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A Quest of Anti-Biofilm Activity of *Zingiber officinale* Root and *Coriandrum sativum* Seed Extract Against Clinical Isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

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Abstract: ***Biofilm formation by Microbe causes a variety of infections which complicates the antimicrobial therapy. In the present study, the Pseudomonas aeruginosa and Staphylococcus aureus strains have been isolated from the clinical samples and characterized. Zingiber officinale root and Coriandrum sativum seed's hot and cold extract was prepared in ethanol and water. Visible eye scoring was given to judge anti-biofilm activity. P. aeruginosa in treatment with Zingiber officinale root extract displayed maximum anti-biofilm activity whereas in study with S. aureus, no encouraging results were obtained.***

Keywords: Pseudomonas aeruginosa, Staphylococcus aureus, Zingiber officinale and Coriandrum sativum

1. Introduction

Extracellular polymeric substances, EPS secreted by microorganisms attached to either an abiotic or a biotic surface to form biofilm community. In past years, studies regarding bacterial structure and behaviour have used planktonic cells that are cultivated in liquid or solid media and recent studies have shown that, naturally most bacteria are attached to surfaces as sessile form especially in biofilm (Costerton, 2005). Many bacteria can exist in planktonic or sessile forms, biofilm formed by aggregation of such sessile bacteria (Watnick, 2000; Costerton *et al*, 1978). With the help of adhesins and extracellular polysaccharides (EPS) organisms attach themselves to form microcolonies. Infection due to biofilms forming pathogen is difficult to eradicate because they resistance to antibiotics and host defence mechanism (Allison *et al*, 2000). Majority of biofilms associated diseases are linked with the implantable devices like, catheters, prostheses, heart valves, or impairment of the host defence systems such as cystic fibrosis patients (Costerton *et al*, 1999). 65% of human infections are involved with biofilms (Potera, 1999).

Some biofilms are beneficial to mankind including sewage treatment on contrary biofilms also pose lot of problems to mankind such as corrosion of pipes, and chocking water filters, infection of medical implants and causing diverse chronic diseases in humans. Wash hand basins in the kitchen, on teeth, contact lenses, water pipes or plumbing lines and gut epithelium are few stations of biofilm. (Coghlan, 1996). Lactobacilli in the vagina prevents the existence of other bacteria because of production of acids, bacteriocins, hydrogen peroxide and biosurfactants (Reid, 2001, Wang, 2000, McLean and Rosenstein, 2000). *Streptococci* and *Actinomyces spp* forms dental plaque which protect other species against colonisation by other bacterial pathogens (Marsh and Bradshaw, 1995; Kolenbrander, 2000). Most common causative agent *S. epidermidis* is associated with implant infections (Rupp and Archer, 1994). Chronic bacterial prostatitis and prostatic calcifications are caused by bacterial biofilms (Mazzoli *et al*, 2009).

2. Literature Survey

UTI (Urinary Tract Infection) is linked to biofilm formation due to indwelling catheters used for treatment (Hatt *et al*, 2008, Mazzoli *et al*, 2009). A scanning electron microscopy study revealed the presence of biofilm on the sinus mucosa of patients infected with *P. aeruginosa* (Cryer *et al*, 2004, Marcus *et al*, 2008). *P. aeruginosa* can cause a wide range of infections like, wound infections, pulmonary infections, medical-device-related infections, bacteremia, and urinary tract infections (Bodey *et al.*, 1983). Due to ability to form biofilm, *P. aeruginosa* tolerate to antimicrobial agents to a large extent (Costerton *et al.*, 1995; Costerton *et al.*, 1999). On the same side *S. aureus* can cause many different infections, that can be mild and superficial to those that are life threatening or fatal. *S. aureus* cause the infection derived from a innate flora, or may be community or hospital acquired thus *S. aureus* can causes of both community and hospital-acquired infections (Boyce, 1997; Projan and Novick, 1997). Infections caused by methicillin resistant *S. aureus* (MRSA) are particularly problematic because of resistance to conventional most antibiotics therefore, leaves few treatment options. *S. aureus* can infect virtually to body site and every human organ system (Archer, 1998). *S. aureus* can also cause rigorous life-threatening infections of the joints, central nervous system, bone, circulatory system, urinary tract, gastrointestinal tract, respiratory tract, brain abscesses, endocarditis, meningitis, and infections of the eyes (Ing *et al.*, 1997; Archer, 1998).

The present study was focused to investigate the anti-biofilm activity of *Zingiber officinale* root and *Coriandrum sativum* seed water and ethanol's hot and cold extracts on *S. aureus*

and *P. aeruginosa*. The present study can help drug designer to choose natural products to overcome the ailments associated with biofilm formation.

3. Methodology

Isolation and Identification of Test Organism

Clinical samples of urine, pus, blood and sputum sample were collected from different regional pathology laboratories. A collected sample was immediately enriched in sterile nutrient broth (at 37 ^{0}C for 48 hrs). After incubation, loopful of culture was plated on selective media. For selection of *S. aureus*, nutrient agar containing one percentage (1 %) of glycerol monoacetate was been used for primary isolation of *S .aureus* whereas, for isolation of enriched broth was streaked on Pseudomonas Isolation Agar (PSI, Himedia- Mumbai). Typical colonies of *S. aureus* and *P. aeruginosa* were picked up and maintained on nutrient agar slant for further identification.

Isolates were identified on the basis of morphological, cultural & biochemical characteristics and the results were compared with Bergey's Manual of Determinative Bacteriology 9th edition.

Inoculums Preparation

A loopful of culture from isolated bacterial slants was inoculated in into fresh sterile nutrient broth (5ml) and incubated at 37 ^{0}C for for 6-8 hrs. Turbidity of nutrient broth was adjusted to 0.5 McFarland standards (1.5×108 CFU/ml) by standard procedure. This suspension was used as inoculum.

Collection and Processing of Plant Material

Plant Material was collected from regional market, identified and processed. Plant material was washed with tap water and dried in shade for a week and pulverized in a mechanical mortar and pestle. Material was stored in air tight container in dark and dry place.

4. Preparation of Herbal Extracts

Cold Extracts

Exact 5 g of each plant material was macerated in 50 ml of methanol and water separately for 24 h with intervals of shaking. After 24 h macerated solvents were then filtered through Whattman No.1 filter paper with suction. The filtrate was evaporated to dryness under reduced pressure at 40^{o}C.

Hot Extract

Soxhlet extraction procedure was adopted for extraction. A 5g ground herb placed in a thimble loaded into the soxhlet extractor installed over constant temperature water bath maintained at 60 ^{0}C. The cycles were continued for 25 h with 200 ml solvent maintained continuously refluxing over the sample. After the extraction the solvent was removed from the solute mixture by reduced pressure with rotary evaporator to obtain final volume of 50 ml.

Powdered extracts was stored in vials at 4 ^{0}C in refrigerator. A stock solution of 0.2 g/ml in dimethyl sulfoxide (DMSO) was made for each extract. Extracts were labeled with following abbreviations, CECE (*Coriandrum sativum* Ethanol Cold Extract), CEHE (*Coriandrum sativum* Ethanol Hot Extract), CWCE (*Coriandrum sativum* Water Cold Extract), CWHE (*Coriandrum sativum* Water Hot Extract), ZECE (*Zingiber officinale* Cold Ethanol Extract), ZEHE (*Zingiber officinale* Hot Ethanol Extract), ZWCE (*Zingiber officinale* Water Cold Extract) and ZWHE (*Zingiber officinale* Water Extract)

Anti-Biofilm Activity Testing

Test was used to perceive the ability of bacteria to adhere to glass tubes. A 5 ml of nutrient broth was inoculated by 100 µl of inoculums (1.5×108 CFU/ml) in glass test tube. The control tube contains nutrient broth only. Inoculated tubes were incubated at 37 °C for 24 hrs. After 24 hrs of incubation, the contents of tubes were decanted and 1ml of plant extracted were add to each tubes after that all tubes were incubated for 24 hrs at 37 °C once again. After final incubation the contents of tubes were emptied and tubes were stained for 1 minute by adding (1 ml) of 0.1% safranin for *P. aeruginosa* and 0.1% Crystal violet for *S. aureus.* Production of slime was visible as a film on walls of tube. Biofilm formation was scored from 0 to 4 according to visible eye examination (Slime absent- score 0; weak, score 1; moderate, score 2; strong score 3; or very strong, score 4).

5. Result and Discussion

In present study, *P. aeruginosa* and *S. aureus* were isolated from clinical samples. Each isolates were confirmed by battery of biochemical, morphological and cultural characteristics. Each isolates were studied for their biofilm production and their inhibition by the extracts of selected herb. Both isolates found to be positive for biofilm formation when studied individually. The triplicate experiments was performed for anti-biofilm activity with CECE (*Coriandrum sativum* Ethanol Cold Extract), CEHE (*Coriandrum sativum* Ethanol Hot Extract), CWCE (*Coriandrum sativum* Water Cold Extract), CWHE (*Coriandrum sativum* Water Hot Extract), ZECE (*Zingiber officinale* Cold Ethanol Extract), ZEHE (*Zingiber officinale* Hot Ethanol Extract), ZWCE (*Zingiber officinale* Water Cold Extract) and ZWHE (*Zingiber officinale* Water Extract). When each extract tested individually for their anti-biofilm activity, results are obtained encouraging for ethanol extract and they are scored (Table 1).

Table 1: Visible eye score of anti-biofilm activity of test extracts

	P. aeruginosa							*S. aureus*						
	Turbidity (Broth)		Score					Turbidity (Broth)		Score				
	Without extract	With extract	0	1	2	3	4	Without extract	With extract	0	1	2	3	4
CECE	+	+	✓					+	+	✓				
CEHE	+	+			✓			+	+	✓				

CWCE	+	+	✓					+	+	✓				
CWHE	+	+	✓					+	+		✓			
ZECE	+	+	✓					+	+	✓				
ZEHE	+	+				✓		+	+	✓				
ZWCE	+	Low					✓	+	+	✓				
ZWHE	+	+		✓				+	+	✓				

Where, Right tick (symbol) denotes anti-biofilm activity corresponding to score, (+) denotes bacterial turbidity with extract same as without extract.

Table 1 shows the visible eye scoring of anti-biofilm activity. The score were fixed by comparing the results with the bio-film on tubes without extract. With *P. aeruginosa*, Extract ZEHE scored 3 showing maximum antibiofilm activity, clear walls of test tube, effect of CEHE was moderate (score 2) and ZWHE displayed weak activity (score 1). Rest of the studied extracts had no anti-biofilm activity but bacterial turbidity was observed in all the tubes. Turbidity in broth with extract shows that the extract exerting antibiofilm activity only not bactericidal or bacteriostatic activity and low turbidity in test broth with ZWCE extract interpreted to be having bacteriostatic/ bactericidal effect hence no biofilm was formed, thus scored higher but this score is not considered for anti-biofilm activity. Study with *S. aureus*, was not encouraging. Only CWHE found to have weak anti-biofilm (score 1) activity.

In the study of Chusri *et al*., 2013 found that plants extracts possibly prevented biofilm formation of on polystyrene and glass surfaces by *Pseudomonas aeruginosa*. Our finding is in harmony with Kim and Park (2013) reports as, ginger extract act negatively against *P. aeruginosa* PA14 biofilm formation. On the same context Yahya *et al.* (2013) reported that the ethanolic extract of *Z. officinale* inhibits *P. aeruginosa* biofilm. Recent searching came with the phenolic compounds isolated from *Z. officinale* having quorum sensing inhibitors and it was verified on *P. aeruginosa* MTCC 2297 by Kumar *et al*., (2014). Study conducted by Razak and Rahim, (2003) proposed that, the aqueous extract of *Piper betle* inhibits adherence by inhibiting glucan production of *Streptococcus mutans* which resembles with the study of Rahim and Khan (2006) were extracts of *Syzygium ormaticum* (aqueous and methanol) shown to adhesion inhibition of *S. mutans* and inhibits the production of glucosyltransferase. Proportion of tannins and flavonoids matters for anti-biofilm activity (Siqueira *et al*., 2012). Adherence of bacterial cell is weakened by sublethal doses of antibacterial agents (Sharma and Sabnis, 2010). Man and O'Toole, (2001) reported *Curcuma longa* contains curcumin as a main key compounds which could delay formation of biofilm by making bacterial cells in a planktonic state. Water and methanol extracts of Peppermint investigated to be antibiofilm against *Listeria monocytogenes, P. aeruginosa* and *Candida albicans* (Sandasi *et al*., 2008). *Z. officinale* in ethanolic extract have anti-biofilm activity against *P. mirabilis* and *P. aeruginosa* ATCC 27853.

The current investigation is of great importance in dealing with the problem of anti-biofilm activity. Extract of *Z. officinale* yield good result to treat their phytochemicals as an anti-biofilm agent. The study of phytochemical profile will guide researcher to target biofilm formation activity.

6. Conclusion

A wide range of influence on bacteria has shown by *Z. officinale* ethanolic extract. Here *Z. officinale* has confirmed its significance of using it in a food. The tested bacteria are often cause of skin and other infections and *Z. officinale* extract had effect against them whereas *Coriandrum sativum* extract was not so effective. Even though there are countless reports available on the antimicrobial potential of plants extracts, there are scanty of reports are available on the anti-biofilm activities of plant extracts. Hence, the present study intended to uncover the anti-biofilm activities of plant extracts.

7. Future Scope

The natural products have tremendous potential to substitute conventional therapy and have gained interest in present scenario. At this period of time, researchers are paying attention and exploring the pharmacological and therapeutic effects of natural products of herbal origin. The most promising reason is that herbal products comparatively safe and have been traditionally practiced in medicines. Identification of phytochemicals of *Z. officinale* will give helpful for targeting medical problems associated with biofilm forming *P. aeruginosa.*

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