Antibiofilm Activity of Polyherbal Formulation Gel Against Human Wound Pathogens

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Abstract- *Natural products from plants have been recognized as a useful resource that may serve as leads to the discovery of new antimicrobial substances with possible new mechanisms of action. The aim of the present study was to develop a polyherbal formulation and study their antibiofilm activity against S. aureus. The aim of the present study was to develop a polyherbal formulation and its antibiofilm activity against human wound pathogens. The polyherbal formulation was extracted using the cold extraction method. The anti-microbial activity and antibiofilm effect were studied using MIC, MBC, and MTP assay followed by EtBr/Ao staining method. The results showed that the minimum inhibitory concentration of polyherbal formulation was found to be 28.22 µg/ml. The MTP assay results showed the anti-biofilm activity of the Polyherbal formulation was found to be 500 51.34 µg/ml. The total minimum bacterial concentration present in the Polyherbal formulation was observed as 7.3× 103 CFU/ml. Antibiofilm activity of polyherbal formulation by EtBr/AO fluorescent imaging showed the induction of apoptosis in Staphylococcus aureus. Taken together, these results confirmed that the polyherbal formulation has antibiofilm activity against Staphylococcus aureus and can be used for the treatment of chronic wounds.*

Keywords- Natural Products, Staphylococcus aureus, polyherbal formulation, EtBr/AO fluorescent imaging

I. INTRODUCTION

Bryophyllum pinnatum (Lamarck) Oken [syn.: Kalanchoe pinnata (Lamarck) Persoon, syn.: Bryophyllum calycinum Salisbury] is a plant of the family Crassulaceae. It is originally from Madagascar but can now be found across tropical Africa, tropical America, India, China, and Australia. The plant is known under various common names, such as life plant, air plant, love plant, Cathedral bells, and Goethe plant. Leaf extracts from B pinnatum have been used in traditional medicine of the regions where it grows and are suggested to have the following properties: sedative, central nervous system depressant, anticancer, analgesic, antipyretic, muscle relaxant, gastro protective, anti-inflammatory, antiseptic, anti-allergic, anti-anaphylactic, immune modulating, and wound healing .

Coleus aromaticus Benth (Family: Labiatas) is a wonder plant and cultivated through out India. The leaves of Coleus aromaticus juice is used in urinary diseases, calculus, dyspepsia, liver disease, chronic cough etc. In our laboratory, it was observed that the leaves of Coleus aromaticus has potentiality on urolithiasis .

India has a great heritage of traditional system of medicines like Ayurveda, Siddha, and Unani, where hundreds of medicinal plants are being used to treat various diseases with their known ethno pharmacological evidence. Sennaa auriculata (L.) Roxb. syn. Cassia auriculata L., family Fabaceae (former Caesal piniaceae) is one of the medicinal plants that has been used traditionally in Ayurveda, Siddha, and Unani since the 15th century .

Biofilms are usually considered from the physiological perspective of the encased microbial cells. However, they can also be considered as biophysical materials, where the cells are equivalent to colloids and the encasing extracellular polymeric slime (EPS) as a cross-linked polymer gel. This framework has allowed parallels to be drawn from soft matter physics, permitting the current understanding of biofilms as viscoelastic materials. Understanding biofilm mechanical properties, and how the biofilm responds to mechanical forces in its surroundings, offers new insight into the establishment and survival of these microbial communities. Measuring a biofilm's mechanical moduli (i.e. Young's, shear, storage or loss; discussed below) offers a way of describing biofilms that is complementary to current quantification parameters from microscopy analysis, such a biomass or roughness.

Biofilm refers to the complex communities of microbes that may be found attached to a surface or may form aggregates without adhering to a surface, as seen in Pseudomonas aeruginosa, Staphylococcus aureus, and some other bacteria and buried firmly in an extracellular matrix (ECM). The biofilm lifestyle allows the bacteria to withstand hostile environmental conditions like starvation, desiccation and makes them capable to cause a broad range of chronic diseases. Hence, it is considered as a major cause of persistent nosocomial infections in immune-compromised patients. Around 50% of the nosocomial infections are confined to the patients by indwelling devices used for the purpose of medical treatments such as catheters, cardiac pacemakers, joint prosthesis, dentures, prosthetic heart valves and contact lenses

These foreign bodies provide an ideal surface for the attachment of bacterial cells. Thus a significant increase in biofilm formation has been observed in the presence of implants. In many cases, the use of antibiotics like imipenem, colistin and many more can only reduce the biofilms but cannot eliminate the entire biofilm. Due to their toxic and side effects it is not possible to reach the minimal concentration of antibiotic in-vivo. The higher values of MIC and MBC for the biofilm bacterial cells have therefore made the antibiotic treatment less adequate.

Anti-biofilm molecules belong to diverse compounds thereby inhibiting the biofilm formation. The identified anti-biofilm compounds are mainly isolated from the natural sources, some synthetic compounds, chelating agents, and antibiotics also have been found to possess anti-biofilm activity.In biofilms, the bacterial cells are enclosed in an extracellular matrix, which is a complex and highly polar mixture of biomolecules including proteins, polysaccharides, nucleic acids and lipids. The matrix provides protection from various stress conditions such as antimicrobial exposure or immune cells attack. However, the matrix of the biofilm does not act as a mechanical barrier for the antimicrobial agent.

Plants are a rich reservoir of compounds that have numerous reported biological activities including antimicrobial properties thereby becoming a good resource to explore for the discovery of useful and novel antimicrobial products. The World Health Organization also recognizes the place of plants as a mainstay of primary health for over half of the world's population especially in resource poor countries. Natural products from plants have been recognized as a useful resource that may serve as leads to the discovery of new antimicrobial substances with possible new mechanisms of action. Bioactive plant-based products have the potential to promote the health of animals when included as feed and food components.

As the predominant pathogen in both community- and hospital-acquired wound infections, Staphylococcus aureus has attracted widespread attention and has been extensively studied. Bacterial colonization, which is defined as the presence of replicating microorganisms adherent to the wound in the absence of tissue damage, is the initial step in the development of wound infection. A variety of surface-associated factors are involved in the attachment and colonization of S. aureus to host cells and extracellular matrix, such as wall teichoic acid (WTA), fibronectin-binding protein A/B (FnBPA/B), iron-regulated surface determinant A (IsdA), clumping factor A/B and collagen-binding protein .

However, colonized bacteria can form complex microbial communities, which are called biofilms, and embed themselves in extracellular polymeric substances (EPS), serving as a shield against host immune cells and antibacterial agents. Polysaccharide intercellular adhesion (synthesized by the enzyme encoded by the ica operon) and extracellular DNA [cooperatively regulated by the holin-like protein CidA (cidA)/antiholin-like protein LrgA (lrgA) network and LysR family regulatory protein CidR (cidR)] are the main components of EPS, exhibiting an important effect on intercellular adhesion and biofilm formation in vitro and in vivo. With the protection of the biofilm matrix, S. aureus proliferates continually to a critical cell density, at which point the agr quorum-sensing system is activated.

Furthermore, we investigated the antibiofilm activity of the extracts on the six pathogens to either prevent formation or destroy pre-formed bacterial biofilms because it is an important strategy for microbial persistence in living and non-living tissues.

II. IDENTIFY, RESEARCH AND COLLECT IDEA

1. Antipathogenic Potential of a Polyherbal Wound-Care Formulation (Herboheal) against Certain Wound-Infective Gram-Negative Bacteria

This study investigated antipathogenic efficacy of a polyherbal wound-healing formulation Herboheal against three multidrug-resistant strains of gram-negative bacterial pathogens associated with wound infectiddthvhgvhgons. Herboheal was evaluated for its quorum-modulatory potential against three different human-pathogenic bacteria, first in vitro through the broth dilution assay and then in vivo in the model host Caenorhabditis elegans. Herboheal at ≥0.1% v/v was able to inhibit (19–55%) in vitro production of quorum sensing-regulated pigments in all these bacteria and seemed to interfere with bacterial quorum sensing by acting as a signal-response inhibitor. This formulation could compromise haemolytic activity of all three bacteria by ∼18–69% and induced their catalase activity by ∼8–21%. Herboheal inhibited P. aeruginosa biofilm formation up to 40%, reduced surface hydrophobicity of P. aeruginosa cells by ∼9%, and also made them (25%) more susceptible to lysis by human serum. Antibiotic susceptibility of all three bacteria was modulated owing to pretreatment with Herboheal. Exposure of these test pathogens to Herboheal (≥0.025% v/v) effectively reduced their virulence towards the nematode Caenorhabditis elegans. Repeated subculturing of P. aeruginosa on the Herboheal-supplemented growth medium did not induce resistance to Herboheal in this mischievous pathogen, and this polyherbal extract was also found to exert a post-extract effect on P. aeruginosa, wherein virulence of the Herboheal-unexposed daughter cultures, of the Herboheal-exposed parent culture, was also found to be attenuated. Overall, this study indicates Herboheal formulation to be an effective antipathogenic preparation and validates its indicated traditional therapeutic use as a wound-care formulation.

2. Antimicrobial, anti-inflammatory and wound healing activity of polyherbal Formulation

According to Ayurveda, individual herbs are insu cient to achieve a desired therapeutic e ect. When it is optimized as multiple herbs composition in a particular ratio it will give a therapeutic e ect in a better way with reduced toxicity. In order to develop such an intervention, the present study was intended to develop a poly- herbal drug from methanolic extracts of Plumbago zeylanica Linn, Datura stramonium Linn and Argemone Mexicana Linn. The study also aimed to evaluate the impact of polyherbalism on antimicrobial and antioxidant e ect,thereafter the ratio of individual plant extracts was optimized accordingly to treat the wound. The poyherbal drug was put on preclinical trial to access the anti-inflammatory and wound healing activity as 2% and 5% polyherbal carbopol-940 gels. The antimicrobial activity was assessed by agar well di usion and broth dilution method while wound healing activity was evaluated by excision and incision wound models. Topical anti-inflammatory activity was assessed by carrageenan induced paw oedema. The findings of the study revealed thesynergistic antimicrobial potential of Polyherbal drug against gram-positive and negative strains. Polyherbal carbopol- 940 gels (2% and 5%w/w) promoted the wound healing and anti-inflammatory e ect. The high rate of wound contraction (< 0.0001), early epithelialization period (< 0.0001) and increased wound breakingstrength (< 0.0001) were observed in 2% and 5% polyherbal gel treated group when compared to the normal control and negative control group. The antimicrobial and anti-inflammatory e ect of Polyherbal drug provokedand promoted the wound healing process through accelerated remodelling of damaged tissue.

3. **Formulation and evaluation of polyherbal gel containing extracts of Azadirachta indica, Adhatoda vasica, Piper betle, Ocimum tenuiflorum and Pongamia pinnata**

More than 80% of the world's population still greatly depends upon traditional medicines for treatment of various skin diseases [1. In the recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries, as herbal medicines have been reported to be safe with minimal side effects especially when compared with synthetic drugs. Herbal treatments applied topically have gained considerable attention due to their widespread use and ill-defined benefit/risk ratio. There are numerous medicinal plants which are widely used in the treatment of skin diseases and also known to possess antimicrobial activity. Topical application of gels at pathological sites offer great advantages in a faster release of a drug directly to site of action as compared to cream and ointment. Azadirachta indica (Neem), is phytochemically rich in steroids, alkaloids, tannins, triterpenes, flavonoid and anthraquinone glycosides. It has been known to be used traditionally for their various therapeutic properties like antibacterial, antimicrobial, antioxidant, skin disorder, and wound healing activity. Also it has been reported to possess various therapeutic properties like anti-inflammatory, antipyretic, antimalarial, antiulcer, antidiabetic,neuropharmacological effect, anthelmintic activity,antimicrobial and antibacterial effect. Ocimum tenuiflorum has been found to exhibit various activities like antioxidant, antidiabetic, chemo preventive effect, anti-ulcer, anticarcinogenic, anti-stress and also useful in modulation of immune response. Adhatoda vasica has been reported to be used traditionally for their various medicinal properties like anthelmintic, antiulcer, anticestodal, antioxidant, antifungal, antimicrobial and antibacterial effect. Piper betle and Pongamia pinnata have been known to possess various therapeutic properties like antioxidant, antifungal, antimicrobial, hypoglycemic and antibacterial effect. As per the literature survey the aforesaid plants have been reported for their antibacterial and antimicrobial effect by different researchers in most of the research articles. Based on this information it was decided to develop a herbal gel containing plant extracts which will possess better activity and prove to be effective against microorganisms. Despite of the fact that these plants possess good antimicrobial action, their use and application on the skin surface in the raw form is difficult. Hence, the present investigation was thus undertaken for preparation of polyherbal gel formulation using ethanolic extracts of Azadirachta indica, Adhatoda vasica, Piper betle, Ocimum tenuiflorum and Pongamia pinnata, so as to facilitate their effective use to exhibit its antimicrobial action. The prepared formulations were thereafter evaluated for their physical appearance, pH, viscosity, spreadability, drug content, skin irritation test, washability and antibacterial activity

III. WRITE DOWN YOUR STUDIES AND FINDINGS

1)Biofilm

The involvement of biofilm in providing resistance was made evident from a study on P. aeruginosa where the mucoid nature of biofilm was found responsible for high resistance toward tobramycin.The metabolic state of biofilm-associated bacteria is another potential reason of antimicrobial resistance. Cells of the nutrient depleted zones (slow growing state) in the biofilm may lead to dormancy like the stationary phase which makes the bacteria insensitive to antibiotics since they divide very infrequently .

Dividing cells are sensitive to some antibiotics including b lactams, thereafter making them unfit for use. Walters et al. reported that antibiotic resistance was also influenced by limited oxygen supply as observed in case of P. aeruginosa, where antibiotic was effective at the air-biofilm interface, the part of the biofilm exposed to oxygen (50– 90 mm in the biofilm).Moreover, studies also demonstrate that biofilm cells undergo a higher rate of mutation than their planktonic counterparts resulting in a 10-fold increase in the efficiency of transfer of plasmid having antibiotic resistance gene, when biofilm is exposed to a sub-lethal concentration of that antibiotic .

2) Biofilm formation

Biofilm formation on any surface involves mainly 3 stages. The first stage involves attachment of cells to a surface followed by assembly of the cells to form microcolonies and finally differentiation of biofilm into a mature structure. After the complete development of biofilm, its disassembly or dispersion takes place through both mechanical and active processes.Deposition of bacteria is especially mediated by sedimentation, Brownian motion and hydrodynamic forces, whereas adhesion to the substratum is governed by Lifshitz–Van der Waals, acid–base, hydrophobic, electrostatic interaction forces.Certain surface associated proteins like OmpA, fibronectin binding proteins, protein A, SasG, biofilm associated protein (BAP)and many other factors are involved in the formation of biofilms, particularly, during initial attachment stages. Some species cannot attach to a surface but can anchor themselves to the matrix or directly to the earlier colonies. Small signalling molecules with the help of cell-cell communication systems mediate this colonization. This phenomenon is generally referred to as quorum sensing.Biofilm formation is a major quorum-sensing controlled phenotype.In biofilms, the bacterial cells are enclosed in an extracellular matrix, which is a complex and highly polar mixture of biomolecules including proteins, polysaccharides, nucleic acids and lipids.The matrix provides protection from various stress conditions such as antimicrobial exposure or immune cells attack. However, the matrix of the biofilm does not act as a mechanical barrier for the antimicrobial agent .

3) Biofilm models

Study of various biofilm model systems enhances the knowledge regarding the biofilm biology. The biofilms are studied using both in-vivo and in-vitro model systems. In-vitro biofilm model systems are broadly classified into 3 major types including closed or static model, open or dynamic models and microcosms. The most frequently used closed model systems are microtitre plate-based model systems which uses static and batch growth conditions.In this model, there is no flow of media, product or waste materials into or out of the reactor, so the experimental conditions changes gradually in the wells like accumulation of signaling components, increase of bacterial population and depletion of nutrients in media. Since, it is cost effective and require small volume of reagents therefore, numerous tests can be performed at a single time. Additionally, microtitre plate-based

3.1. Antimicrobial activity

It was determined by measuring the diameter of zone of inhibition. The results obtained in the evaluation of the antimicrobial activity of Formulation A, B, C and control(base) against the selected microorganisms are shown in Table 4 and Figure 2A, 2B, 2C and 2D. Base showed zone of inhibition in the range of 8.98±0.7943 to 9.76±0.8798 against S. aureus, E.coli B. subtilis and A. niger. Formulation C showed better zone of inhibition in the range of 15.87±0.7804 to 19.01±0.6542 as compared to Formulation A, B and base. Thus, formulation C exhibited maximum activity against selected strains due to high amount of herbal extracts in comparison to others. The results were found to be statistically significant ($p < 0.05$). models can be used to differentiate between biofilm-deficient mutants and biofilm forming wild type strain.

IV. RELATED WORK

1) Bryophyllum pinnatum

Extracts of Bryophyllum pinnatum, a plant with a variety of common names in English (life plant, air plant, resurrection plant, Canterbury bells, cathedral bells, Mexican love plant) have been used by modern physicians mainly as a psychiatric sedative. Identified active ingredients include bufadienolides, flavonoids, glycosides, steroids and organic acids, while in vitro effects in rodent tissue include sedation, positive inotropism, antimicrobial activity and H1 antagonism (ileum, peripheral vasculature, bronchial muscle).

2) Coleus aromaticus

Coleus aromaticus is used for seasoning meat dishes and in food products, while a decoction of its leaves is administered in cases of chronic cough and asthma. It is considered to be an antispasmodic, stimulant and stomachic and is used for the treatment of headache, fever, epilepsy and dyspepsia. It is used to treat conditions such as indigestion, diarrhoea, nervous tension, insect bites, toothache, earache, rheumatism, whooping cough, and bronchitis. The plant also finds prominent importance in modern medicine.

3) Senna auriculata

Senna auriculata known as ‘Avaram’ in Tamil is a shrub belonging to the family Fabaceae found throughout India in open forests. The flowers of this plant are used in preparation of tea, which is prescribed in diabetes. The plant possess antidiabetic, antioxidant, hepatoprotective, antipyretic, anti-inflammatory and antimicrobial properties. Therefore, keeping in view of the above mentioned pharmacological properties, the phytochemicals of Senna auriculata floral extracts were screened and tested for antibacterial and antidiabetic properties.

V. OBSERVATION

Antibiofilm activity of acetone leaf extracts of nine underinvestigated south African Eugenia and Syzygium (Myrtaceae) species and their selectivity indices

Collection of plant material, drying and storage Healthy leaves of nine species from the Myrtaceae family were collected in the summer of 2017, at the Lowveld National Botanical Garden in Nelspruit, Mpumalanga, South Africa. The identities of the plants were verified by Ms. Magda Nel from the Department of Plant and Soil Science, University of Pretoria. Voucher specimens were prepared and deposited in the HGWJ Schweickerdt Herbarium of the University of Pretoria and voucher specimen numbers (PRU) were obtained. The plants selected for this study were: Eugenia erythrophylla Strey (PRU 123616), Eugenia natalitia Sond. (PRU 123613), Eugenia woodii Dummer (PRU 123615), Eugenia untamvunensis A.E.van Wyk (PRU 123618), Eugenia zeyheri (Harv.) Harv. (PRU 123617), Syzygium legatii Burtt Davy & Greenway (PRU 123619), Syzygium masukuense subsp. masukuense (PRU 123623), Syzygium species A (PRU 123622) and Syzygium gerrardii (Harv. Ex Hook.f.) Burtt Davy (PRU 123620). Syzygium species A could not be identified by the plant taxonomists and may be a new species. Several of these species may be threatened or endangered. Therefore, we did not collect plant material in the veld but only in good botanical gardens with known provenance of the plants. Furthermore, we only investigated leaf material to ensure that it is a sustainable resource. Methods which were previously developed in the Phytomedicine Programme were used to process the plants. Briefly, leaves were harvested and transported in open mesh loose woven bags into the laboratory. Leaves attacked by microbes or insects were removed and the rest were air-dried indoors at room temperature with good airflow to reduce any microbial attack as well as to facilitate grinding. The dried leaves were then ground to a fine powder using a Janke and Künkel homogenizer Model A10 mill. The leaf powders were weighed and stored in closed glass containers in the dark at room temperature. Extraction Acetone was used as the solvent of choice to extract the powdered leaves as well as to prepare the plant concentrations for the bioassays. Extraction with acetone is considered the best choice because it can extract compounds of a wide range of polarities, it is nontoxic to bioassay systems and easy to remove from extracts . Two grams of ground dry leaf samples were extracted with 20 mL acetone. The mixture was sonicated for 20 min, vigorously shaken, and then poured into a 50 ml polyester centrifuge tube and centrifuged at 4000 x g for 10 min (Hettich Centrifuge, Rotofix 32 A, Labotec, Johannesburg, South Africa). The supernatant was collected and filtered through Whatman No. 1 filter paper into pre-weighed glass vials and concentrated by drying under a stream of cold air. The dried extracts were weighed and the yield obtained by dividing the mass extracted by the initial mass. A concentration of 10 mg/mL (stock solution) in acetone was prepared for use in the assays.

VI .CONCLUSION

The selected plant extracts had varying antimicrobial activity against bacteria which indicates the potential of the plant species against bacterial pathogens. The findings from this study will provide researchers with the use of a polyherbal formulation that is not only cost-effective but also of herbal origin. Anti-biofilm activity of polyherbal formulation by EtBr/AO fluorescent imaging) revealed that it induces apoptosis in cells thereby causing cell death in Staphylococcus aureus. Therefore, the polyherbal formulation can be used for the treatment of wound infections caused by Staphylococcus aureus.

REFERENCES

[1] Harrington CB, Hansen JA, Moskowitz M, Todd BL, Feuerstein M. It's not over when it's over: long-term symptoms in cancer survivors—a systematic review. Int J Psychiatry Med. 2010;40:163-181.
[2] Kamboj A, Saluja A. Bryophyllumpinnatum (Lam.) Kurz.: phytochemical and pharmacological profile: a review. Pharmacognosy Rev. 2009;3:364-374.
[3] Ghosh, R.B., Sur, T. K., Maity L.M. and Chakraborty S.C. Antiurolithiatic activity of Coleus aromaticusBenth. in rats. Ancient Science of Life, XX(1+2), 44-47, (2000).
[4] Gupta, S., Sharma, S. B., Singh, U. R., and Bansal, S. K. (2011). Salutary Effect of Cassia auriculata L. Leaves on Hyperglycemia-Induced Atherosclerotic Environment in Streptozotocin Rats. Cardiovasc. Toxicol. 11, 308–315. doi:10.1007/s12012-011-9120-4
[5] Stoodley P, Cargo R, Rupp CJ, Wilson S, Klapper I. Biofilm material properties as related to shear-induced deformation and detachment phenomena. J IndMicrobiolBiotechnol 2002;29:361–7.
[6] Wu H, Moser C, Wang HZ, Hoiby N, Song ZJ. Strategies for combating bacterial biofilm infections. Int J Oral Sci 2015; 7:1-7; PMID:25504208.