder was weighed and mixed in bottles (amber color) containing 500 ml of the respective solvents (aqueous, methanol, dichloromethane, and hexane) with different polarities (water- 1.000, methanol- 0.762, dichloromethane- 0.309, n-hexane- 0.009). Then the bottles were stored at room temperature (25℃) for 3- 7 days with frequent mixing. Following 3- 7 days, mixtures were filtered using muslin clothes separately, into clean containers. Then the filtrates were concentrated by evaporation using a rotary vacuum evaporator [15].

2.3 Evaluation of *in vitro* Anti-Inflammatory Activity

The anti-inflammatory activity was evaluated by two *in-vitro* methods,

1. Heat-induced egg albumin denaturation assay
2. Hypotonicity-induced HRBC membrane stabilization method

2.3.1 Preparation of concentration series of plant extracts and reference drug (Diclofenac Sodium)

In both *in vitro* assays plant extracts and reference drug (Diclofenac Sodium) were diluted to obtain a concentration series of 15.625, 31.25, 62.5, 125, 250, 500, 1000, and 2000 µg/ml. To dissolve the plant extract Dimethyl sulfoxide (DMSO) was utilized. Serial dilutions were prepared by diluting in distilled water. A freshly prepared PBS (Phosphate Buffered Saline) was used in both *in vitro* assays.

2.3.2 Heat-induced egg albumin denaturation inhibition assay

2.3.2.1 Principle of the egg albumin denaturation inhibition assay

Inflammation is a complex process that typically involves pain and includes events such as increased vascular permeability, increased protein denaturation, and membrane modification. Protein denaturation is the process by which proteins lose their tertiary and secondary structures due to external stress or molecules such as strong acid or base, a concentrated inorganic salt, an organic solvent, or heat. When biological proteins are denatured, they lose their biological function. Protein denaturation is a well-known cause of inflammation. As a result, the capacity of a drug or a plant product to prevent protein denaturation may also aid in the prevention of inflammatory conditions. Egg albumin denaturation inhibition assay is a type of protein denaturation inhibition assay. Here, the protein required for the assay is obtained by egg white separated from an egg. When the mixture is heated, heat denatures the proteins (egg albumin) and increases the turbidity which results in the increment of absorbance. Drug or plant products that have anti-inflammatory activity will inhibit the protein denaturation and decrease the turbidity which results in a decrease in absorbance [16].

2.3.2.2 Egg albumin denaturation inhibition assay method

As the test mixture, 2.8 mL of phosphate-buffered saline (PBS) with pH adjusted to 6.4, 0.2 mL of egg albumin (egg white separated from

fresh hen's egg), and 2 ml of root extracts were gently mixed to obtain a final volume of 5 mL. A concentration series of 15.625, 31.25, 62.5, 125, 250, 500, 1000, and 2000 µg/ml was prepared from root extracts.

As the standard mixture, 2.8 mL of PBS (pH 6.4), 0.2 mL of egg albumin (egg white separated from fresh hen's egg), and 2 mL of reference drug solution (Diclofenac Sodium) were gently mixed to obtain a final volume of 5 mL. A concentration series of 15.625, 31.25, 62.5, 125, 250, 500, 1000, and 2000 µg/ml was prepared from Diclofenac Sodium crystalline powder.

As the positive control, 2.8 mL of PBS (pH 6.4), 0.2 mL of egg albumin (egg white separated from a fresh hen's egg), and 2 mL of distilled water were gently mixed to obtain a final volume of 5 mL.

As the negative control 5 mL of distilled water was used.

All the mixtures were then incubated at a temperature of 37 ± 2 ℃ for 15 minutes. Then the temperature was gradually heated up to 70 ℃. Once reached the temperature the mixtures were kept for another 5 minutes at 70 ℃. Then all the mixtures were allowed to cool down for 15 – 20 minutes. After cooling the absorbance of each reaction mixture was measured at a wavelength of 660 nm by a spectrophotometer. The test was carried out in triplicates.

The protein denaturation inhibition percentage of each reaction mixture was calculated using the following formula:

$$\% \text{ Inhibition of egg albumin denaturation} = \frac{(Vc - Vt)}{Vc} \times 100$$

(V_c = absorbance of the positive control, V_t = absorbance of the test) [16, 17].

2.3.3 HRBC membrane stabilization methods

2.3.3.1 Principle of the HRBC membrane stabilization assay

During inflammation, lysosomal membrane lysis might occur, releasing enzymes that cause a range of illnesses. Nonsteroidal anti-inflammatory medications (NSAIDs) and plant products show potential anti-inflammatory work by either inhibiting the release of lysosomal enzymes or by stabilizing lysosomal membranes. When red blood cells are exposed to harmful substances like hypotonic medium, heat, methyl salicylate, and phenyl hydrazine lysis of the red cell membrane may occur. Human red blood cell membranes are thought to be similar to lysosomal membranes, inhibition of hypotonicity-induced lysis of red blood cell membranes will be used to assess the anti-inflammatory effect of various substances [16].

2.3.3.2 Assay method

As the test mixture, 2 mL of hypotonic saline, 1 mL of PBS (pH 7.4), 0.5 mL of test extract at various concentrations, and 0.5 mL of 10% v/v HRBC were used. A concentration series of 15.625, 31.25, 62.5, 125, 250, 500, 1000, and 2000 µg/ml was prepared from root extracts.

As the standard solution, 2 mL hypotonic saline, 1 mL PBS (pH 7.4), 0.5 mL standard drug solution (Diclofenac Sodium) of varying concentrations, and 0.5 mL 10% v/v HRBC were used. A concentration series of 15.625, 31.25, 62.5, 125, 250, 500, 1000, and 2000 µg/ml was prepared from the standard drug.

As the control, 2 mL of distilled water, 1 mL of PBS (pH 7.4), and 0.5 mL of 10% v/v HRBC suspension were mixed.

Distilled water was used as the negative control.

All reaction mixtures were incubated at a temperature of 37 °C for 30 minutes. Then they were centrifuged at 3000 rpm for 5 minutes. After centrifugation, the supernatant was separated to measure the hemoglobin content at a wavelength of 560 nm. The test was carried out in triplicates.

The protection against hemolysis of the HRBC membrane was calculated as a percentage using the formula given below.

$$\text{Percentage protection} = 100 - \left(\frac{\text{optical density of the sample}}{\text{optical density of the control}}\right) \times 100$$ [18, 19].

2.4 Evaluation of *in vitro* Antibacterial Activity

2.4.1 Test microorganisms used

American Type Culture Collection (ATCC) strains of bacteria that are more prevalent in causing wound infections were obtained from Medical Research Institute, Colombo 08, Sri Lanka.

Pseudomonas aeruginosa (ATCC 27853), *Escherichia coli* (ATCC 25922), and *Staphylococcus aureus* (ATCC 25923) were used in the study as the test strains. The bacterial strains were transported to the laboratory in nutrient agar slants. They were subcultured and stored at 4℃ for further experiments.

2.4.2 Evaluation of antibacterial activity by agar disc diffusion method

This procedure was carried out according to the guidelines of the Clinical and Laboratory Standard Institute [20]. A standard cell suspension was prepared by dissolving a few isolated bacterial colonies of the organism in sterile saline and the turbidity of the cell suspension was adjusted according to the 0.5 McFarland turbidity standard. Sterile cotton swabs were used to prepare lawn cultures of test organisms on MHA. Then sterile filter paper discs (6mm diameter) obtained from Whatman No. 01 filter papers were placed on the agar surface. Five discs were placed on each agar plate for each organism for the disc diffusion method. Therefore, for each extract, three agar plates were used. The discs were labeled as 2 mg, 1 mg, 0.5 mg, 0.25 mg, and 0.125 mg, respectively. The concentration of the positive control (Gentamicin) was 0.125mg. As the negative control, distilled water was used.

E.g., To obtain a 2mg disc, 5µL from the 400mg/mL plant extract was loaded using a sterile micropipette. The plates were then incubated for 37℃ overnight. The test was carried out in triplicates. After incubation, the inhibition zones were observed [21, 22].

2.4.3 Evaluation of antibacterial activity using agar well diffusion method

A standard cell suspension was prepared by dissolving a few isolated bacterial colonies of the organism in sterile saline and the turbidity of the cell suspension was adjusted according to the 0.5 McFarland turbidity standard. Sterile cotton swabs were used to prepare lawn cultures of test organisms on MHA. Four wells were made on the inoculated MHA plates with the base of a sterile 1000 µL pipette tip. The bottom of the prepared wells was sealed with one drop of 1% molten MHA agar using a sterile pipette and allowed to set. For the well diffusion method, a serial dilution of the plant extract was made yielding concentrations of 25, 50, 100, 200, and 400 mg/mL. 2 plates were used for each organism, therefore for each extract 6 agar plates were required. The plates were labeled as follows (Fig. 1),

Fig. 1. Labelling of plates in well diffusion method (triplicated results)

Wells were then filled with plant extracts. Two negative controls were used, distilled water and the reagent itself. As the positive control, 25 mg/ml of Gentamicin was used. Plates were then incubated at 37℃ aerobically. Test performed in triplicates. After incubation, zones were measured [22, 23].

2.5 Statistical Analysis

All the investigations were done in triplicates and expressed as mean value ± SEM (Standard Error of the Mean). *P* value (P = 0.05), $R^{2,}$ and all the dose-response curves were obtained by Graph Pad Prism 9 (Version 9.5.1).

3. RESULTS AND DISCUSSION

Medicinal plants are used in the treatment of many diseases and disorders. Many current drugs involved in routine medications were originally extracted from plants, and now they are produced synthetically [12]. Herbal remedies are increasingly used to treat inflammation and pain due to their effectiveness, fewer side effects, availability, and low cost [24]. The *in vitro* anti-inflammatory activity was observed using two methods to comprehend the results of each method separately.

3.1 Anti-Inflammatory Properties of Tuberous Roots of *M. jalapa*

According to the above Fig. 2, all 4 extracts that have been used in this experiment, show a significant degree of anti-inflammatory activity. The curve of the methanol extract shows a higher potency in lower concentrations when compared with the curve of Diclofenac Sodium. However, when the concentration increases, the methanol extract's potency seems to be lower than the potency of Diclofenac Sodium. The aqueous extract shows an almost similar curve shape to the reference drug Diclofenac Sodium.

According to the Fig. 3 details, all 4 extracts that have been used in this experiment, show a significant degree of anti-inflammatory activity. Fig. 3 shows that the aqueous extract shows the highest anti-inflammatory potency while the hexane extract shows the lowest.

When comparing the results obtained by both *in vitro* assays, it is observed that they comprehend each other regarding the anti-inflammatory potency of *M. jalapa* tuberous roots. However, it is observed that in the egg albumin denaturation assay methanol extract showed the highest potency with an IC_{50} value of 137.9µg/mL while in the HRBC stabilization method aqueous extract showed the highest anti-inflammatory potency with an IC_{50} of 197.4 µg/mL. We assume that the above observation can be due to the difference in principles in the two assays. The discrepancies shown in the results for each extract can be caused by variations in disulfide, electrostatic, hydrogen, and hydrophobic bonding that occur as a result of denaturation mechanisms [25, 26, 27].

Table 1. A summary of dose-response curve details of aqueous and organic solvent (methanol, dichloromethane, and hexane) extracts of *M. jalapa* tuberous roots and reference drug in egg albumin denaturation inhibition assay

Tubular results	Reference drug (Diclofenac Sodium)	Aqueous extract	Methanol extract	Dichloromethane extract	Hexane extract
IC_{50} (µg/ml) (Half maximal inhibitory concentration)	150.8	146.4	137.9	243.7	306.7
R-squared	0.9918	0.9870	0.9724	0.9781	0.9349
P value	0.9312	<0.0001	0.0004	<0.0001	<0.0001

In egg albumin denaturation inhibition assay, Diclofenac Sodium exhibits an IC_{50} value of 150.8µg/ml with a high R-squared value (R^2 = 0.9918), conveying a strong positive relationship with the inhibitory percentage and log concentrations. The following information was gathered when comparing the IC_{50} values of tuberous root extracts. Extracts according to the decreasing potencies can be ordered as methanol (137.9 µg/ml) > aqueous (146.4 µg/ml) > dichloromethane (243.7 µg/ml) > hexane (306.7 µg/ml). In the comparison of all extracts, the methanolic root extract (137.9 µg/ml) of M. jalapa exhibited the highest potency. However, the aqueous extract has exhibited an almost similar potency (146.4 µg/ml) to the standard drug (Diclofenac Sodium) (150.8 µg/ml)

Table 2. A summary of dose-response curve details of aqueous, methanol, dichloromethane, and hexane extracts of *M. jalapa* tuberous roots and reference drug in HRBC membrane stabilization assay

Tubular results	Reference drug (Diclofenac Sodium)	Aqueous extract	Methanol extract	Dichloromethane extract	Hexane extract
IC_{50} (µg/ml)	258.7	197.4	294.9	208.9	237.0
R-squared	0.9893	0.9641	0.9079	0.8807	0.9037
P value	0.0005	<0.0001	<0.0001	<0.0001	0.9306

In the HRBC membrane stabilization method, Diclofenac Sodium exhibits an IC_{50} value of 258.7 µg/ml with a high R-squared value (R^2 = 0.9893), conveying a strong positive relationship with the inhibitory percentage lysis and log concentrations. The following information was gathered when comparing the IC_{50} values of tuberous root extracts. Extracts according to the decreasing potencies can be ordered as aqueous (197.4 µg/ml) > dichloromethane (208.9 µg/ml) > hexane (237.0 µg/ml) > methanol (294.9 µg/ml). In the comparison of all extracts, the aqueous extract (197.4 µg/ml) of M. jalapa exhibited the highest potency and higher inhibition of HRBC membrane lysis than the standard drug, Diclofenac Sodium (258.7 µg/ml)

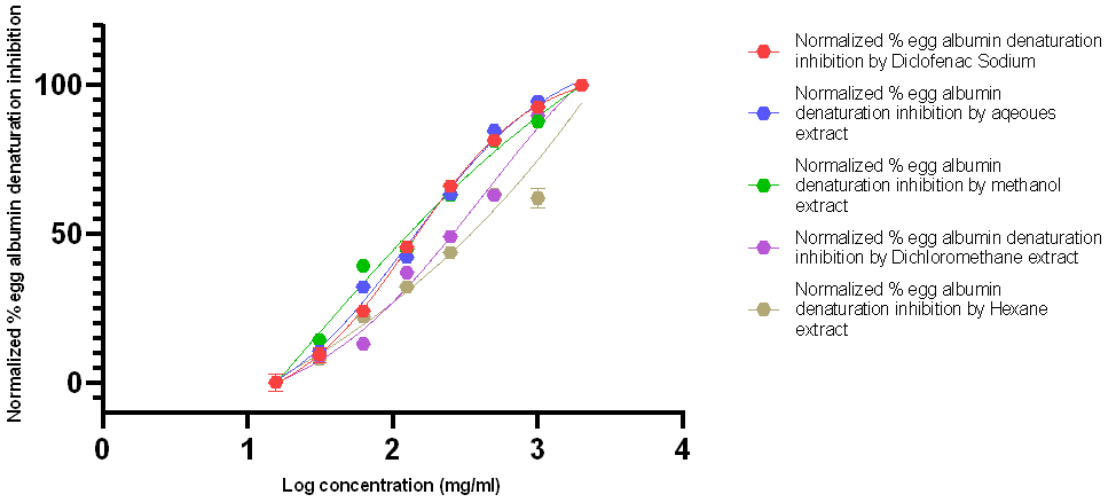

Fig. 2. Dose-response curves of aqueous and organic solvent (methanol, dichloromethane, and hexane) extracts of *M. jalapa* tuberous roots and the reference drug in the egg albumin denaturation inhibition assay

3.2 Antibacterial Properties of Tuberous Roots of *M. jalapa*

There was no significant antibacterial activity shown by any extract of *M. jalapa* against the selected pathogens under the used concentrations. However, there were some zones of inhibition shown in the well diffusion method against *S. aureus*. (The inhibitory zones are expressed as mean inhibitory zone ± SEM (Standard error of the mean)).

In the well diffusion method, *S. aureus* showed inhibitory zones of 15.33 ± 0.33 mm, and 11.00 ± 0.58 mm against the dichloromethane extract at a concentration of 400 mg/ml and 200 mg/ml, respectively. In the meantime, an inhibitory zone of 14.00 ± 2.08 mm was shown by the hexane extract at a concentration of 400 mg/ml. However, no inhibitory zones were observed in the disc diffusion method against *S. aureus*.

Even though our study does not show significant antibacterial potential from *M. jalapa* tubers, a study which was conducted in Tunisia using *M. jalapa* tubers has shown contradictory results. That study reveals that the aqueous extract has a very promising antimicrobial potency, Hajji et al., 2022 [27,28]. It is possible to obtain contradictory findings for the same experimental study because different quantities of secondary metabolites in plant parts can affect the variation of biological activities like antibacterial potency. For this present study, tuberous roots were obtained during the daytime in October 2022, from an estate in Puttalam District, in North-

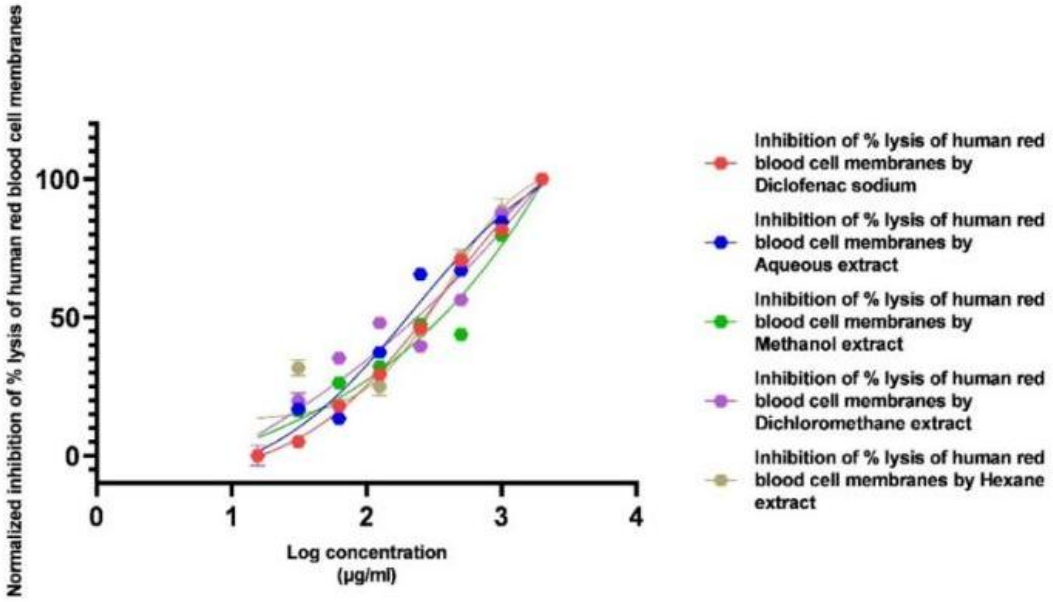

Fig. 3. Dose-response curves of aqueous and organic solvent (methanol, dichloromethane, and hexane) extracts of *M. jalapa* tuberous roots and the reference drug in the HRBC membrane stabilization assay

western Province, Sri Lanka, and in Hajji et al., 2010, tuberous roots were obtained from Kerkenah island (Sfax, Tunisia). Environmental and abiotic factors are considered to be different because of the difference in locations of the above-mentioned instances. Environmental factors such as CO_2 availability, light intensity, humidity, minerals, temperature, and water availability are considered to have a greater impact on the development and secondary metabolite production of a plant. Abiotic factors like cold stress, drought stress, salt stress, heavy metal stress, temperature variations, the influence of polyamines, the influence of plant growth regulators, nutrient stress, and the influence of climate change also affect the production of secondary metabolites [29]. The change in the quantities of different secondary metabolites can be a reason to obtain contradictory results. The extraction method and the solvents employed in the extraction process may have a significant impact on getting these contradictory results.

4. CONCLUSION

This study clearly shows that there is strong anti-inflammatory activity in the tuberous roots of *M. jalapa*, with no significant antibacterial activity against the selected pathogens. Therefore, further studies are very necessary to determine the mechanism and active constituents responsible for the anti-inflammatory activity while finding out the discrepancies in antibacterial activity results done in other similar studies on *M. jalapa* tubers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Appendix A

Percentage inhibition values for aqueous and organic solvent (methanol, dichloromethane, and hexane) extracts of *M. jalapa* tuberous roots and reference drug in egg albumin denaturation inhibition assay

Concentration (µg/mL)	Reference Drug (Diclofenac Sodium)	Aqueous extract	Methanol extract	Dichloromethane extract	Hexane extract
2000	82.62 ± 0.66	76.25 ± 0.16	71.10 ± 0.61	59.16 ± 0.62	37.80 ± 0.78
1000	77.00 ± 0.57	72.55 ± 0.20	62.86 ± 0.89	53.44 ± 0.46	24.54 ± 1.13
500	68.52 ± 0.88	65.57 ± 0.94	58.45 ± 0.55	38.59 ± 0.66	25.01 ± 0.37
250	56.82 ± 1.26	50.77 ± 0.46	46.00 ± 0.41	30.87 ± 0.45	18.13 ± 0.41
125	41.17 ± 1.26	35.97 ± 1.13	33.6 ± 1.8	24.17 ± 0.42	14.10 ± 0.82
62.5	24.87 ± 0.70	29.09 ± 0.49	29.93 ± 0.20	10.82 ± 0.87	10.59 ± 0.37
31.25	13.58 ± 1.95	14.00 ± 0.45	13.02 ± 1.33	8.52 ± 0.53	5.62 ± 0.33
15.625	6.51 ± 2.14	6.70 ± 0.66	3.37 ± 0.98	59.16 ± 0.62	2.86 ± 0.20

Appendix B

Percentage inhibition values for aqueous and organic solvent (methanol, dichloromethane, and hexane) extracts of *M. jalapa* tuberous roots and reference drug in HRBC membrane stabilization assay

Concentration (µg/mL)	Reference drug (Diclofenac Sodium)	Aqueous extract	Methanol extract	Dichloromethane extract	Hexane extract
2000	85.09±0.44	66.23±0.88	61.84±0.76	33.33± 0.44	27.19± 0.44
1000	70.61±1.16	56.58±0.76	49.56±0.88	29.39± 0.44	24.12± 1.16
500	61.84±0.76	45.61±1.16	28.07±1.16	19.74± 0.76	19.74± 0.76
250	42.54±0.44	44.74±1.32	30.26±0.76	14.47± 0.76	12.72± 1.16
125	29.39±1.16	27.19±0.44	21.05±0.76	17.11± 0.76	7.46± 0.88
62.5	20.18±0.44	12.28±0.88	17.54±0.88	13.16± 0.76	5.70± 0.44
31.25	10.09±0.44	14.47±0.76	11.40±0.44	8.33± 0.88	9.21± 0.76
15.625	6.14±0.44	3.95±0.76	1.75±0.44	2.19± 1.16	0.88± 0.44

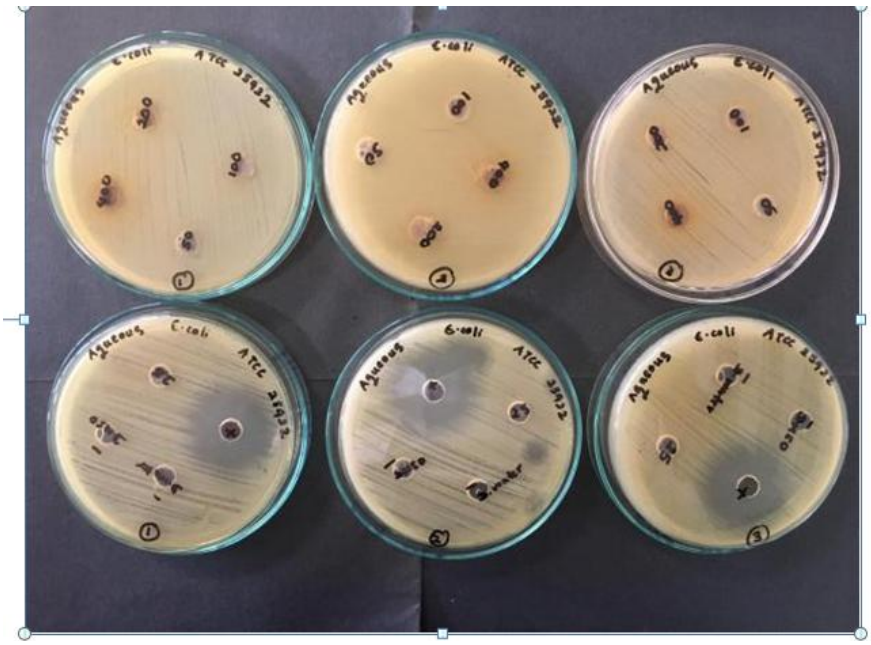
Appendix C

Antibacterial activity results of aqueous, methanol, dichloromethane, and hexane extracts of tuberous roots of *M. jalapa* against *S. aureus*, *E. coli,* and *P. aeruginosa*.

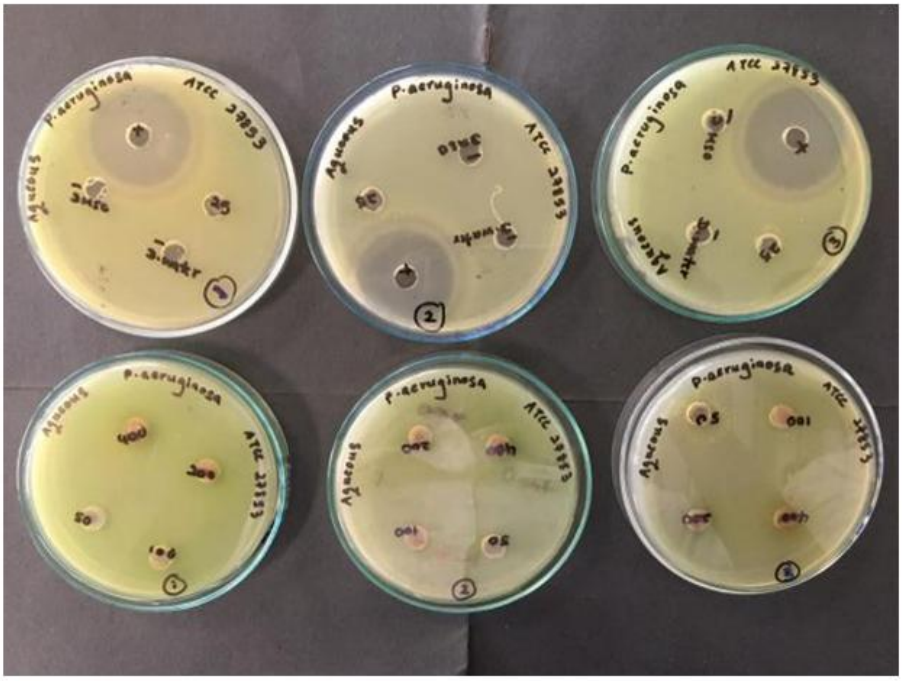
1.1 Antibacterial activity results of the aqueous extract against *S. aureus*, *E. coli,* and *P. aeruginosa*

1.1.1 Antibacterial activity results of the aqueous extract against *S. aureus*, *E. coli,* and *P. aeruginosa* in well diffusion method.

Aqueous extract against *S. aureus*

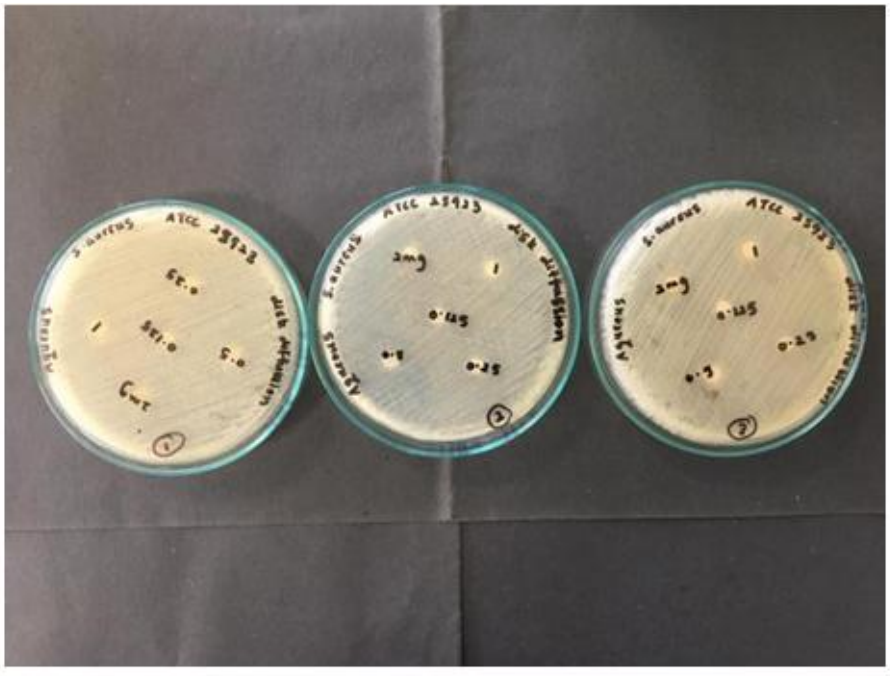


Aqueous extract against *E. coli*

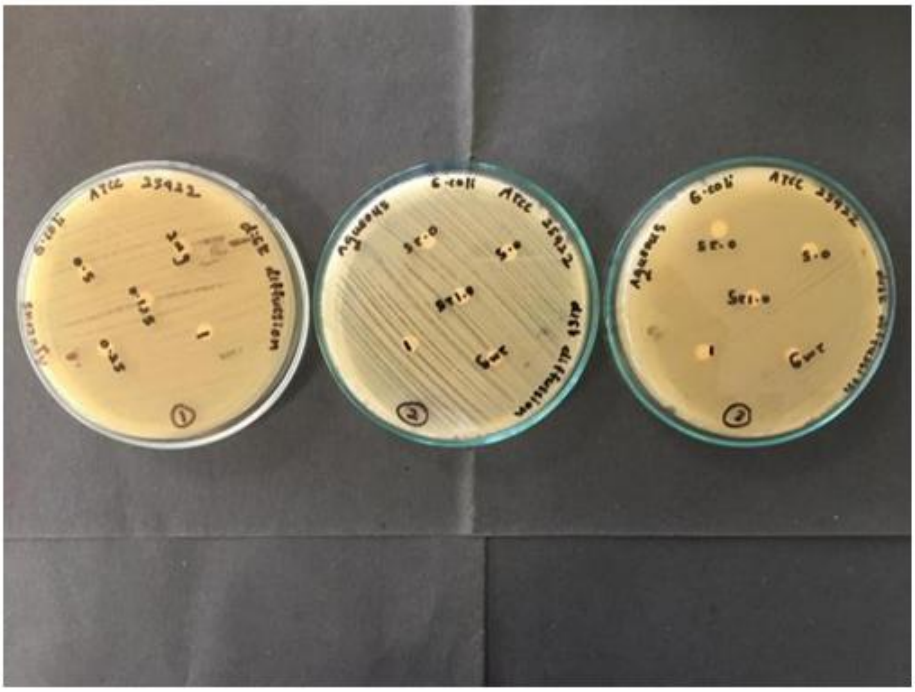


Aqueous extract against *P. aeruginosa*

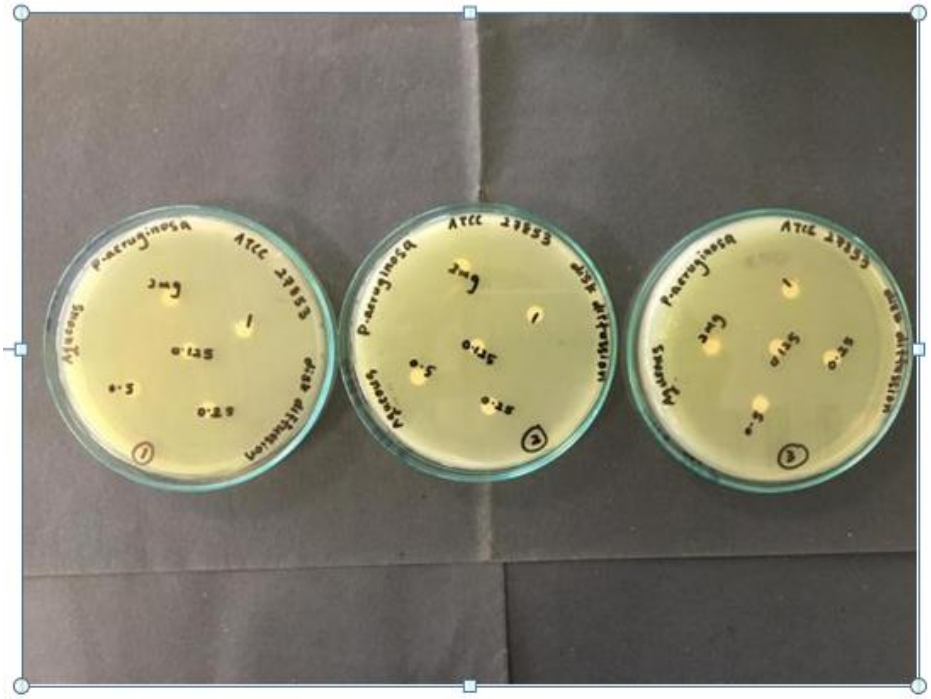
1.1.2 Antibacterial activity results of the aqueous extract against *S. aureus*, *E. coli,* and *P. aeruginosa* in the disc diffusion method



Aqueous extract against *S. aureus*



Aqueous extract against *E. coli*.

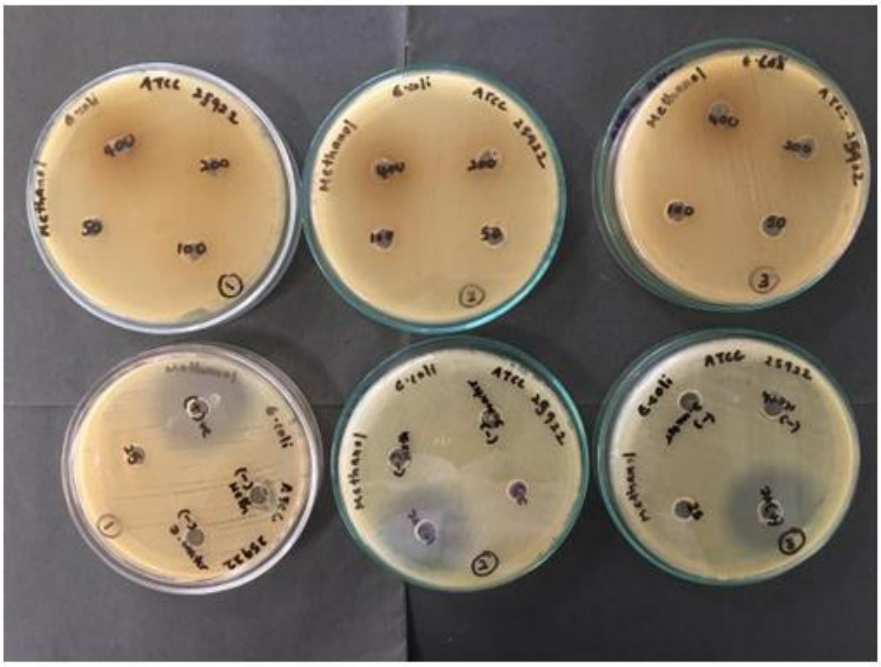


Aqueous extract against *P. aeruginosa*

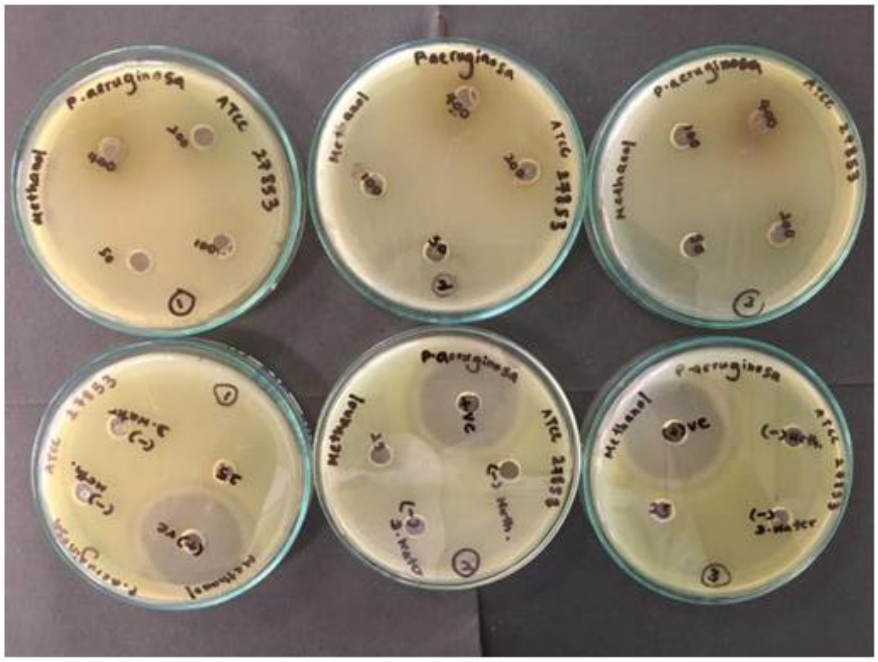
1.2 Antibacterial activity results of the methanol extract against *S. aureus*, *E. coli,* and *P. aeruginosa*

1.2.1 Antibacterial activity results of the methanol extract against *S. aureus*, *E. coli,* and *P. aeruginosa* in well diffusion method

Methanol extract against *S. aureus*

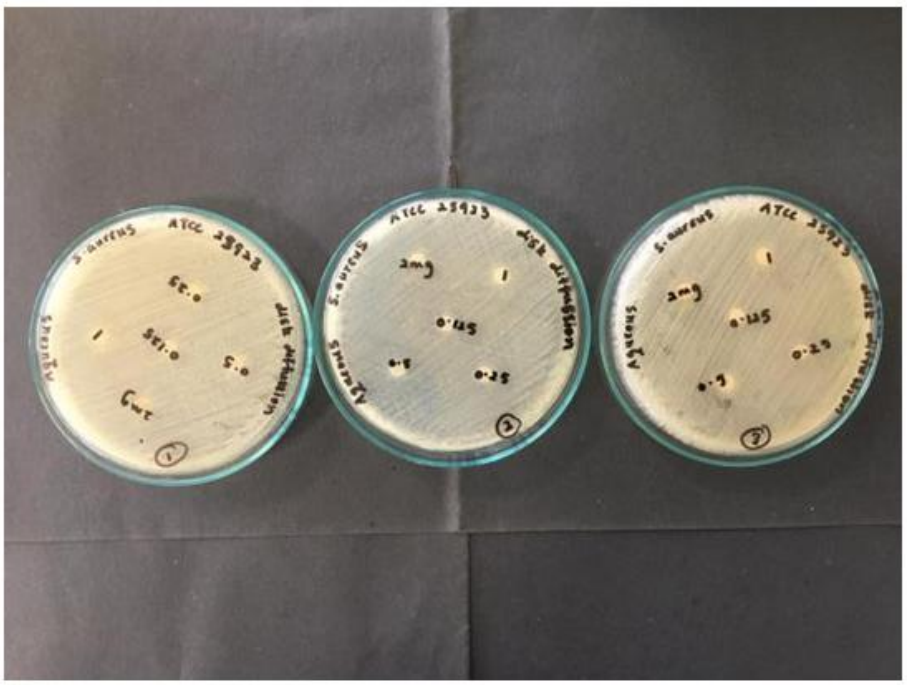


Methanol extract against *E. coli*

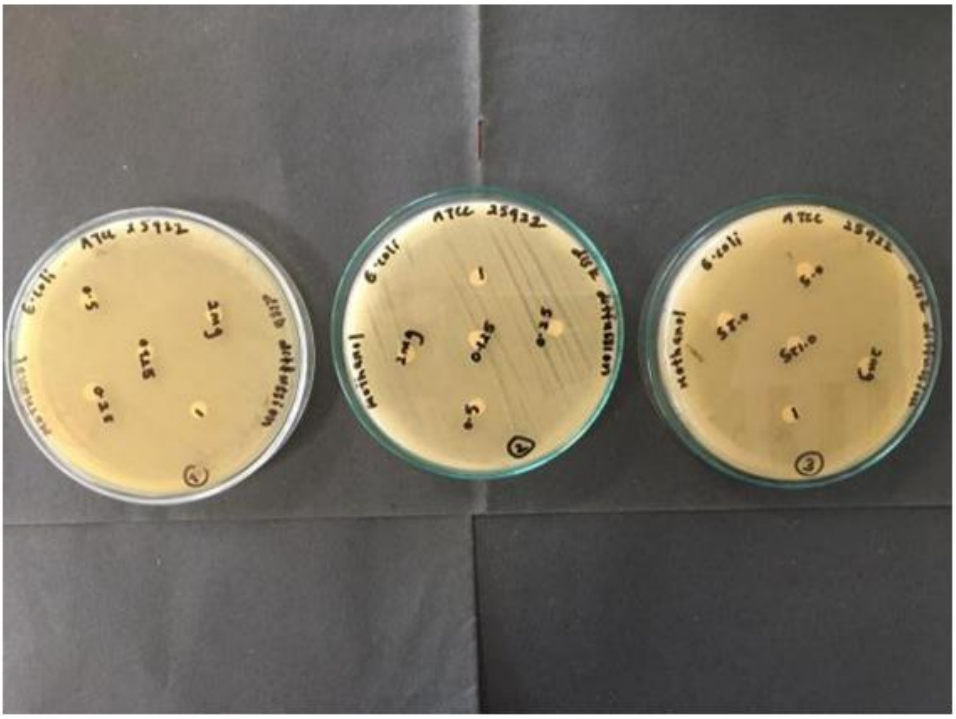


Methanol extract against *P. aeruginosa*

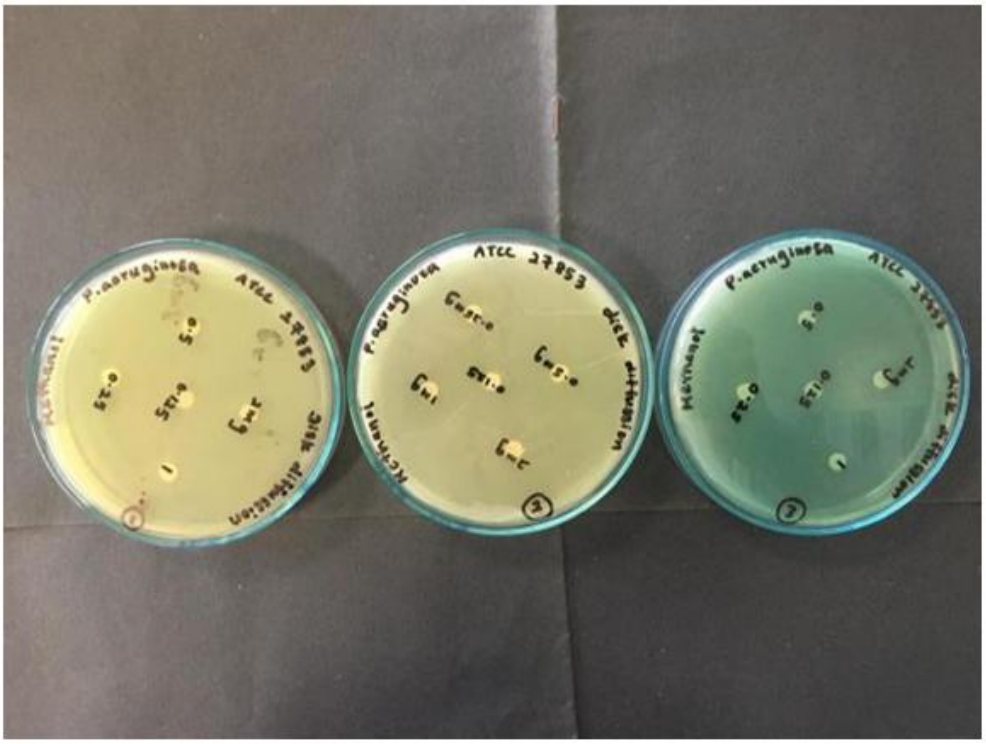
1.2.2 Antibacterial activity results of the methanol extract against *S. aureus*, *E. coli,* and *P. aeruginosa* in the disc diffusion method



Methanol extract against *S. aureus*



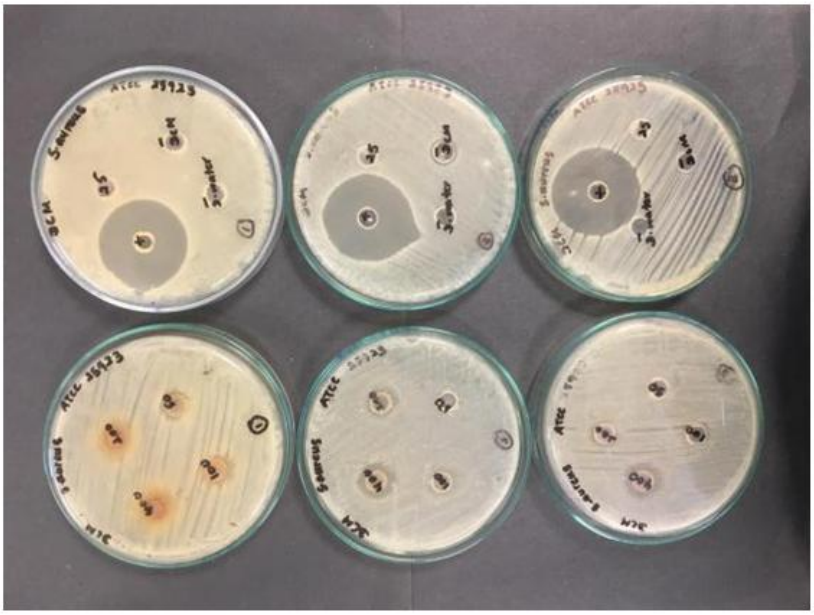
Methanol extract against *E. coli*



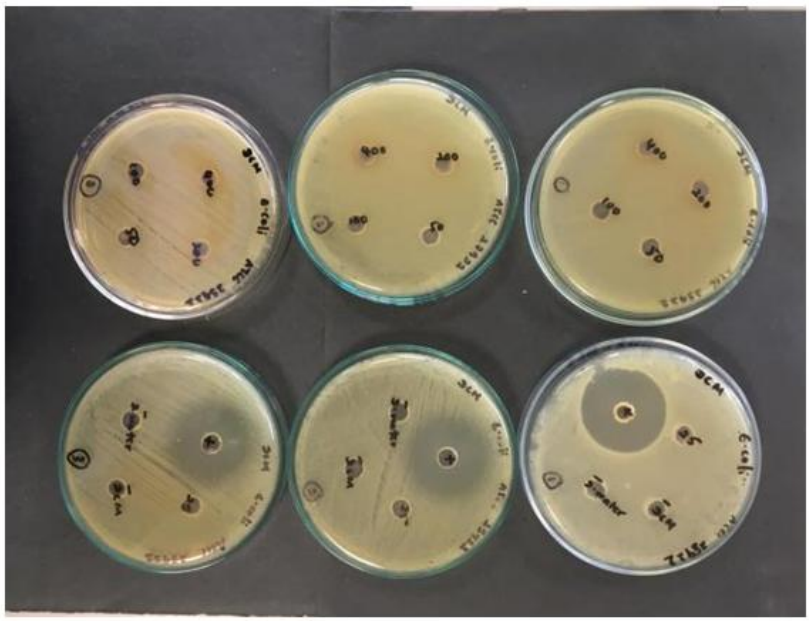
Methanol extract against *P. aeruginosa*

1.3 Antibacterial activity results of the dichloromethane extract against *S. aureus*, *E. coli,* and *P. aeruginosa*

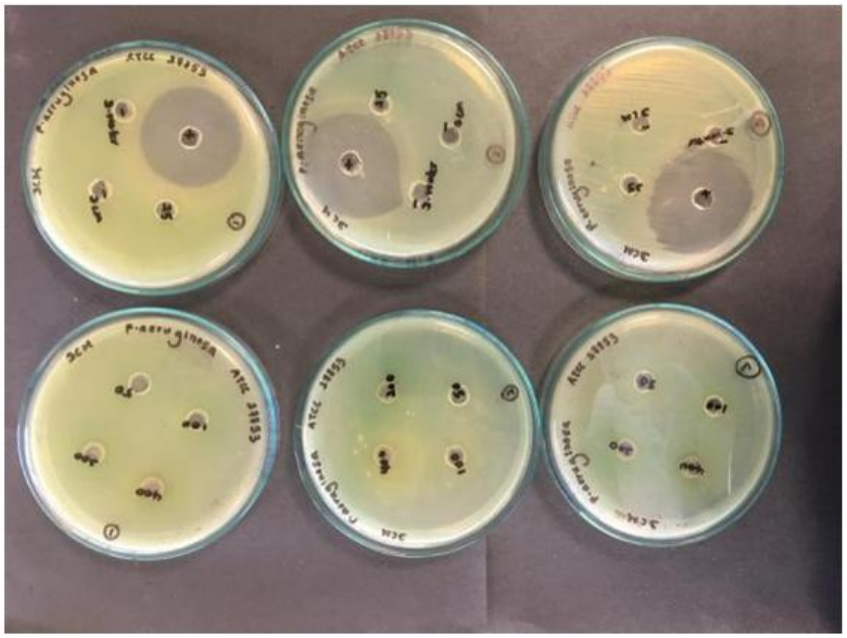
1.3.1 Antibacterial activity results of the dichloromethane extract against *S. aureus*, *E. coli,* and *P. aeruginosa* in well diffusion method



Dichloromethane extract against *S. aureus*

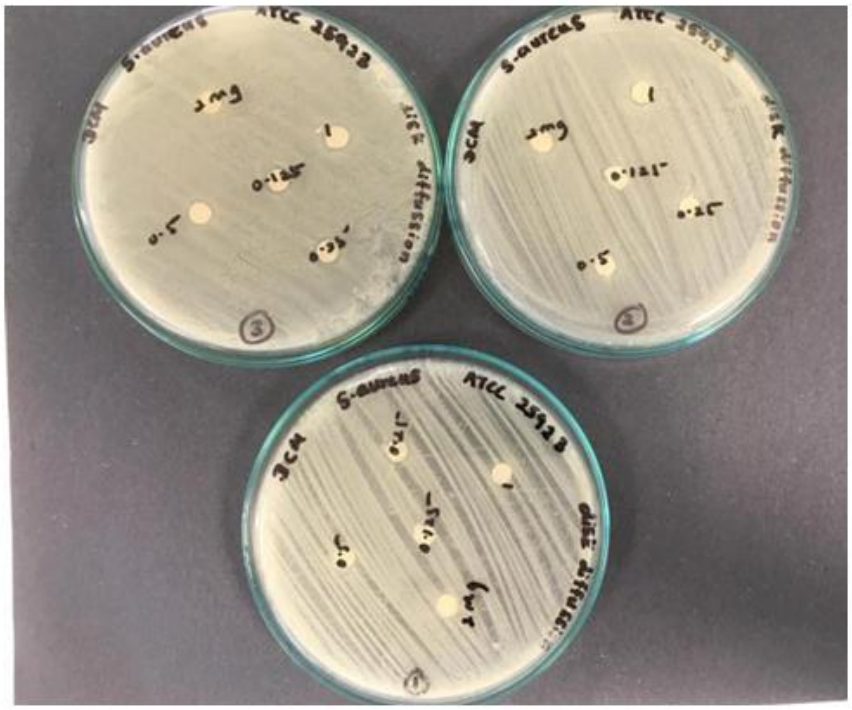


Dichloromethane extract against *E. coli*



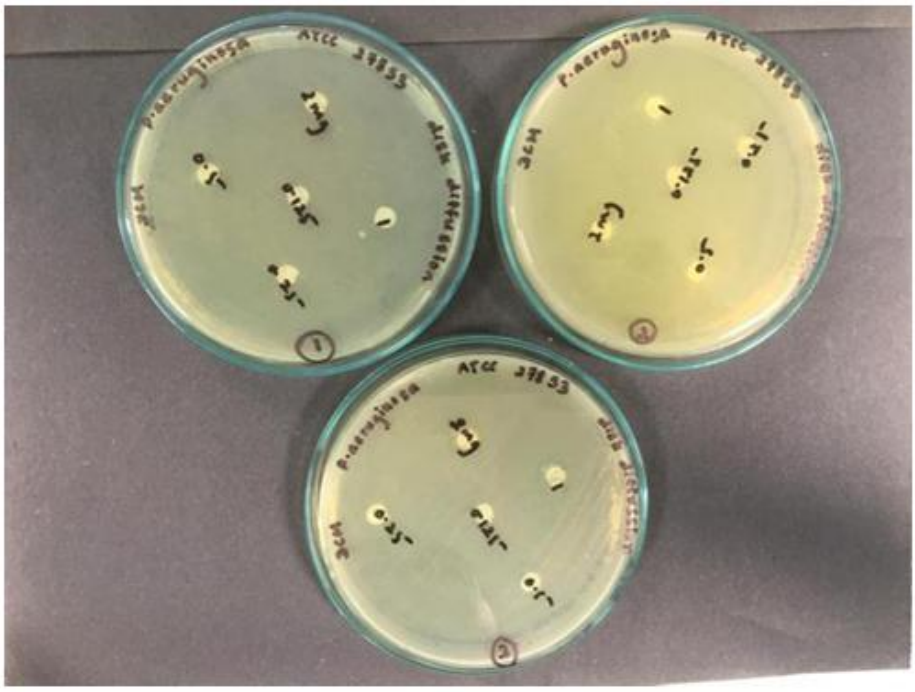
Dichloromethane extract against *P. aeruginosa*

1.3.2 Antibacterial activity results of the dichloromethane extract against *S. aureus*, *E. coli,* and *P. aeruginosa* in the disc diffusion method



Dichloromethane extract against *S. aureus*

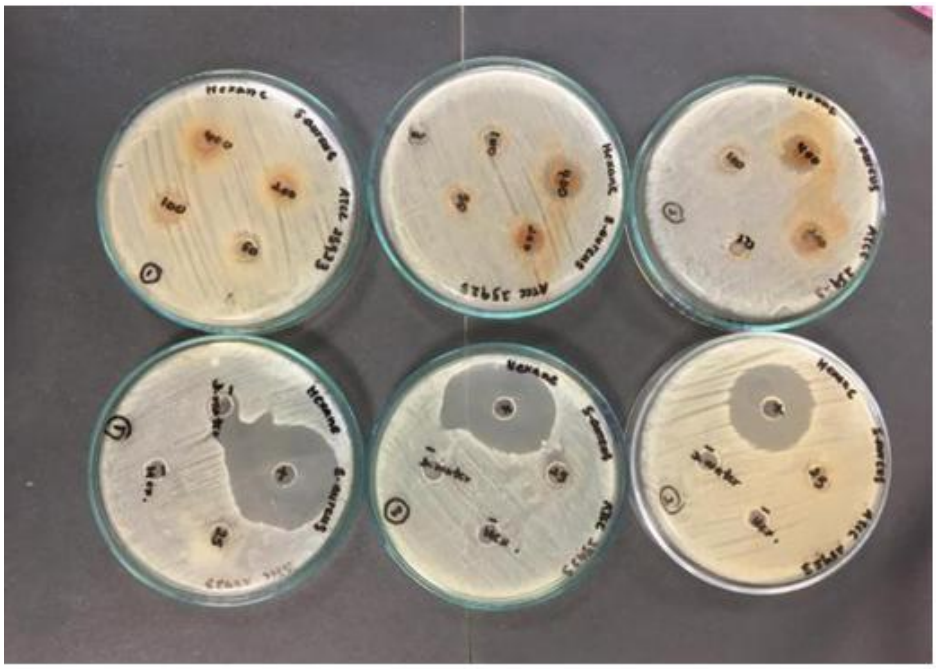
Dichloromethane extract against *E. coli*



Dichloromethane extract against *P. aeruginosa*

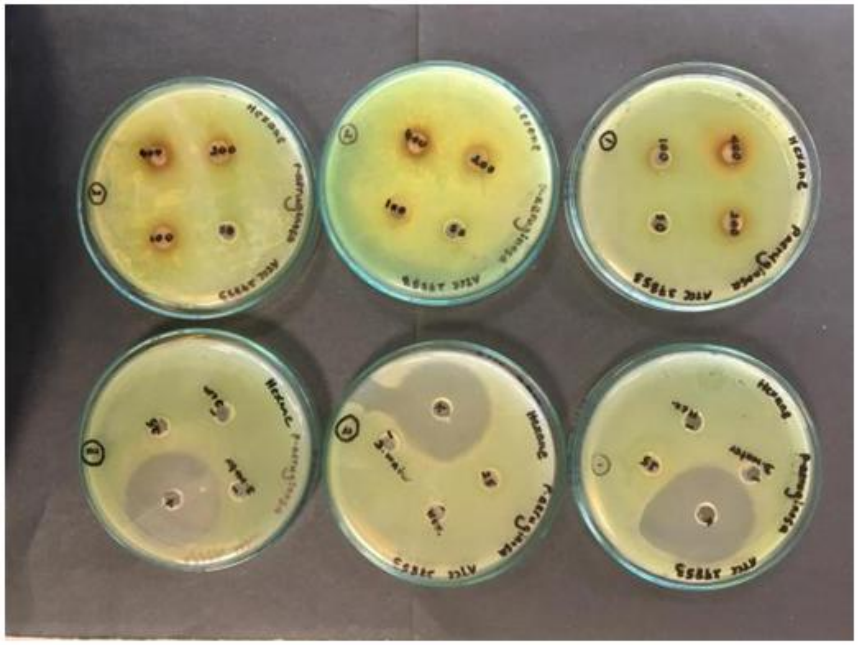
1.4 Antibacterial activity results of the hexane extract against *S. aureus*, *E. coli,* and *P. aeruginosa*

1.4.1 Antibacterial activity results of the hexane extract against *S. aureus*, *E. coli,* and *P. aeruginosa* in the well diffusion method



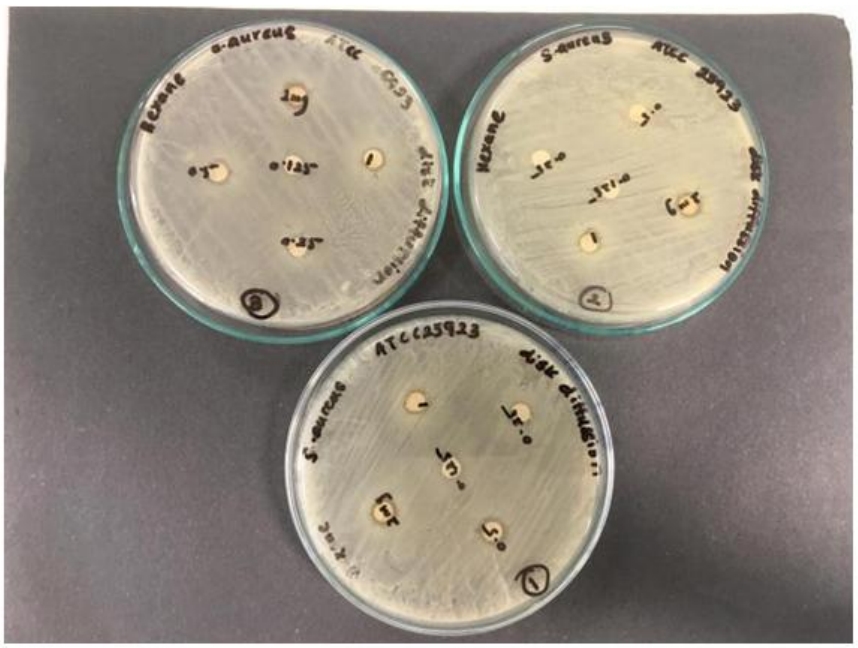
Hexane extract against *S. aureus*

Hexane extract against *E. coli*

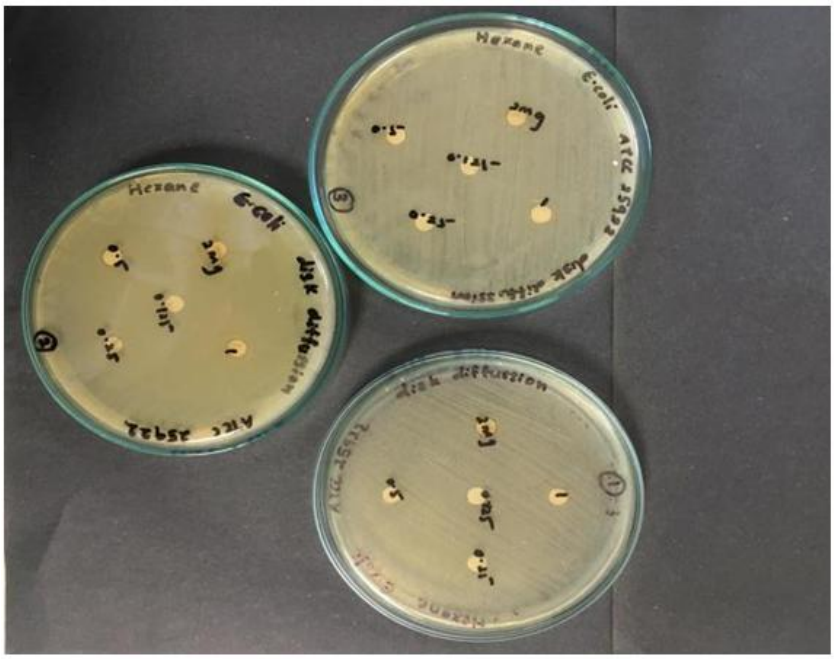


Hexane extract against *P. aeruginosa*

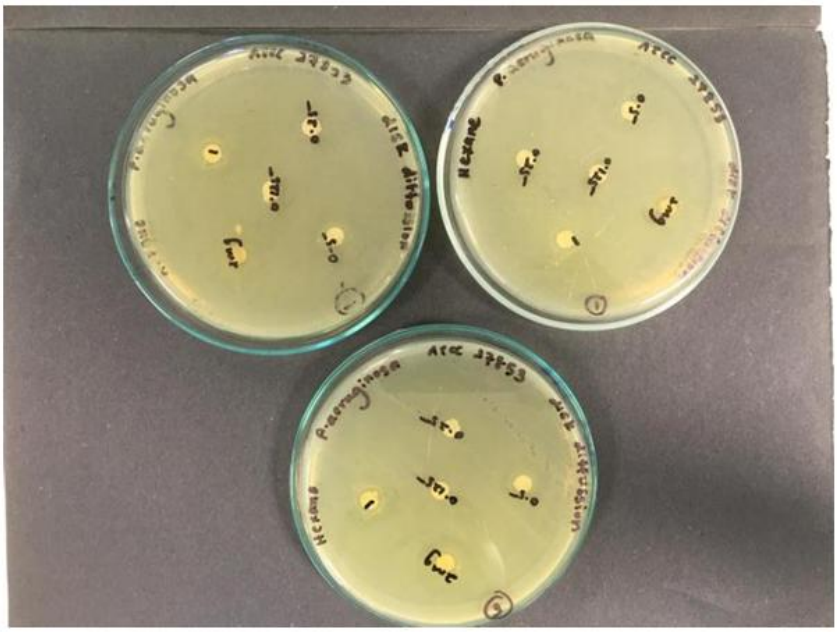
1.4.2 Antibacterial activity results of the hexane extract against *S. aureus*, *E. coli*, and *P. aeruginosa* in the disc diffusion method



Hexane extract against *S. aureus*



Hexane extract against *E. coli*



Hexane extract against *P. aeruginosa*

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