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# Phytochemical Screening and Antibacterial Activity of the Pilasthiri Kalimbu Prescribed to Cure Wound by the Traditional Siddha Practitioner of Kanyakumari District, India

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***Abstract:*** ***The polyherbal formulation of the Siddha Kalimbu comprised of 15 traditionally used herbs explore for the treatment of wound. The present investigation was mainly focused on the scientific assessment of phytochemical and antibacterial activities. The presence of phytochemicals constituents in the herbal formulation revealed positive response of significant secondary metabolites. The unexplored area of Siddha Pilasthiri Kalimbu towards their antioxidation effect in aqueous, chloroform and ethanol extracts indicated promising antioxidant activities of the crude extract.***
***Key words: Polyherbal formulation, wound, antioxidation, Qualitative analysis, Kalanchi, Samoolam***

## I. INTRODUCTION

Medicinal plants are important for pharmacological research and drug development not only as plant constituents used directly as therapeutic agents but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. Phytochemicals with adequate antibacterial efficacy will be used for the treatment of the bacterial infections (Krishnaraju *et al.*, 2005). Biomolecules of plant origin appear to be one of the alternatives for the control of antibiotic resistant human and plant pathogens (Raghavendra *et al*., 2006). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Iwu *et al*., 1999; Colombo & Bosisio, 1996). The potential for developing antimicrobials from higher plants appears rewarding lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Parekh & Chanda, 2007). Traditional medicine is widespread and plants still presents a large source of natural antioxidants that might serve as leads for the development of novel drugs. Several anti-inflammatory, digestive, anti-necrotic, neuroprotective and hepatoprotective drugs have recently been shown to have an antioxidant or anti-radical scavenging mechanism as part of their activity.

### *A. Materials and Methods*

The ingredients required for the formulation of medicines were procured from commercial Siddha raw drug store was authenticated and prepared by the Traditional Medicinal Practitioner. The herbal formulation were prepared as prescribed in the written scripts, books and palm leaf parchments of my Forefathers and Grandpa –Traditional Vadiyars.

### *B. Analysis of Antibacterial activity*

Antibacterial activity was conceded out for the Siddha Kalimbu used to cure wound was tested against 4 bacterial pathogens. 500g of Kalimbu/ointment was bought from the Siddha Practitioner for the preparation of different solvent extracts was selected for the antibacterial activity test using different solvents via ethanol, aqueous and chloroform**.** Kalimbu is prepared by the combination of medicinal plants in the form of Kalanchi.

### *C. Preliminary Phytochemical Analysis*

Preliminary phytochemical screening of the crude extracts was determined following the standard procedure (Brinda *et al*. 1981


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*D. Preparation of solvent extracts*

The solvent extract of ethanol, aqueous and chloroform of 5g Kalimbu were completely dissolved in 5 ml of 0.5% Tween 80 and preserved at 5°C in airtight bottles until further use (Lin *et al.*, 1999). All the extracts were subjected to antibacterial activity assay.

*E. Antimicrobial Susceptibility Test*

Four human pathogens selected for the antibacterial assay are *Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vulgaris and Klebsiella pneumonia.* Muller - Hinton agar is used as the nutrient medium for the selected bacterial strains. General methodology was followed for the preparation and sterilization of agar medium. Disks of 4 mm diameter were cut from Whatman No.4 filter paper for inhibitory study. The discs were taken in culture tubes and sterilized using autoclave at 121°C under 15 lbs pressure for 15 minutes. Disc diffusion method was used to screen the antibacterial activity. The empty sterile disc was dipped in the respective extracts and dried in room temperature placed on the inoculated agar medium in petriplates with the sterilized forceps (Lennette *et al*., 1985). Then the plates were incubated at 37° C for 24 hours. The antibacterial activity of Kalimbu was observed through the zone of inhibition around the disc was measured in millimeter and tabulated.

## II. RESULTS AND DISCUSSIONS

Green plants synthesis and preserve variety of bio-chemical products are extracted and used as chemical feed stocks or as raw material for various scientific investigations. The World Health Organization (WHO) estimated that 80% of the World population depends on traditional system of medicine. Preliminary phytochemical screening of different metabolites via steroids, alkaloids, sugars, phenolic compounds, flavonoids, saponins, tannins, anthroquinone and aminoacids in aqueous, ethanol and chloroform extracts of Siddha Chooranam revealed the presence or absence of different metabolites.

*A. Qualitative Analysis of Siddha Chooranam and Extracts*

Phytochemical analysis of the Siddha Chooranam revealed the presence of alkaloid, phenol and tannin whereas, the absence of steroids, sugars, flavonoids, saponins, tannins, anthroquinone and aminoacids. Aqueous extract revealed the presence of phenolic constituents alone whereas, the absence of steroids, alkaloids, sugars, flavonoids, saponins, tannins, anthroquinone and aminoacids. The ethanolic extract revealed the presence of tannin whereas, the absence of steroids, alkaloids, sugars, phenolic compounds, flavonoids, saponins, anthroquinone and aminoacids. The phytochemical analysis of chloroform extract revealed the presence of phenol and flavanoid constituents whereas, the absence of steroids, alkaloids, sugars, saponins, tannins, anthroquinone and aminoacids (Table: 1). Alkaloid can be used for treating wound and plant phenolics are a major group of compounds that act as a primary antioxidant (Potterat, 1997).

Table – 1 Phytochemical Screening of Siddha Chooranam and Extracts

| S.No | Phytochemicals | Observations | | | |
|---|---|---|---|---|---|
| | | Kalimbu | Aqueous | Ethanol | Chloroform |
| 1 | Steroids | - | - | - | - |
| 2 | Alkaloids | + | - | - | - |
| 3 | Sugar | - | - | - | - |
| 4 | Phenol | + | + | - | + |
| 5 | Flavonoids | - | - | - | + |
| 6 | Saponins | - | - | - | - |
| 7 | Tannins | + | - | + | - |
| 8 | Anthroquinone | - | - | - | - |
| 9 | Aminoacid | - | - | - | - |

+ Presence - Absence

*B. Antibacterial Activity*

The clinical isolates of *Proteus vulgaris*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were used for the antibacterial assay. The antibacterial activity of the selected microorganisms was determined using Disk diffusion method. The extract loaded disks were measured after 24 h of incubation. The antibacterial activity of Siddha medicine prescribed for Wound (Chooranam) showed both positive and negative activity. Chooranam showed the maximum zone of inhibition (24mm) against



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*Pseudomonas aeruginosa*, whereas, *Staphylococcus aureus* and *Proteus vulgaris* showed the maximum zone of inhibition (16mm) and minimum zone of inhibition (5mm) against *Klebsiella pneumonia*. The antibacterial activity of ethanolic extract of the chooranam showed the maximum zone of inhibition (13mm) against *Proteus vulgaris* whereas, *Staphylococcus aureus* showed the maximum zone of inhibition (9mm) and minimum zone of inhibition (2mm) against *Pseudomonas aeruginosa* and *Klebsiella pneumonia.* The antibacterial activity of aqueous extract of the chooranam showed the maximum zone of inhibition (15mm) against *Pseudomonas aeruginosa* minimum zone of inhibition (2mm) against *Staphylococcus aureus* and *Proteus vulgaris.* On the other hand, aqueous extract of Chooranam fail to inhibit the growth of the bacteria *Klebsiella pneumonia*. The antibacterial activity of chloroform extract of the chooranam showed the maximum zone of inhibition (5mm) against *Pseudomonas aeruginosa* (3mm) against *Staphylococcus aureus* and minimum zone of inhibition (2mm) against *Proteus vulgaris* and *Klebsiella pneumonia* (Table: 2

Table- 2 Antibacterial activity of Siddha Chooranam and Extracts

| Siddha Medicine and Extracts | Inhibition Zone in diameter (mm) | | | |
|---|---|---|---|---|
| | Pa | Sa | Pv | Kp |
| Chooranam | 24mm | 16mm | 16mm | 5mm |
| Ethanol | 2mm | 9mm | 13mm | 2mm |
| Aqueous | 15mm | 2mm | 2mm | - |
| Chloroform | 5mm | 3mm | 2mm | 2mm |
| Amikacin | 31mm | 18mm | 19mm | 8mm |

*Pseudomonas aeruginosa* (Pa), *Staphylococcus aureus* (Sa),
*Proteus vulgaris* (Pv) *Klebsiella pneumonia* (Kp)

## III. CONCLUSION

The phytochemical screening of the Siddha Kalimbu showed the presence of secondary metabolites like alkaloid, phenols and tannin constituents. The assessment of phytochemical constituents and anti-bacterial activity of the Siddha Kalimbu prescribed to cure wound revealed the importance to create awareness about the significance of unexplored medicinal practice and value of its perpetuation. The present work carried was a basic approach to find out the antimicrobial activity in Siddha medicine. Further works on the purification of individual groups of bioactive components might be able to reveal the exact potential of the kalimbu to inhibit several pathogenic microbes.

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