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Original Article

Antibacterial activities of Leaf extracts of Indian Bay leaf (Cinnamomum tamala) against Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa

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Abstract

Background: : Natural herbal remedies have shown promising antimicrobial properties and fewer side effects than synthetic antimicrobial agents. ***Objective:*** The purpose of the present study was to investigate the antibacterial activities of Indian bay leaf extracts against Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa. ***Methodology:*** This experimental study was carried out from July 2019 to June 2020 in the Department of Pharmacology and Therapeutics with the collaboration of the Department of Microbiology at Mymensingh Medical College, Mymensingh, Bangladesh. The antibacterial activity was tested at different concentrations (20, 10, 5,2.5, 1.25 & 0.625 mg/ml) of both spice extracts by using the disc diffusion & broth dilution method. The extracts were prepared by using solvents aqueous & methanol. The test microorganisms were also tested for their activity against a standard antibiotic Gentamicin (80 mg) by broth dilution method. The result was compared with that of Aqueous and Methanolic extracts. ***Results:*** Salmonella typhi, Staphylococcus aureus, and Pseudomonas aeruginosa were all inhibited by ALE at doses of 15 mg/ml and higher. Pseudomonas aeruginosa was inhibited in the case of MLE at concentrations of 15 mg/ml and higher. At doses of 10 mg/ml, Staphylococcus aureus and Salmonella typhi began to exhibit noticeable activity. The Zone of Inhibition (ZOI) for ALE using the disc diffusion technique varied from 6 to 25 mm at various extract concentrations. Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa had minimum inhibitory concentrations (MIC) of 10, 15, and 15 mg/ml in ALE and 5, 5, and 7.5 mg/ml in MLE, respectively. Additionally, this finding was compared to that of the common antibiotic Gentamicin, whose MICs were lower than those of ALE and MLE. The current investigation revealed that leaf extracts, both aqueous and methanolic, exhibited antibacterial properties against Salmonella typhi, Pseudomonas aeruginosa, and Staphylococcus aureus. ***Conclusion:*** The study observed a definite antibacterial effect of both the aqueous and methanolic extract of leaves of Cinnamomum tamala against Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa. The physiologically active components of Cinnamomum tamala that give it its antibacterial properties need to be identified and isolated via more research.

Keywords: :Antibacterial activity; Cinamomum tamala; Staphylococcus aureus; Salmonella typhi; Pseudomonas aeruginosa; Zone of Inhibition; Minimum inhibitory concentration; Broth dilution and disc diffusion; Aqueous; methanolic extracts

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Introduction

One of medicine's greatest triumphs of the twentieth century has been the development of antibiotic chemotherapy. This medicine is commonly used to treat a variety of microbiological illnesses, although Fleming cautioned that overuse of antibiotics may cause bacteria to become resistant. Worldwide public health is at increased risk from these drug-resistant types of microorganisms[1]. These days, because plant-based medications are inexpensive and have few adverse effects, research is being done to determine their effectiveness in traditional medicine. Approximately 80.0% of people worldwide still primarily use herbal treatments, according to the WHO[2]. The plant C. tamala, sometimes called Indian bay leaf, is a member of the Lauraceae family and genus Cinnamomum. It goes by several other names in India, including Tejpat and Tejpatta. There are up to 350 species of this plant in the world. The plant C. tamala is an evergreen plant of modest size. There are a lot of leaves on its grey-brown trunk, and its bark is delicate and wrinkled[3].

Cinnamomum tamala dried leaves are used to add taste to various culinary preparations. Aromatic essential oils found in plant bark and leaves include phenolic chemicals that have several medicinal benefits against diabetes, arteriosclerosis, Alzheimer's disease, and arthritis[4]. The most often used portion of Cinnamomum Tamala is the leaf. It has a variety of components, but the main ones are the essential oils, which include curcumenol (2.3%), β-caryophyllene (6.6%), sabinene (4.8%), germacrene D (4.6%), and furanogermenone (59.5%)[4]. It contains other ingredients including eugenol and cinnamon aldehyde, which are found in the barks and give them their scent[5]. The oil's diuretic, carminative, and anti-flatulent qualities make it useful in medicine[6]. The extract fractions of Cinnamomum Tamala contain phenol, alkaloids, and terpenoids. The spice plant has phytochemicals that are used to develop antibacterial agents[7].

Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa are important pathogens for causing hospital acquired infection. Strong antibacterial action against gram-positive and gram-negative bacteria as well as fungi is demonstrated by C. tamala. Strong antibacterial action was demonstrated by oil and its constituents against Helicobacter pylori, Pseudomonas aeruginosa, Bacillus subtilis, Aspergillus fumigatus, and methicillin-resistant Staphylococcus aureus. Essential oil has shown growth-inhibiting properties against Escherichia coli and Mycobacterium tuberculosis[8].

Cinnamomum tamala leaf methanolic extract also significantly lowers blood glucose, blood glycosylated hemoglobin, LPO, serum AST, and ALT levels while significantly raising antioxidant enzymes like superoxide dismutase. It could be applied as a diabetic adjunct treatment[9]. Asthma, colic, blood dysentery, diarrhea, constipation, flatulence, indigestion, jaundice, hyperacidity, anorexia, dysmenorrhea, leucorrhea, postpartum hemorrhage, high fever, skin conditions, sore throat, sexual weakness, and tuberculosis have all been treated with this plant for a long time[10].

It has a very promising role in having diverse activities. Therefore, the present study was aimed to evaluate the antibacterial effects of Aqueous and Methanolic extract of Leaves of Indian bay leaf against some food-borne pathogens.

Methodology

Study Settings and Population: From July 2019 to June 2020, this experimental study was conducted in the Department of Pharmacology & Therapeutics in cooperation with the Department of Microbiology at Mymensingh Medical College, Mymensingh, Bangladesh.

Study Procedure:

Tested Bacterial Strains: Bacterial strains, Staphylococcus aureus ATCC 25923, Salmonella typhi ATCC 14028 and Pseudomonas aeruginosa (ATCC 27853) were used in the study. The Department of Microbiology at Mymensingh Medical College in Mymensingh provided the pure cultures of these.

Maintenance of bacterial culture and inoculum preparation: On nutrient agar slants and plates, pure cultures were routinely maintained and replenished. Following a 24-hour incubation period at 37°C, the cultures were streaked on sterile nutrient agar plates and then refrigerated at 4°C. To prevent contamination, bacterial cultures were replaced every one to two weeks. The pure bacterial culture was grown in nutrient broth at 37°C for the whole night to create the inoculum.

Plant Material: The fresh tender leaves were purchased from a rural area of Mymensingh.

Preparation of Aqueous Leaf Extract: The leaves were washed and dried in the shade at room temperature for six to seven days. Finally, dried materials were pulverized into fine powdered substances by a grinder. 50 gm of powder of Indian bay leaves were weighed with the electric balance and transferred into one conical flask. Then 500 ml distilled water in the flask was added. The solution was kept at room temperature for at least 24 hr. The aqueous extract was then

filtered by using a muslin cloth. The filtrate was again filtered using Whatman no.1 filter paper under strict aseptic conditions. The resulting filtrate was collected in previously tared sterilized Petri plates and dried in a rotary flash evaporator at 450 C for proper dehydration. After the complete removal of the solvent, the Petri plates were weighed and then the net weight of the dried extract was determined and used. 1gm dried extract was then dissolved in 50ml sterilized distilled water.

For the preparation of the aqueous stock solution, 1gm of ALE was dissolved in 50 ml of D/W to get a concentration of 0.02 gm/ml i.e. 20mg/ml which was labeled as stock solution. From the above stock solution, different concentrations such as 10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml, and 0.625mg/ml were prepared with appropriate volumes of D/W.

Table 1:Preparation of the ALE solutions of different concentrations

Amount of Solution (ml) taken from Stock Solution	Amount of Distilled Water (ml)	Concentration (milligram /ml)
1	31	0.625
1	15	1.25
1	7	2.5
1	3	5
1	1	10
1	00	20

Preparation of Methanolic Leaf Extract: The leaves were washed and dried in the shade at room temperature for six to seven days. Finally, dried materials were pulverized into fine powdered substance by a grinder. 50 gm of powder of Indian bay leaves were weighed with the electric balance and transferred into one conical flask. Then 500 ml 100% Methanol in the flask was added. The solution was kept at room temperature for at least 24 hr. The methanolic extract was then filtered by using a muslin cloth. The filtrate was again filtered using Whatman no.1 filter paper under strict aseptic conditions. The resulting filtrate was collected in previously tared sterilized petriplates and dried in a rotary flash evaporator at 45℃ for proper dehydration. After the complete removal of the solvent, the petriplates were weighed and then the net weight of dried extract was determined and used. 1gm dried extract was then dissolved in 50ml sterilized distilled water. For the preparation of the methanolic stock solution, 1gm of MLE was dissolved in 50 ml of D/W to get a concentration of 0.02 gm/ml i.e. 20mg/ml which was labeled as stock solution. From the above stock solution, different concentrations such as 10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml, and 0.625mg/ml were prepared with appropriate volumes of methanol.

Table 2: Preparation of the MLE Solutions of Different Concentration

Amount of solution (ml) taken from a stock solution	Amount of Distilled Water (ml)	Concentration (milligram /ml)
1	31	0.625
1	15	1.25
1	7	2.5
1	3	5
1	1	10
1	00	20

Antibacterial Sensitivity Testing Using Disc Diffusion Method: Antibacterial sensitivity test was performed by Kirby-Bauer disc diffusion technique. Filter paper disc of 6mm diameter using Whatman No[1]. filter paper was prepared and sterilized. After matching with 0.5 McFarland standards for each isolate, a sterile cotton swab was dipped into bacterial suspension and streaked in three directions on the surface of Mueller Hinton Agar plates and then left for 5-10 minutes at room temperature. The blank discs were aseptically placed over the Mueller Hinton agar plates seeded with the test microorganisms. Then with the help of micropipette 10µl 20mg/ml, 10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml, and 0.625mg/ml concentrations of Aqueous & methanolic leaf Extracts were transferred to different disc aseptically. while 10µL of distilled water & 100% methanol were added in a sterile filter paper disc as a negative control in both extracts. Plates were incubated at 37°C for 24 hours. After 24 hours the results were recorded. The antibacterial activity results were expressed in terms of the diameter of the zone of inhibition <9mm zone was considered inactive; 9-12mm was partially active; while 13-18mm was active and >18mm was very active as described in Gupta et al[11].

Determination of Minimum Inhibitory Concentration (MIC) of *Cinnamomum tamala* Leaves Extract Against Test Bacteria by Broth Dilution Method

Preparation of ALE Stock and Working Solutions: As described before, 1gm Aqueous extracts powder was dissolved in 50 ml D/W in which 1 ml of solution contained .02 gm or 20 mg of ALE powder and it was the stock solution used to prepare ALE working solutions. Sets I, II, III, IV, V, VI, and VII respectively were made in different test tubes by mixing the measured amount of ALE stock solution with the measured amount of nutrient broth

medium. The concentrations of these sets were 15mg/ml, 10mg/ml, 7.5mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml, and 0.625mg/ml ALE respectively. Set-VIII (Control-1) was . made with ALE stock solution. Set-IX (Control-2) was made with a nutrient broth medium. Set-X (Control-3) was made with nutrient broth medium in test tubes.

Preparation of MLE Stock and Working Solutions: As described before, stock solution 20 mg/ml. Different sets of working solutions & controls were prepared as described before.

Inoculation of Bacterial Suspension to Working Solutions of ALE & MLE in Test Tubes: After matching the turbidity of bacterial suspension with 0.5 McFarland standards, 20µl of bacterial suspension of *Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa* were separately added to each concentration of working solutions of ALE & MLE in separate test tubes. These inoculums were also added to the Control-1 and 2, but not to Control-3. These were Incubated at 370 C for 18-24 hours.

Examination of Growth of Test Organisms in Different Concentrations of ALE & MLE: After 18 to 24 hours of incubation, the growth of test organisms in different preparations of ALE & MLE were examined and compared against that of controls by matching their turbidity. The clear preparations were considered as "No growth" of bacteria and turbid ones, as "Growth of bacteria". The MIC was reported as the lowest concentration of ALE & MLE required to prevent the visible growth of test organisms.**Testing Antimicrobial Activity of a Standard Antibiotic:** The test microorganisms P. aeruginosa were also tested for their activity against the antibiotic Gentamicin (inj. 80mg) by broth dilution method.

Statistical analysis: Findings were recorded and analyzed. Collected data were checked and edited first and processed with the help of the software Statistical Package for Social Sciences (SPSS) version 21 and analyzed. Statistical analyses were done by using appropriate statistical tools. Statistical significance was assessed at the 0.05 level for all analyses.

Ethical Clearance: Ethical Clearance: Institutional Review Board (IRB) clearance, Memo no. MMC/IRB/2020/240, Dated 11/02/2020. This is to certify that the thesis protocol entitles Antibacterial activities of Indian bay leaf (Cinnamomum tamala) leaves extracts against *Staphylococcus aureus, Salmonella typhi,* and *Pseudomonas aeruginosa* submitted by Dr. Shompa Sharmin Rasna as a student of M. Phil. (Pharmacology) part- Final has been reviewed and approved by the Institutional Review Board (IRB) of Mymensingh Medical College.

Table 3 : Preparation of the MLE Solutions of Different Concentration

No. of Sets	ALE Solution (ml)	Nutrient broth medium (ml)	Total (ml)	Concentration of ALE (mg/ml)	Test Organism (µl)
Set- I	7.5	2.5	10	15	20
Set- II	5	5	10	10	20
Set- III	3.75	6.25	10	7.5	20
Set- IV	2.5	7.5	10	5	20
Set- V	1.25	8.75	10	2.5	20
Set- VI	0.625	9.375	10	1.25	20
Set- VII	0.3125	9.6875	10	0.625	20
Set- VIII C-1	10	0	10	20	20
Set-IX C-2	-	10	10	-	20
Set-X C-3	-	10	10	-	-

Table 4: Composition and Different Concentrations of Working MLE Solutions and the Controls

No. of Sets	MLE stock Solution (ml)	Nutrient broth medium (ml)	Total (ml)	Concentration of MLE (mg/ml)	Test Organism (µl)
Set- I	7.5	2.5	10	15	20
Set- II	5	5	10	10	20
Set- III	3.75	6.25	10	7.5	20
Set- IV	2.5	7.5	10	5	20
Set- V	1.25	8.75	10	2.5	20
Set- VI	0.625	9.375	10	1.25	20
Set- VII	0.3125	9.6875	10	0.625	20
Set- VIII C-1	10	0	10	20	20
Set-IX C-2	-	10	10	-	20
Set-X C-3	-	10	10	-	-

Table 5: Composition and Different Concentrations of Working Gentamicin Solutions and the Controls

No. of Sets	Gentamicin Stock Solution-2 (ml)	Nutrient Broth Medium (ml)	Total (ml)	Concentration of Gentamicin (µg/ml)	Test Organism (µl)
Set-I	2	8	10	2	20
Set-II	1.5	8.5	10	1.5	20
Set-III	1	9	10	1	20
Set-IV	0.75	9.25	10	0.75	20
Set-V	0.5	9.5	10	0.5	20
Set-VI	0.25	9.75	10	0.25	20
Set-VII (C-1)	-	10	10	-	20
Set-VIII (C-2)	-	10	10	-	-

Results

Indian bay leaves were shown to be efficient against the test bacterial strains in this investigation. When using the disc diffusion technique, the zones of inhibition for ALE Staphylococcus aureus, Salmonella typhi and Pseudomonas aeruginosa were 18 mm, 21 mm, and 25 mm respectively, at doses of 20 mg/ml. The greatest zone of inhibition against Pseudomonas aeruginosa (25 mm) was seen at a dosage of 20 mg/ml. Salmonella typhi and Staphylococcus aureus also began to exhibit distinct activity at 15 mg/ml conc, while Pseudomonas aeruginosa did the same. At 20 mg/ml concentrations, the zone of inhibition for MLE Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa was 21 mm, 25 mm, and 26 mm, respectively against the aforementioned microorganisms. Pseudomonas aeruginosa showed the largest zone of inhibition at a dosage of 20 mg/ml (26 mm).

Pseudomonas aeruginosa begins to exhibit clear activity at concentrations of 20 mg/ml, whereas Salmonella typhi and Staphylococcus aureus begin to show clear activity at 10 mg/ml conc. There was no zone against any bacterium on the negative control disc, which contained solely D/W and methanol. Pseudomonas aeruginosa was determined to represent the test organism most vulnerable to ALE and MLE. As seen in the image, the outcomes of the broth dilution method for extracting leaves were also contrasted with those of the common antibiotic Gentamicin. The MICs of the aqueous extract for Salmonella typhi (15 mg/ml), Pseudomonas aeruginosa (15 mg/ml) and Staphylococcus aureus (10 mg/ml) were determined using the broth dilution method. Pseudomonas aeruginosa 7.5 mg/ml, Salmonella typhi 5 mg/ml and Staphylococcus aureus 5 mg/ml are the MICs of the Indian bay leaf methanolic extract for the test organisms. Gentamicin had minimum inhibitory concentrations (MICs) of 1µg/ml, 1µg/ml, and 1.5µg/ml against Salmonella typhi, P. aeruginosa and S. aureus respectively According to CLSI (2016), standard sensitive MICs of Gentamicin for test bacteria were ≤4µg/ml, consistent with my study results[12].

Table 6: Antibacterial Activity of Different Concentrations of ALE Measured in Zone of Inhibition

Concentrations of ALE solutions in milligram	Zone of Inhibition (ZOI) in mm		
	Staphylococcus aureus	*Salmonella typhi*	*Pseudomonas aeruginosa*
20	18	21	25
10	08	08	08
5	07	07	07
2.5	06	06	06
1.25	06	06	06
0.625	06	06	06
Control	06	06	06

Table 7: Antibacterial Activity of Different Concentrations of MLE Measured in Zone of Inhibition

Concentrations of MLE solutions in miligram/ml	Zone of Inhibition (ZOI) in mm		
	Staphylococcus aureus	*Salmonella typhi*	*Pseudomonas aeruginosa*
20	21	25	26
10	15	21	09
05	10	08	07
2.5	07	07	6.5
1.25	06	06	06
0.625	06	06	06
Control	06	06	06

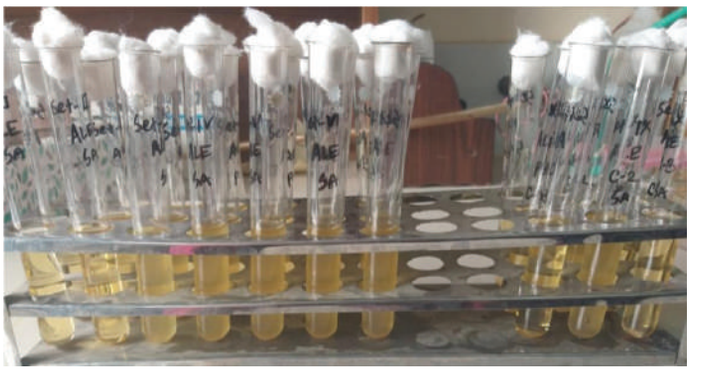

Figure II: Determination of MIC of ALE against test organisms

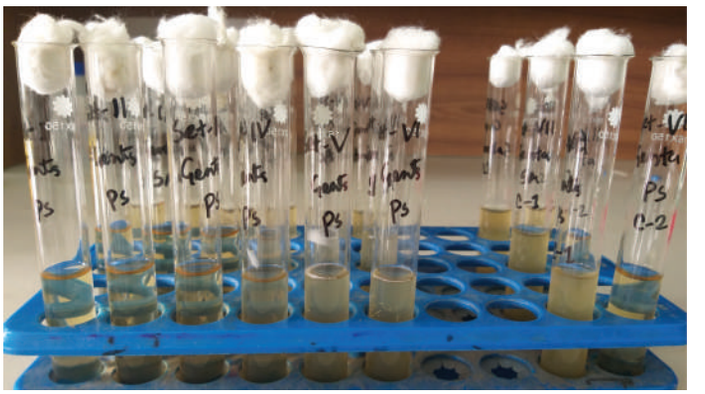

Figure III: MIC of Gentamicin

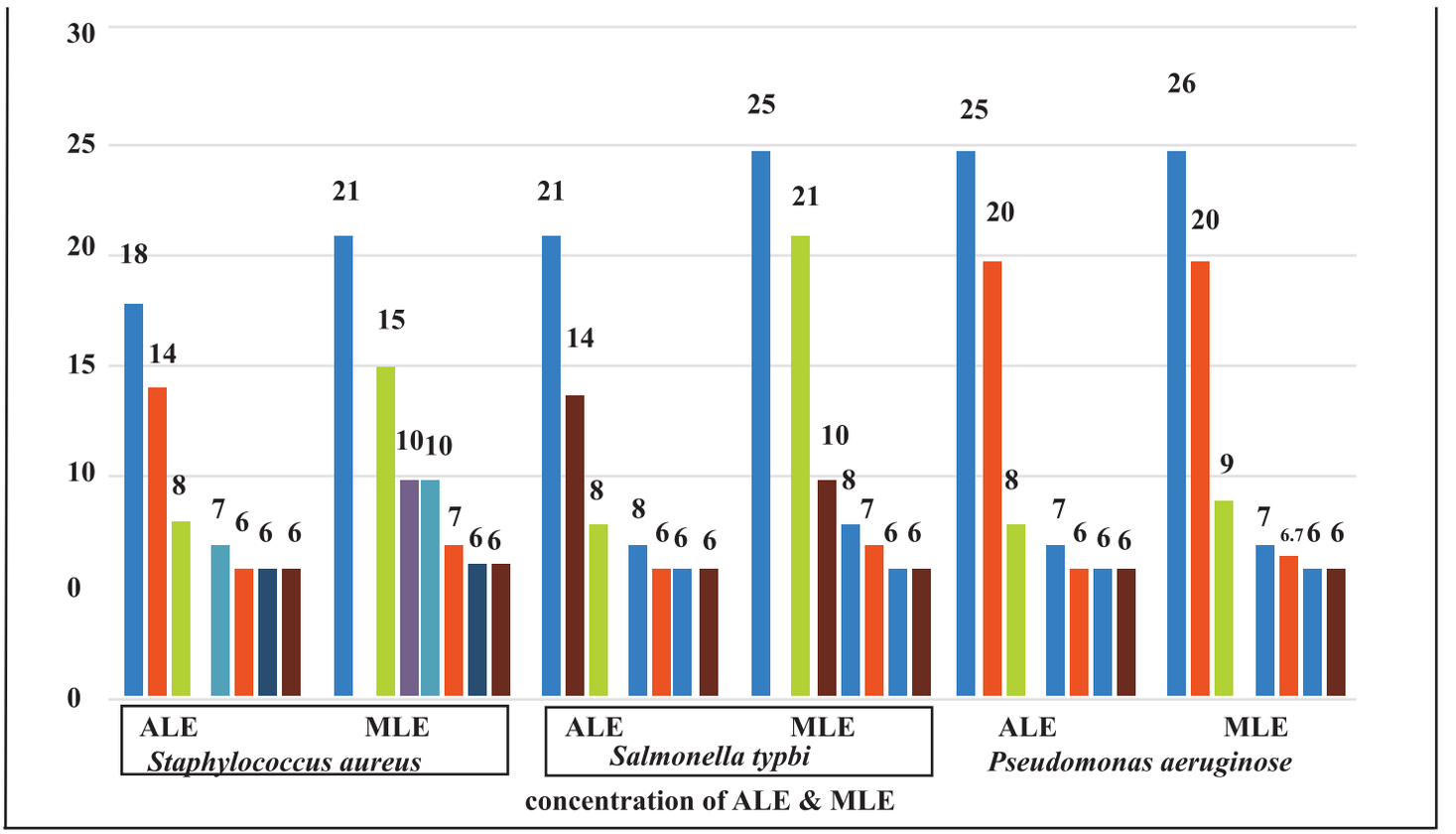

Figure I: Multiple bar diagram showing comparison of Zone of Inhibition (ZOI) between ALE & MLE

Table 8: MIC of ALE against *Staphylococcus aureus*, S*almonella typhi,* and *Pseudomonas aeruginosa*

No. of sets	Concentrations (ALE) mg/ml	*S. aureus*	*S. typhi*	*P. aeruginosa*
Set-I	15	No growth	No growth	No Growth
Set-II	10	No growth	Growth	Growth
Set-III	7.5	Growth	Growth	Growth
Set-IV	05	Growth	Growth	Growth
Set-V	2.5	Growth	Growth	Growth
Set-VI	1.25	Growth	Growth	Growth
Set-VII	0.625	Growth	Growth	Growth
	Controls			
Set-VIII C-1	20	No growth	No growth	No growth
Set-IX C-2	NB media + Bacteria	Growth	Growth	Growth
Set-X C-3	NB media + No Bacteria	No growth	No growth	No growth

Table 9: MIC of MLE against *Staphylococcus aureus*, *Pseudomonas aeruginos*, and *Salmonella typhi.*

Number of Sets	Concentrations (MLE) mg/ml	*S. aureus*	*S.typhi*	*P. aeruginosa*
Set-I	15	No growth	No growth	No growth
Set-II	10	No growth	No growth	No growth
Set-III	7.5	No growth	No growth	No growth
Set-IV	5	No growth	No growth	Growth
Set-V	2.5	Growth	Growth	Growth
Set-VI	1.25	Growth	Growth	Growth
Set-VII	0.625	Growth	Growth	Growth
	Controls			
Set-VII C-1	20	No growth	No growth	No growth
Set-IX C-2	NB media +Bacteria	Growth	Growth	Growth
Set-X C-3	NB media +No Bacteria	No growth	No growth	No growth

Table 10: Comparison between MICs of ALE, MLE, and Gentamicin against *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Salmonella typhi*

MIC of	Test Organisms		
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Salmonella typhi*
ALE	10 mg/ml	15 mg/ml	15 mg/ml
MLE	5 mg/ml	7.5 mg/ml	5 mg/ml
Gentamicin	1 µg/ml	1 µg/ml	1.5 µg/ml

Discussion

In the present study, the in vitro antibacterial activity of ALE and MLE was quantitatively evaluated based on the zone of inhibition by disc diffusion method, and the MIC was assessed by broth dilution technique. Different concentrations of the extract exhibited varying degrees of inhibitory effect. Several studies have been conducted to evaluate the antibacterial properties of Cinnamomum tamala

Waseem et al[13] analyzed the antimicrobial activity of Cinnamomum tamala leaves against *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi,* and other different bacteria by agar well diffusion method using different solvents such as aqueous, hexane, isobutanol, crude extract. In the aqueous extract, ZOI for *Staphylococcus aureus* was 14 mm and 15 mm, and for P. aeruginosa 17 mm and 19 mm at 6µl and 12µl concentration respectively. There was no effect observed in aqueous leaf extract against Salmonella typhi. This finding against *S. aureus* was somewhat similar to the present study in which ZOI against *S. aureus* was 14mm at 15 mg/ml. *Pseudomonas aeruginosa* showed 20 mm ZOI at 15 mg/ml of the extract. There was little difference in result which may be due to extraction procedure or different organism strain. Shete and Chitanand[14] examined the antimicrobial activity of some commonly used Indian spices against pathogenic organisms including *Staphylococcus aureus, Salmonella typhi,* and *Pseudomonas aeruginosa* using aqueous, ethanolic, and methanolic extract by well diffusion methods. There was no effect observed against *Pseudomonas aeruginosa.* In the aqueous extract for *S. aureus* ZOI was 12 mm and for *S. typhi* was 12 mm. In the present study for *S. aureus* and *S. typhi* was 14 mm at 15 mg/ml concentration of extract. This a bit difference in result may be due to extraction procedures or different organism strains.

In methanolic extract, ZOI for *Staphylococcus aureus* was 12 mm, for *Pseudomonas aeruginosa* was 15 mm and for *Salmonella typhi* was 14 mm. In the present study, ZOI for *Staphylococcus aureus* was 15 mm at 10 mg/ml, *Pseudomonas aeruginosa* had 20 mm at 15 mg/ml and *Salmonella typhi* showed 21mm at 10 mg/ml concentration of extract. The difference in results may be attributed to the difference in the concentration of extract or the difference of strains of the organism by the aforementioned researchers.

Another study was carried out by Sukumar et al[15] to assess of bioactivity of cinnamomum tamala against *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and other bacteria by broth dilution method using aqueous and methanolic extract. In the aqueous extract, the MIC for *Staphylococcus aureus* was 10 mg/ml, for *Salmonella typhi* MIC was 13 mg/ml and the MIC for *Pseudomonas aeruginosa* was 13 mg/ml. In the present study the MIC for S*taphylococcus aureus* was 10 mg/ml, for *Salmonella typhi* was 15 mg/ml, and for *Pseudomonas aeruginosa* was 15 mg/ml which is similar to that study.

Sukumar et al[16] examined the antibacterial activities of Cinnamomum tamala against *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and other bacteria by broth dilution method using aqueous and methanolic extract. In the methanolic extract, the MIC for *Staphylococcus aureus* was 2.25 mg/ml, for *S. typhi* MIC was 9 mg/ml and the MIC for *Pseudomonas aeruginosa* was 9 mg/ml. In the present study, the MIC for *S. aureus* was 5 mg/ml, *Salmonella typhi* was 5 mg/ml and *Pseudomonas aeruginosa* was 7.5 mg/ml. This finding of the present study is somewhat similar to that study.

Another study was carried out by Sarita et al[17] to assess the antibacterial activity of some medicinal plants such as Cinnamomum tamala against human pathogenic bacteria including *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa*. In methanolic extract MIC for *Staphylococcus aureus* was 12.5 mg/ml whereas there was no effect against *Salmonella typhi* and *Pseudomonas aeruginosa* in the study which did not coincide with the present study where the MIC for S*taphylococcus aureus* was 5 mg/ml for *Salmonella typhi* was 5 mg/ml and for *Pseudomonas aeruginosa* was 7.5 mg/ml. This dissimilarity may be attributed to the difference in the procedure for preparation of extract or difference of strains of organisms by the aforementioned researchers.

Conclusion

Extracts from Indian bay leaf have the potential to be developed as a medicinal agent in preventing bacteria-related disorders. Additional research is needed to identify and isolate the biologically active components in Indian bay leaves that are responsible for this antibacterial action. The practice of employing medicinal plants like Indian bay leaf as supplementary or alternative medicine in underdeveloped nations may lower not only the clinical burden of drug resistance development but also the side effects and cost of the treatment when compared to manufactured drugs.

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