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Antibiofilm Activity of Chromatographic Fractions from Extracts of *Mangifera indica* and *Mentha piperita* Leaves against the Clinical Isolates of *Staphylococcus aureus*

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Abstract

Bacterial biofilms contribute significantly to antimicrobial resistance and therapeutic failure. This study investigated the inhibitory effects of chromatographically fractionated *leaf extracts of Mangifera indica (mango) and Mentha piperita (mint) against biofilm formation by clinical isolates of staphylococcus aureus. Methanolic extracts of both plant materials were subjected to column chromatography to obtain distinct fractions, which were subsequently evaluated for antibiofilm activity using microwell diffusion assays in tryptic soy broth. Ciprofloxacin served as the positive control while DMSO was employed as the negative control. Among the mango extract fractions F3A demonstrated the most potent antibiofilm activity with 98.14 ≤ 0.53 % inhibition at 50 mg/ml. Similarly, the mint extract fraction F5 exhibited the highest biofilm inhibition at 86.94 ± 0.51 %. Notably, fraction 1 yielded the highest percentage recovery among all fractions tested. These results indicate that M. indica and M. piperita leaf extracts contain bioactive compounds with significant antibiofilm properties against S. aureus. The finding support the traditional antimicrobial applications of these plants and suggest their potential as sources of novel antibiofilm agents. Further studies to identify thespecific bioactive compounds aand elucidate their mechanisms of action are required.*

Keywords: Antibiofilm: Chromatography: *S. aureus*: Resistance: Bioactive

INTRODUCTION

Adhered cell is a complex structure of micro biome with different bacteria colonies or single type of cells in a group, adhered to the surface (Divakar *et al*., 2019). Microbial adhesion is considered the first step for biofilm formation. This structure constitutes a protective milieu against environmental stresses and human host defenses. The architecture of biofilms have positively charged ions, such as Ca^{2+} or Mg^{2+}, which form supportive cross bridges between polymers and allow biofilms to grow to thicknesses of up to 300 µm (2021). *Staphlococcus aureus* is a gram positive bacteria found in grape-like (staphlo-) clusters hence, the name staphylococcus. The natural habitat of *S. aureus* in humans is the skin and nasopharynx. It can cause a wide variety of infections involving skin and soft tissues, endovascular sites and internal organs. *S. aureus* is an important pathogen in the community and in hospitals, causing high morbidity and mortality. *S. aureus* is one of the common bacteria that forms biofilm to protect themselves from external forces (Noor *et al*., 2025). The first step in biofilm formation is the process where planktonic bacteria get close to the material surface through physical forces which include attractive van der Waals and repulsive electrostatic forces and chemical forces which involves the cohesion between microbial cells (Ghazay.and Mamdouh, 2021). The appendages (e.g., fimbriae, pili, and flagella) strengthen the attachment between the bacterial cell and the surface once the bacteria get in touch with the material surface (S. *aureus* skin infection is a frequent and recurrent problem in children with the common inflammatory skin disease atopic dermatitis (A.D.). The first step during colonization and infection is bacterial adhesion to the cornified envelop of corneocytes in the outer layer, the stratum corneum (Krista *et al*., 2022).

Medicinal plants are important sources of therapeutic remedies of various diseases. .*Mangifera indica* contains pharmacologically active chemical constituents. Mint is a name for over a dozen plants species, including peppermint and spearmint that belongs to the genus *Mentha.* The plant contains bacteriostatic properties and anti-adhesive activity which makes it an interesting component of soft drinks to counteract the formation of bacterial cell aggregates, flocks, haziness, and biofilms. It is also important in extending the shelf –life of soft drinks. Research has shown that several health benefits of mint come from applying it to the skin, inhaling its aroma or taking it as a capsule because the plants have relatively no toxic or adverse effects when used by humans or animals (Irum *et al*., 2022). *S. aureus* have

the ability to develop biofilms as well as communicate using quorum sensing in a bacterial cell density-dependent manner (Yuriko *et al*., 2024).

This research work is aimed at determining the Antibiofilm Activity of Chromatographic Fractions from Extracts of *Mangifera indica* and *Mentha piperita* Leaves against the Clinical Isolates of *Staphylococcus aureus*

MATERIALS AND METHODS

Plant Sample Collection and Identification

Mango Leaf and Mint Leaf

Mango leaf was harvested fresh from a garden in Maiduguri road, Kaduna metropolis while fresh mint leaf was purchased from a local market at Taiwo road, Kaduna metropolis. Fresh mango and mint leaves were identified in the department of Biological Sciences, Kaduna State University, KASU and assigned voucher specimen numbers of KASU/BSH/554 and KASU/BSH/673, respectively. The leaves were air dried for 3 weeks and pulverized differently using pestle and mortar and then stored at room temperature (25°C) prior to the extraction.

Preparation of Methanol Extracts of Mango and Mint Leaves

The extraction was done using methanol and aqueous as solvents and in accordance with the method described by Prashant *et al*., 2011. Exactly 70 g and 60 g of grounded mango and mint leaves were macerated in 300 L and 250 L of methanol (99.8% purity), respectively. Then kept at room temperature with occasional shaking for 7 days. After 7 days the solution was filtered using muslin cloth which is further filtered using what man filter paper and funnel to get a clear extract. The filtered solution was transferred to the beaker and then put in the water bath (model HH420 Pec Medica USA) to evaporate the methanol. The solution was evaporated to dryness and stored at room temperature for further use.

Biochemical Identification of Clinical Isolate *of Staphylococcus aureus*

Clinical isolate of *Staphylococcus aureus* was obtained from the laboratory unit of St. Gerard's hospital, Kakuri, Kaduna and identified using catalase and methyl red tests as described by MacFaddin, 2000. *Staphylococcus aureus* is known to possess catalase enzyme which converts hydrogen peroxide into oxygen and water. Formation of bubbles indicate a positive test for *S. aureus*. Methy red test for *S. aureus* is based on production of sufficient acid during the fermentation of glucose. It involves growing of *S. aureus* in a broth medium containing glucose. A positive result is shown by utilization of glucose with production of stable acid and changes in the colour of methyl red from yellow to red

In the methyl red test, the test bacteria was grown in a broth medium containing glucose. If the bacteria has the ability to utilize glucose with production of a stable acid, the color of the methyl red changes from yellow to red, when added into the broth culture.

Column Chromatographic Separation of Bioactive Components from Crude Methanolic Extracts of Mango and Mint Leaves

Column chromatographic seperation of bioactive components from crude methanol extracts of mango and mint leaves were carried out concurrently according to the method described by Devika and Koilpillai (2015). Two solvent systems were used for the fractionation. About 50 g of silica was made into slurry with ethylacetate and the placed in a cylindrical tube that is plugged at the bottom by a piece of glass wool using a funnel. The mango and mint extracts were dissolved separately in ethylacetate. A pipette was used to add the sample to the top of the column. The stopcock was opened and the solvent was allowed to drain by opening the stopcock. A very small amount of solvent was used to wash down any sample that may have clung to the sides of the column. This additional solvent was also drained. The sample was eluted through the column by using a pipette to add 4ml of the solvent. A funnel was placed at the top of the column and the remainder of the column was filled with the solvent in each set up. The mobile phase was collected as it drained from the column into test tubes. Additional solvent with increase polarity starting from absolute ethylacetate (100%), ethylacetate : Methanol (4:1), 3:2, 2:3,1:4), and absolute Methanol (100 %) were added to the top of the column as needed until all the desired compounds have eluted from the column.

The rate of the movement of the components is expressed as:

R_f = the distance travelled by solute/ the distance travelled by the solvent

R_f is the retardation factor.

Fractions with the same Rf values were pooled together for antimicrobial assay against clinical isolates of *S. aureus*

Formation and Inhibition of Adhered Cells Assay

Formation and inhibition of adhered cells were performed according to the method described by Ting-Yu *et al*. (2022). All fractions were reconstituted using DMSO (Dimethyl sulfoxide) to prepare stock solution of 200 mg/ml and then two fold dilution to get 100 mg/ml and 50mg/ml of the fractions. Standard drug (ciprofloxacin) was used as positive control. Plate containing only the adhered cells culture was used as negative control.

After preparation of the different concentrations of each fraction recovered, 100 µL of tryptic soy broth was added to each reaction well of the 96-well micro titre plate. Then 100 µL of the *S. aureus* isolate equivalent of 0.5 McFarland standard (1.0×10^{-8} CFU/ml) was added into the wells. The plate was incubated at 37 ºC for 90 minutes to allow for adhesion of microbial cells then, 100 µL of phosphate buffer saline (pH 7.4) was added to each well to wash and remove non-adherent cells.Then 100 µL broth was added along with 100µL fractions of mango and mint extracts, and then incubated for 48 hours for the eradication of adherent cells. Positive and negative control was done using ciprofloxacin and DMSO respectively.

After 48 hours of incubation, phosphate buffer saline was added, and then aspirated again before the addition of crystal violet for the cells to bound, and then ethanol was added to make the cells dissolve. The absorbance was taken at 630 nm.

Statistical Analysis

Results are mean values $\pm$ Standard deviation. The data was statistically analyzed by one-way ANOVA using online software for Graph pad prism version 7. A 95 % confidence interval was used to determine the statistical difference between the mean values.

Results and Discussion

The fractions with same RF values were pulled together resulting into eleven (11) fractions from both mint and mango extracts. F1 had the highest percentage recovery of 35.14 as shown in table 1.

The fractions of crude methanol extracts of mint and mango leaves showed inhibition against adhered cells of *S.aureus* at 25 50 and 100 mg/ml. F3A at 25 mg/ml and F2 at 100 mg/ml showed very high inhibition against adhered cells formed *S. aureus* which was not significant ($P \geq 0.05$) to the adhered cells inhibition by the standard drug F7. There was significant ($P \leq 0.05$) increase in the percentage inhibition of adhered cells formed by *S. aureus* by F5 (crude methanol extract of Mint) compared to F6 crude methanol extract of Mango) this complies with the findings of Matasyoh *et al.*, 2007. All fractions of methanol extracts of mango and mint leaves showed levels of efficacy against *S. aureus*, however, at different concentrations this conforms with the findings of Azwanida 2015 and Zhang *et al.*, 2019 where they reported the antibacterial efficacy pattern of plant extracts at 50 mg/ml. It is possible that the fractionated extract of mango and mint leaves have active components that interfered with the synthesis or expression of filament-associated adhesins on the surface of the cells, thereby reducing induced adhesion and total biomass, this is in conformity with the report of Maimuna *et al*., 2020 on biofilm eradication.

Table 1. Percentage Recovery of Fractions of Methanol Extract of Mango and Mint Leaves

S/NO.	FRACTIONS	% RECOVERY
1.	F1A	15.48
2.	F2A	16.10
3.	F3A	4.38
4.	F4A	10.94
5.	F5A	18.84
6.	F6A	6.64
7.	F7A	3.64
8.	F1	35.14
9.	F2	27.14
10.	F3	27.14
11.	F4	9.74

KEY: F1, F2, F3and F4 are pooled fractions from mint extract while F5A, F6A, F7A, F1A, F2A, F3A and F4A are pooled fractions from mango extract

Table 2: % Inhibition of Different Concentrations of Chromatographic Fractions of Methanol Extracts of Mango and Mint Leaves against Adhered Cells of *S. aureus*

S/No.	FRACTIONS	CONC.(25 mg/ml) (% Eradication)	CONC. (50 mg/ml) (% Eradication)	CONC. (100 mg/ml) (% Eradication)
1.	F1A	20.66 ± 0.165^{i2}	21.54 ± 0.068^{i2}	38.50 ± 0.174^{f1}
2.	F2A	27.45 ± 0.081^{gh3}	36.47 ± 11.704^{h2}	51.50 ± 0.1069^{e1}
3.	F3A	81.34 ± 0.639^{b2}	70.29 ± 0.996^{b3}	98.14 ± 0.539^{a1}
4.	F4A	29.72 ± 0.570^{h3}	54.62 ± 0.395^{d2}	60.01 ± 0.207^{d1}
5.	F5A	39.22 ± 0.209^{g2}	35.70 ± 0.133^{h3}	73.80 ± 0.050^{c1}
6.	F6A	55.62 ± 0.361^{c1}	59.06 ± 0.561^{d2}	64.70 ± 0.564^{d2}
7.	F7A	41.98 ± 0.094^{ef3}	71.04 ± 3.970^{b2}	84.69 ± 0.046^{b1}
8.	F1	41.62 ± 0.274^{ef2}	40.78 ± 4.754^{g2}	60.36 ± 0.596^{d1}

9.	F2	45.56 ± 1.731^{a1}	62.53 ± 40.237^{e3}	97.05 ± 1.501^{d2}
10.	F3	39.54 ± 8.443^{e2}	49.14 ± 0.279^{g3}	71.37 ± 24.391^{c1}
11.	F4	42.32 ± 0.317^{f2}	73.83 ± 21.101^{b1}	72.92 ± 0.363^{c1}
12.	F5	64.25 ± 0.485^{b2}	75.33 ± 0.263^{c3}	86.94 ± 0.510^{b1}
13.	F6	41.19 ± 0.649^{cd1}	49.84 ± 0.541^{f2}	50.91 ± 0.369^{e1}
14.	Ciprofloxacin	100.01 ± 0.619^{a1}	100.00 ± 0.230^{a1}	100.00 ± 0.447^{a1}
15.	Dimethyl sulfoxide	14.94 ± 9.338^{c1}	21.24 ± 2.391^{h3}	24.57 ± 1.915^{f2}

Values having different superscripts (number) across the row and (alphabets) down the column are significantly different ($P\leq0.05$).

KEY:F1, F2, F3and F4 are pooled fractions from mint extract while F5A, F6A, F7A, F1A, F2A, F3A and F4A are pooled fractions from mango extract. F5 Mint methanol (crude extract) F6 Mango methanol (crude extract) ,Ciprofloxacin : positive control , Dimethyl sulfoxide : negative control.

CONCLUSION

Fraction of mango extract (F3A) inhibited the formation of *adhered cells formed by S. aureus at 100 mg/ml by* 98.14±0.53 % while the most potent fraction of the mint extract (F5) had the highest % inhibition of adhered cells at 86.94 ± 0.510 %. Therefore, both mango and mint leaves have the potential to inhibit the formation of adhered cells by *S. aureus*. The most potent fractions of the mango and mint fractions should be characterized to reveal the bioactive components present.

AUTHORS CONTRIBUTIONS

Zubairu Maimuna conducted the analysis on formation and inhibition of adhered cells while Ahmed Aisha prepared the extracts and Biochemical identification of the microbes. Fractionation of the extract using column chromatography was carried out by both authors.

CONFLICT OF INTEREST

There was no any conflict of interest between the authors.

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