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EFFICACY OF AQUEOUS EXTRACT OF KAMPILLAKA (MALLOTUS PHILIPPENSIS) AGAINST PATHOGENIC BACTERIA FROM DUSHTAVRANA (NON-HEALING ULCER)

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ABSTRACT

Vrana ropana or wound healing is a natural process occurring in the body. It gets delayed and transfigured to Dushtavrana, due to the vitiation of Doshas and microbial action, are analogous with Non-healing ulcer. Chronic non-healing ulcers affect as an economic burden to the healthcare system, by reducing the quality of life for those who are affected and often leading to serious events such as limb amputations or even premature deaths. Kampillaka is a drug mentioned for Krimihara and Vranaropana and studies have shown its anti-microbial effect against various micro organisms. In the current study the aqueous extract of Kampillaka (Mallottus philippensis) against pathogenic bacteria is evaluated in pus samples of subjects diagnosed with Dushtavrana by culture and sensitivity. With the current study it is evident that the mean zone of inhibition of aqueous extract of Kampillaka possess antimicrobial action against Staphylococcus spp., Pseudomonas spp. and E.coli. Further it is also obvious that as the concentration of aqueous extract of Kampillaka increases, the zone of inhibition also increases.

KEYWORDS: *Non-healing ulcer, Dushtavrana, Kampillaka, Pus culture and sensitivity, Staphylococcus spp., Pseudomonas spp. and E.coli*

INTRODUCTION

Vrana ropana or wound healing is a natural process occurring in the body. It gets delayed and transfigured to *Dushtavrana,* due to the vitiation of Doshas[1] and microbial action. The symptoms of *Dushtavrana* mentioned by *Acharya Susruta* are analogous with Non-healing Ulcer like *Deergha kaalanubandhi* (chronic-6 weeks), *atyartha vedana* (severe pain), *paka*(suppuration), *amanonja gandha* (unpleasant odor), *shotha* (inflammation), *puyasrava*[2](pus discharge).

One of the Indian studies on the epidemiology of chronic wounds, estimated the prevalence at 4.5/1000 population[3]. Popularity towards traditional, complementary and alternative medicines are increasing globally[4]. At this situation, more Ayurvedic drugs with wound healing properties has to be exploited with evidence-based study. In Ayurvedic Classics, lot of drugs are mentioned for its *Vrana ropana* and *Krimighna* actions but a very few has been evaluated for their actions through in vitro study.

Kampillaka is one such drug which is mentioned as both *Vrana nashana* and *Krimihara*[5] The extract is known to contain flavonoids, glycosides, tannins, proteins and amino acids[6]. Hence in present work, culture and sensitivity is taken as a tool to evaluate the concept of *upashaya* and *anupashaya* in vitro to revalidate the activity of *Kampillaka* against the Pathogenic bacteria by culture and sensitivity in *Dushtavrana* with special reference to Non-healing Ulcer.

AIMS AND OBJECTIVES

To evaluate the sensitivity of aqueous extract of *Kampillaka (Mallotus philippensis* Muell. Arg.*)* against Pathogenic bacteria from Pus sample of *Dushtavrana* (Non-healing Ulcer) patients by culture and sensitivity in vitro.

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MATERIALS AND METHODS

Thirty patients fulfilling diagnostic and inclusion criteria, of non-healing ulcer of at least more than six weeks duration with *Puyasrava* and with or without following *Dushtavrana lakshanas:Kandu, Amanojna gandha, Atisamvruta, Atimrudu, Atyavasanna, Rakta, Krishna, Pandu varna*, covered with *Putimamsa , Shotha, Paka, Unmargi vrana*, excessive *Dushta shonita* discharge, within 18-70 years of age, from a Tertiary Ayurvedic Hospital in Hassan was selected for the study. Diagnosed subjects of varicose vein ulcer, tubercular and malignant ulcers and with any other complications which may interfere with the course of study were excluded from the study.

RESEARCH DESIGN

An observational experimental study

METHODOLOGY

The extracts of *Kampillaka* was prepared by hot extraction method by Soxhlet extraction. 150 grams of *Shodhita Kampillaka (Phala raja)* coarse powder placed inside a thimble in a filter paper, which was loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor was placed into a flask containing 750 ml of distilled water. Extraction done for 2 days. The extract liquid then placed on water bath and collected the extract and weighed. 5.25 grams of *Kampillaka* was obtained by this procedure.

Early morning pus sample was collected from the subjects fulfilling the diagnostic and inclusion criteria. Further culturing was done on McConkey and Muller Hinton Agar (MHA) plates by streaking method using one loop full of inoculum. The plates were then kept for 24–48 hours culture in incubator at 37^0C. After 24-48 hours of incubation, the cultural characteristics like colony morphology were studied[7,8,9]. Different concentrations of aqueous extract of *Kampillaka* were prepared by dissolving 3gm of aqueous extract in 9ml of distilled water that gave a stock solution carrying 3000µl of drug concentration. From the stock solution, different concentrations like 2000µl, 1000µl, 500µl, 100µl, of the aqueous extract were prepared and used distilled water as a control. MHA plates were uniformly swabbed with sterile non-toxic cotton swab (lawn culture). The different concentrations of drug were then subjected to antibacterial sensitivity test by agar well diffusion method. Six equidistant wells were made on the plates with the help of a sterile cork baurer. 3000µl of aqueous extract of different concentrations were poured into labelled wells on different plates, including the control (distilled water). All the plates were incubated at 37℃ for 24 hours and then zone of inhibition was measured with a ruler in mm.

Table No.1: Organisms Identified

Organism	Frequency	Percentage
Staphylococcus spp	12	40.0
Pseudomonas spp	11	36.7
E coli	7	23.3

Table No. 2: Colony characteristics of *Staphylococcus spp.*

Culture Characters	Organism identified
Size- 2.2 mm Shape – Round Surface –Smooth Elevation- Raised Edge – Entire Opacity- Opaque Colour of colony- Yellow Consistency – Buttery	*Staphylococcus spp.*

Table No. 3: Colony characteristics of *E-coli*

Culture Characters	Organism identified
Size- 2.2-3 mm Shape – Round Surface –Smooth Elevation- Raised Edge – Entire Opacity- Opaque Colour of colony- Grey to white Consistency – Buttery	*E-coli*

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Table No. 4: Colony characteristics of *Pseudomonas spp.*

Culture Characters	Organism identified
Size- 2.2 mm Shape – Oval Surface –Smooth Elevation- Raised Edge – Entire Opacity- Opaque Colour of colony- Bluish green Consistency – Buttery	*Pseudomonas spp.*

Assessment Criteria

If the drug is sensitive, a clear 'halo' (zone of inhibition) appears around the well that denotes the absence of bacteria which indicates the drug is effective against that bacterium.

Observation and Result

Invitro anti-bacterial activity of aqueous extract of *Kampillaka* was evaluated by agar well diffusion method and zone of inhibition was measured as shown in table.

Table No. 5. Mean values of zone of inhibition at different concentrations of aqueous extract of *Kampillaka* against pathogenic bacteria

Different concentration of aqueous extract of *Kampillaka*	3000µl	2000µl	1000µl	500µl	100µl	Control
Total number of patients (N)	30	30	30	30	30	30
Mean zone of inhibition in mm	16.6	13.77	11.4	9.93	8.2	0

The present study shows that the susceptibility of pathogenic bacteria's like *Staphylococcus, Pseudomonas* and *E.coli* against the aqueous extract of *Kampillaka* is fairly evident between 20 to 18 mm,hence it can be considered as sensitive. 16 to 12 is considered as moderately sensitive. 10 to 08 is considered as resistant. Therefore, with the current study it is evident that the pathogenic organisms like *Staphylococcus spp., Pseudomonas spp. and E.coli* are moderately sensitive to 3000 µl and 2000 µl and resistant to 1000 µl ,500 µl and 100 µl of aqueous extract of *Kampillaka.* The distilled water, which was used as control does not show any sensitivity and it proves the efficacy of the drug in comparison to the various other concentrations, used.

DISCUSSION

For early and uncomplicated healing of *Vrana,* local treatment should given utmost importance along with oral medications. Once the healing of the Ulcers is initiated, then the area should be kept free from further ulcerations. A chronic ulcer with copious discharge and slough is considered as *Dushtavrana*.

Curative action is shown by most of the plant drugs by their *Krimighna* (anti-microbial) property. Sensitivity test for existing *Ayurveda* drugs is important as it direct the use of these drugs within a narrow spectrum of activity, thus specific indication. Present study is on culture and sensitivity of various pathogenic bacteria from pus sample of patients suffering from *Dushtavrana* (Non healing ulcer) with aqueous extract of *Kampillaka.* Therefore before conducting the culture and sensitivity test, pharmaceutical preparation of aqueous extract of *Kampillaka* was essential. Pharmaceutical processing is a technique which converts natural products into therapeutically potent dosage form, which is easily absorbable in the biological system. In this study the aqueous extract of *Kampillaka* was prepared using Soxhlet extraction method.

Acharya Charaka enlist *Kampillaka* as one of *Phalini dravya*[10] with *Katu rasa, Laghu, Rooksha, Teekshna guna, Ushna veerya and Katu vipaka*[11] possess *Vrana ropana* action. *Acharya Susruta* quoted *Kampillaka in Shyamadi varga*[12] with special indication in *Dushtavrana*[13]. The drug is also mentioned in various *Nighantus* such as *Kaiyyadeva Nighantu, Dhanvantari Nighantu, Raja nighantu* and in *Rasa Ratna Samuchaya*[14]. It is *Krimighna, Vranaapaha, Virecanopaga.* Thus implementation of novel approaches like culture and sensitivity methods would reinforce existing *Ayurvedic* knowledge and help in achieving improved diagnostic and curative abilities. Hence present study is undertaken to study various attributes of different pathogenic bacteria as laboratory diagnosis, its culture by pus culture from *Dushtavrana* (Non healing ulcer) patients and sensitivity against *Kampillaka.*

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In aqueous extract, better sensitivity was observed in higher concentration because of more concentration of active molecules. As the concentration decreases, the active molecule content also decreases which might not be capable to destroy the capsule or disrupt the cell membrane or act against the antigens produced leading to the resistance of the organism towards it. Depending on the type of strain of organism, virulence level may vary. Cell constituents help the bacteria to attach to the host cell and prevention of phagocytes from other immune modulator helper cells. While conducting sensitivity, phytochemical constituent of the extracts interact with enzymes and proteins of the cell membrane causing its disruption to disperse a flux of protons toward the cell exterior which will cause cell death or inhibit amino acid biosynthesis of microbial cell. On other hand hydrophobic characteristics of these extracts enable to react with protein of microbial cell membrane and mitochondria to disturbing their cell structures and permeability. Different mechanism such as altering the surface tension of the extracellular medium of cell, ability to complex with extracellular and soluble proteins, to obtrude with bacterial DNA etc. likewise for different strains of bacteria, it has been proposed that the mechanism of antimicrobial effects involves the inhibition of various cellular processes which lead to an increase in plasma membrane permeability and further ion leakage from the cells. Meanwhile for various concentration of the same drug, it might show different zone of inhibition. Because the different components diffuse at different rates may have been responsible for the varying zone of inhibition against microorganisms. The difference in susceptibility of various microorganisms may be attributed to their intrinsic properties and permeability of cell surface to the extracts. Still active phytochemical constituents fails to thrive with antimicrobial action because of cytological characteristics of organisms. Porosity of cell membrane varies from cell to cell via different conditions and the membrane inhibits cell structure perturbations because of its defence to phytochemical components. It is further evident from the zero zone of inhibition obtained by using distilled water as a control against aqueous extract. This clearly emphasis the antimicrobial action the drug *Kampillaka.*

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