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Conclusion and Future Perspectives

The rise of infectious diseases is of primary health concern which is mostly attributed to the evolution of drug resistant microorganisms. Microorganisms demonstrate the property of resistance due to misuse and overuse of antibiotics. A primary concern of present-day health care system is the rise of methicillin resistant *Staphylococcus aureus* which due to its nosocomial activities have been able to infect a sizeable population. Therefore, the need arises for the exploration of alternative drugs from the natural resources such as medicinal plants.

Chemical compounds isolated from plants are significant and differ in structures and activity against various bacterial pathogens. Plant-derived products are attractive due to their natural, affordable, non-toxic, and active at low concentrations properties and have wide spectrum activity against bacteria, fungi, and viruses. Essential oil obtained from plants have been found to have anti-bacterial properties against Gram positive bacteria including resistant strains.

A major role of the present work was to explore local traditional knowledge pertaining to the use of *Kaempferia galanga* L. in folk medicine and its wide availability as a crop from Ri-Bhoi district, Meghalaya. The plant *Kaempferia galanga* L. was investigated for its anti-staphylococcal activity with a purpose of development of a product- A wound healing ointment. The extraction of the essential oil was carried out using Soxhlet apparatus and hexane as the solvent. The yield of the essential oil obtained was higher with the percentage yield of 7.56%. Phytochemical studies of the essential oil indicated the presence of flavonoids and phenolic compounds. The presence of phenolics and flavonoids support the anti-oxidant activity of the essential

oil. The essential oil was also subjected to haemolysis assay, which demonstrated its non-toxicity towards the erythrocyte cells.

The essential oil from *K. galanga* L. inhibited *S. aureus* ATCC 6538 with a zone of inhibition of 27±0.21mm as against 37±1.15mm demonstrated by the positive control chloramphenicol. The essential oil also demonstrated anti-biofilm activity against *Staphylococcus aureus* which is of great importance as biofilm formation by *S. aureus* during wound infection has severe complexity in treatment of the wounds and leads to much greater expense. The antibiofilm activity was found to increase with the increase in the concentration of the essential oil. The maximum inhibition was found to be about 50%.

The GC-MS was used in the characterization of active compounds from theessential oil of *K. galanga* L. that led to the characterization and identification of eleven compounds. The main constituents detected were γ-Murrolene and Germacrene D (11.04%) and citral (1.81%). All the identified compounds belonged to various chemical groups and are known to possess significant biological and pharmacological activities. The Lipinski's Rule of 5 and ADMET analysis revealed that the characterized compounds from the essential oil of *K. galanga* L. have drug-like properties and have been found to possess better water solubility, have better clearance and non-hepatotoxic in nature, without any disturbance in the normal habitat of human internal conditions.

The identified compounds were also found to inhibit the target proteins which are responsible for formation of biofilm in *S. aureus.* Biofilm formation is a major issue in drug resistant strains. CrtM and SarA, the two target proteins selected are responsible for the biosynthesis of staphyloxanthin and virulence factor respectively. *In silico* docking against these two targets by the identified molecules from the essential oil revealed the binding affinity of the compounds. Out of the eleven compounds, γ-Elemene and caryophyllene were found to best docked molecules against the target proteins CrtM and SarA respectively. Further molecular simulation demonstrated the stability and binding affinity of the molecules and the PCA analysis demonstrated the conformational stability and changes in the structure of the molecules.

The wound healing activity of the topical ointment prepared from essential oil of respective concentration. Of the four different concentrations prepared, two of the concentrations were selected for the present study. The 4% and 10% w/w, was

evaluated using a circular excision wound model in Wistar Albino rats in which the open wounds were infected with *Staphylococcus aureus* ATCC 6538. Of the two concentrations the formulated ointment (10% w/w) demonstrated the highest efficacy on wound models when compared to the positive control, mupirocin, a marketed product. On days 4, 10, and 14, wound contractions rate was 48.55%, 94.47%, and 100% for 4% (w/w) and 53.29%, 96.51% and 100% for 10% (w/w) test groups, and 41.23%, 84.40%, and 97.15% for the standard group. The 10% (w/w) concentration dose was much more effective in healing of the wound as compared to positive control which was confirmed through histology studies. The rate of contraction analysis revealed the positive correlation as observed in the wound healing experiments. Histopathological studies reported herbal formulation (10% w/w), produced a skin with significant healing property with proper regeneration, re-epithelization, and improved reformation of collagen fibres, fibroblast cells, inflammatory cells in a newly formed skin and an increased rate of tissue perfusion. The formulation has shown no sign of skin irritancy, erythema and oedema in either intact or abraded sites of all rats and scored zero based on Draize score system for acute dermal toxicity. Our results, thereby support the use of *Kaempferia galanga L.* essential oil formulated ointment in the development of pharmaceuticals for the management of wounds, and/or inflammation-related diseases exhibiting biofilm inhibitory property and anti-staphylococcal activity.

Future Perspectives

- Formulations of essential oil-based ointment along with other sustained release agents may be studied.
- Essential oil of other related species can be studied and compared to understand the diversity.
- Further *in-silico* validation against other target proteins may be investigated so that the mechanism of inhibition can be demonstrated.

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