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IN-VITRO EVALUATION OF *CATHARANTHUS ROSEUS* FOR POTENTIAL ANTIBACTERIAL ACTIVITY AND INHIBITION OF CELL SURFACE HYDROPHOBICITY OF BACTERIAL ISOLATES

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ABSTRACT

The present work aims to evaluate the antimicrobial potential of solvent extracts of *Catharanthus roseus* on some food pathogens. Agar well diffusion method has been used to determine the antimicrobial activities against biofilm and betalactamase producing bacteria. Among the 3 solvent extracts, methanol extract showed exciting activity against all bacterial isolates. The lowest MIC value was observed while using methanol extract; 0.25mg/ml was MIC for *P.aeruginosa, K.pneumoniae* and *S.aureus.* Furthermore, isolates of cell surface hydrophobicity were reduced with those plant extracts. Among the solvent extracts, acetone extract showed better activity against bacterial hydrophobicity. This study suggested the suppressive potential of plant extracts against the bacterial isolates and their hydrophobicity, hence can be used as natural alternative preventives to control the bacterial isolates.

Keywords: Food pathogens, Biofilm, Betalactamase, *Catharanthus roseus,* Hydrophobicity

INTRODUCTION

Drug resistance in every corner of the world is growing to alarmingly high levels. New mechanisms of resistance are evolving and expanding throughout the world, endangering our capacity to treat increasing infectious diseases. A growing list of infections, such as pneumonia, tuberculosis, gonorrhea, and foodborne diseases is difficult to treat and sometimes impossible because antibiotics are less effective. Where in antibiotics can be acquired without a prescription for human or animal use, this way spread of resistance becomes worse. Similarly, in a number of countries where there are no formal treatment protocols, antibiotics are often over-prescribed and over-used by health care staff and veterinarians. Furthermore, a lack of new drug development by the pharmaceutical industry due to decreased monetary incentives and tough regulatory requirements **[1]**.

The medical organization of Centers for Disease Control and Prevention (CDC) has announced a number of microorganisms as imparting urgent, serious, and regarding threats, many of which are already accounted for setting a huge medical and monetary burden on the health care system, patients, and their families **[2]**. There is much need for coordinated efforts to revive this war, renew its research efforts, and take steps to manage the crisis. Steps should be taken at all levels of society to reduce impact and restrict resistance spread with drugs from plant sources **[3]**.

As the percentage of multiple antibiotic resistance isolates increases, an alternative is needed for the eradication. The World Health Organization (WHO) estimated that more than 80% of the world's population still depends upon conventional drug treatments for various diseases **[4]**. Products from medicinal plants do not have the opposite effect but rather work for durable and effective trendy fitness.

Catharanthus roseus is belonged to the family of Apocynaceae, which is commonly available in the subcontinent. *Catharanthus roseus* has a variety of medicinal properties, such as antibacterial, antifungal, antiviral **[5]** and anticancer activity **[6]**. The different parts of *C. roseus* (leaf, stem, flower, and root) had antibacterial activity against gram positive and negative bacteria **[7]**. Despite the antibacterial evaluation, there have been fewer studies to date on the inhibition of biofilm producing bacteria with the leaf of *C. roseus*. The present investigation is targeted on the screening of leaf extracts of the *C. roseus* for its antibacterial capability adopting the usual antibacterial assay method.

MATERIALS AND METHODS

Collection of food samples

The homogenate meat samples were streaked on the chromogenic media and incubated aerobically at 37°C for 24 hours. The loop full of fruit juice samples also inoculated in to same media. The isolated bacteria were identified based on colony morphology, Gram staining reaction, and biochemical characteristics using established standardized methods according to Bergey's Manual of determinative bacteriology.

Isolation of biofilm producing isolates

The agar medium was prepared by adding 37g of the BHI powder, 50 g of sucrose and 10 g of agar, and Congo red (0.8 g/L) in 1 L of distilled water. The mixture was then autoclaved for 15 min at 121°C. Then the media was poured into the petri plates and allowed to solidify. Once the media had solidified, the plates were inoculated with the microorganisms and incubated at 37°C for 24 h. The plates were observed the next day, the organisms were considered positive (biofilm-producers) when they produced black colonies on the agar and negative (non-biofilm producers) when they produced pink, or red-orange colonies on the Congo red agar **[8]**.

Isolation of betalactamase producing isolates

Penicillin solution was dispensed in 0.5ml volume in small test tubes. Test bacteria were removed with a loop from an overnight culture on solid medium and suspended in the Penicillin solution (1000U) to give a density of at least 10^4CFU/ml. After one hour at room temperature, two drops of the starch indicator were added to the suspension, followed by one drop of Iodine reagent. A positive reaction was indicated by the disappearance of blue color immediately. Persistence of blue color for longer than 10 minutes constituted a negative test **[9]**.

Collection of plant material and extraction

The leaf of *Catharanthus roseus* has been used in the present study and which were collected from the Salem area, Tamilnadu, India. The dried leaf was powdered and extract with soxhlet extractor successively with 200 ml of Methanol, Acetone, and Chloroform until colourless extract was obtained at the top of the extractor. Each extract were concentrated at 50 °C under reduce pressure in a vacuum to obtain each plant extract. Extracts were maintained at a temperature between 2 - 8°C for further studies **[10]**.

Antimicrobial activity assay

The antibacterial activity studies were carried out by the agar well diffusion method **[11]**. The Mueller Hinton Agar (MHA) media was sterilized at 121°C under 15 lbs pressures for 30 minutes. After cooling to about 65°C, 20ml of the medium was poured in Petri-dish. Then, 5 wells (6mm diameter) were made by using a sterile cork borer. The 4 different concentrations (1mg, 2mg, 3mg, and 4mg) of the plant extracts were loaded in the wells. Ampicillin and DMSO were served as control. The plates were then incubated at 37°C for 24h. After incubation, the inhibition diameter was measured.

Determination of MIC

Minimal inhibitory concentrations (MIC) of different solvent extracts were determined using the resazurin-based method described by Pulipati *et al*., **[12]** with some modifications. Change of color was observed and recorded. The lowest concentration prior to colour change was considered as the Minimum Inhibitory Concentration (MIC).

Statistical analysis

The values represented in the results are mean ± standard deviation (SD) of each measurement.

Determination of Surface hydrophobicity after plant treatment

Bacterial surface hydrophobicity was measured using the bacterial adhesion to hydrocarbon (BATH) assay as previously described Hui and Dykes **[13]** with modifications. The overnight culture was centrifuged and the pellet was washed twice in phosphate buffer saline (PBS) pH 7.4 then the pellet was resuspended in same buffer with plant extract (5mg/ml) to an optical density at 550 nm. The same procedure was followed and no added herb extracts which were as control. Each bacterial suspension was then incubated at room temperature for 1 h. Then a 3-ml sample of each suspension was collected and the A_0 at 550 nm was determined using PBS as the blank. A volume of 1 ml of xylene was added to the 3-ml cell suspension, and the mixture was vortexed for 2 min. The phases were then allowed to separate for 1 h. The absorbance of the aqueous phase (A) was again determined. Results were expressed as **% attachment to xylene = (1 - A/ A_0) X 100.**

RESULTS AND DISCUSSION

Totally 22 isolates of 6 genera were observed from meat (chicken, goat, and fish) samples and fresh fruit juice (Apple, orange and Mosambi) samples. Among the samples, the meat had the highest predominant bacterial genera than fruit juices. In the case of genera vice, *E.faecalis* and *K.pneumoniae* were highly predominant **(Figure 1)**. The different bacterial genera from poultry meat, goat meat, and fish meat were observed in 2018, by Nagarajan *et al*. **[14]**. This bacterial contamination was mainly due to the unhygienic condition of the butcher’s store. Another previous study Shrestha *et al* **[15]** observed the bacterial genera from chicken meat samples.

Biofilm is an extra-polysaccharide matrix produced by bacteria that plays an important role in the avoidance of the host immune system and resistance to antimicrobial agents, leading to persistent and persistent infections. In this present study, all isolated bacterial genera were subjected to biofilm assay with Congo red agar plate method. Among the 22 isolates, 10(45.4%) of were biofilm producers. The highest count of producers was observed in *E.faecalis* and *K.peumoniae*. Several researchers have been found the biofilm producing isolates of K*.pneumoniae, Salmonella* sp and *E.coli* from meat samples **[16-18]**. Moreover, betalactamase producing isolates also observed in the present study, out of 22, 11(50%) were showed positive for betalactamase producers.

The coexistence of biofilm along with both beta-lactamases producing strains was found to be 32%. The biofilm matrix has been reported to enhance the expression of resistant genes like beta-lactamase. Several studies have documented that biofilm-producing strains generally produce the betalactamase, and that they have been found to have high antibiotic resistance. In a study conducted by Subramanian *et al* **[19]** in India, biofilm producing isolates were reported to produce betalactamase, which compared to non-biofilm producing isolates.

The biofilm producing isolates are difficult to inhibit **[20]** and which helps both the proliferation of microbes and the aggressiveness of infections **[21]**. Therefore, the strains that cause these adverse effects must be weeded out by the use of sharp substitutes. Plant-derived antimicrobials are natural and highly impervious to their protective functions, this is considered safer than synthetic compounds **[22]**. In view of this point, our study was carried out with the leaf of the plant aimed at preventing food bacterial pathogens and that produce adverse effects.

In the present study, both virulence of biofilm and betalactamase producing isolates were subjected to antimicrobial activity with the three different solvents extract of a leaf of *C. roseus*. Among the three solvent extracts, methanol showed the highest antibacterial activity against all isolates, the zone of inhibition was ranged from 9.66±0.471mm to 21.16±0.632mm. The highest zone of inhibition was observed against to *P.aeruginosa* and followed by *K.pneumoniae* and *S.aureus*. Currently, all isolates except *E.faecalis* have been suppressed with extract concentration of 3mg/ml **(Table 1)**. The present results were similar to the earlier results of Shanmugaraju and Bhakyaraj **[23]**. They were inhibited the *S.aureus* with a leaf of *C. roseus.* In a previous study of Kabesh *et al*., **[24]** and Voukeng *et al* **[25]** also inhibited the *P.aeruginosa* with the leaf of *C. roseus*. At the same time, according to Govindasamy and Srinivasan's study **[26]**, bacterial isolates were not inhibited with a leaf of methanol extract, especially *S.aureus* and *E.coli* were not well suppressed, and this was completely contrary to our present study.

The MIC was carryout for all isolates; 0.25mg/ml was MIC for *P.aeruginosa, K.pneumoniae* and *S.aureus*. Among the 5 genera, the highest MIC was observed against *E.faecalis* (2.5mg/ml). The MIC values reported here are less than the ones found by Goyal **[6]** for leaf extract of methanol against *K. pneumonia*, at the same time their *S.aureus* MIC result agrees with the results of our study.

Acetone extract performs a second function in inhibitory activity, which exhibiting the zone of inhibition was ranged from 11±0.816mm to 18.83±0.849mm **(Table 2)**. The *E.coli* was slightly suppressed with acetone extract and all isolates were suppressed only with 4mg concentration of extract. The previous study of Sathiya *et al*., **[27]** were inhibited the *B. subtilis, Klebsiella sp., Staphylococcus aureus* and *Streptococcus* sp, they have inhibited the strains with 250mg/ml concentration of acetone extract. The lowest MIC was observed against *S.aureus* (1.0mg/ml), and 2.0mg/ml MIC was for *P.aeruginosa* and *K.pneumoniae.*

In the case of chloroform extract, better activity was observed against to *K.pneumoniae* and *E.coli*, which exhibiting the zone of inhibition was 15.16±0.632mm **(Table 3)**. While using 3mg/ml concentration of extract, 2 genera were suppressed; all isolates were suppressed while using 4 mg concentration. The best MIC value (2.0mg/ml) for the chloroform extract was seen against *E.coli* and *K.pneumoniae*. The MIC values of acetone and chloroform extract were not correlated to Gaur *et al*, **[28]**, they were observed lower than in our study.

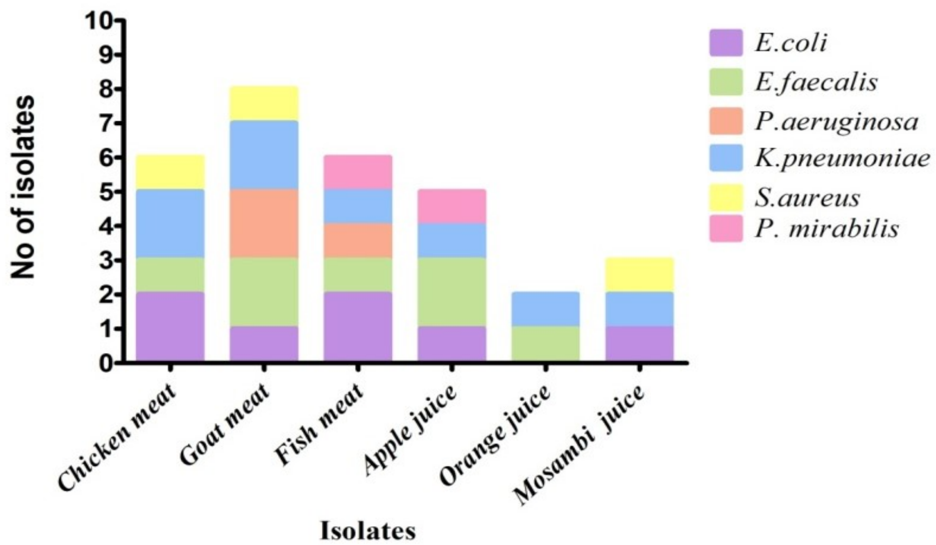

Figure 1: Prevalence of bacterial isolates from various food samples

Table 1: Antibacterial activity of methanol extracts of *Catharanthus roseus*

S. No.	Isolates	Different concentration of Leaf extracts (mg/ml)				Ab	DMSO
		1	2	3	4		
1	*E.coli 2*	-	10±0.816	13.16±0.632	16±0.816	9±0.816	-
2	*E.coli 4*	11±0.816	14.5±0.707	16.33±0.471	18.83±0.849	10±0..471	-
3	*E.faecalis 3*	-	-	9.66±0.471	12.16±0.849	-	-
4	*P.aeruginosa 5*	15.16±0.632	17.5±0.408	21±0.816	21.16±0.632	-	-
5	*K.pneumoniae 1*	10±0.816	11.83±0.632	15±0.408	17±0.816	-	-
6	*K.pneumoniae 2*	-	13.16±0.632	15.33±0.471	19.5±0.408	-	-
7	*S.aureus 1*	10.33±0.942	12.33±0.632	15±0.816	19.33±1.247	-	-

Table 2: Antibacterial activity of Acetone extracts of *Catharanthus roseus*

S. No	Isolates	Different concentration of Leaf extracts (mg/ml)				Ab	DMSO
		1	2	3	4		
1	*E.coli 2*	-	-	-	11.66±0.849	9±0.816	-
2	*E.coli 4*	-	-	-	11±0.816	10±0.471	-
3	*E.faecalis 3*	-	-	-	11.66±0.471	-	-
4	*P.aeruginosa 5*	-	-	11.5±0.408	16±0.816	-	-
5	*K.pneumoniae 1*	-	-	11.16±0.632	14±0.816	-	-
6	*K.pneumoniae 2*	-	-	11.83±0.632	15±0.816	-	-
7	*S.aureus 1*	-	12.5±0.408	16±0.816	18.83±0.849	-	-

Table 3: Antibacterial activity of chloroform extracts of *Catharanthus roseus*

S. No	Isolates	Different concentration of Leaf extracts (mg/ml)				Ab	DMSO
		1	2	3	4		
1	*E.coli 2*	-	-	12.5±0.408	15±0.816	9±0.816	-
2	*E.coli 4*	-	-	11.6±0.632	15±0.816	10±0..471	-
3	*E.faecalis 3*	-	-	-	12.66±0.471	-	-
4	*P.aeruginosa 5*	-	-	-	13.33±0.471	-	-
5	*K.pneumoniae 1*	-	-	11.5±0.408	15.16±0.632	-	-
6	*K.pneumoniae 2*	-	-	12.66±0.471	15±0.816	-	-
7	*S.aureus 1*	-	-	-	14.33±0.471	-	-

In this study, no one isolates were suppressed while using control agents of ampicillin (except *E.coli*) and DMSO. Among the 3 solvents, chloroform extracts showed less activity than other solvent extracts. In 2013, Chaman *et al* **[29]** determinate the chloroform extract of the flower part has not inhibited that microbe. This was seen in different studies into the polarity of antibacterial compounds crucial to their activity **[6]**. Jeyaseelan and his coworkers explain that the inhibitory activity depended on the extraction procedure, plant part, physiological and morphological state of the plant, extraction solvent and microorganism tested **[30]**.

For many bacterial infections, various pathogenesis stages can be responsible, such as mucosal surface adhesion, tissue colonization, and subsequent invasion. Adhesive strains often possess high cell surface hydrophobicity (CSH) as determined by SAT and BATH assays method. The hydrophobic properties are important for bacterial attachment and aggregation of isolates. Previously some antibiotics were utilized for the affect of CSH and tissue adhesive properties of some bacterial pathogens with antibiotics, but it was a chance to development of antibiotic resistance character. In this situation, the combined therapy with medicinal plant extracts may help to postpone the issue **[31]**.

In the present study, all isolates were subjected to BATH assays and all isolates were displayed from different hydrophobicity to each other. The percentage of hydrophobicity was ranged from 63.38 % to 72.91%. The CSH was reduced while treated with plant extract of *C. roseus*. The highest reduction was observed while using the methanol extract, especially CSH highest reduced in *P.aeruginosa*. The second most percentage of hydrophobicities was reduced while using acetone extract. In the case of chloroform extract, slightly reduced the hydrophobicity value than other solvent extracts. The earlier study was demonstrated the antimicrobial activity of essential oil and disruption of bacterial hydrophobicity **[32]**. According to an earlier report of WHO (2014), *C. roseus* was breaking of peptidoglycan chain of bacterial cell wall and transpeptidase, which catalyzes the cross-linking of peptidoglycan in the cell wall **[33]**.

Decreases in hydrophobicity and affixment have been attributed to the ravagement of bacterial adhesive structures, such as fimbriae or flagella (both are highly hydrophobic), by compounds such as tannins, saponins, and flavoniods. Antiglucosyltransferase in flavonols prevents insoluble glucan and formation of stickiness, which encourages bacteria to stick to surfaces **[13]**. In this present study, above-mentioned phytochemicals were obtained from 3 different solvents extracts.

CONCLUSIONS

As present findings that extract of methanol solvent from *C. roseus* has high potential antimicrobial activity against biofilm and betalactamase producing isolates, rather than other extracts. In addition, the extracts in this study suppressed the hydrophobicity of bacterial isolates, leading to a reduction in bacterial adhesion from the host. In the next part of the investigation, the main ingredient will be extracted from this plant extract and used against different bacterial species, and these ingredients may be used as a substance in commercial sanitizer to prevent bacterial adherence.

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