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EFFICACY OF ALCOHOLIC EXTRACT OF *KAMPILLAKA* (MALLOTUS PHILIPPENSIS MUELL. ARG.) AGAINST PATHOGENIC BACTERIA BY PUS CULTURE AND SENSITIVITY IN *DUSHTAVRANA* (NON-HEALING ULCER)

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ABSTRACT

An ulcer is a discontinuity of an epithelial surface (skin or mucous membrane). Chronic non healing ulcers are the wounds that fail to heal, in general they have a fibrotic margin and a bed of granulation tissue which may include areas of slough (necrotic tissue). Although the healing of the vrana is a natural process in the body, it can be delayed due to the *Dosha dushti* and due to *krimi (microorganisms).* The definition of *Vrana* implies the damage of a part of the body leading to discoloration. Hence, for early and uncomplicated healing of *Vrana,* local treatment should give utmost importance along with oral medications. Once the healing of the Ulcers is initiated, then the area should be kept free from further ulcerations. The symptoms of *Dushta vrana* mentioned by *Acharya Susruta* are analogous with that of Non-healing Ulcer. In the current study, the action of alcoholic extract of *Kampillaka* (Mallotus philippensis) against pathogenic bacteria like *Staphylococcus spp., Pseudomonas spp. and Escherichia coli* is evaluated in the pus of subjects diagnosed with *Dushtavrana* (Non-healing ulcer)by culture and sensitivity. With the current study, it is evident that the mean zone of inhibition of alcoholic extract of *Kampillaka* possesses anti-microbial action against the bacterium *Staphylococcus spp., Pseudomonas*

spp., and E. coli. Further, it is also obvious that as the concentration of alcoholic extract of *Kampillaka* decreases, the zone of inhibition increases.

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

INTRODUCTION

Vrana ropana or wound healing is a natural process occurring in the body. It gets delayed and transfigured to *dushta vrana,* due to the vitiation of Doshas **[1]** and microbial action. The symptoms of *Dushta vrana* mentioned by *Acharya Susruta* are analogous with Non-healing Ulcer like *Deergha kaalanubandhi* (chronic), *atyartha vedana* (severe pain), *paka*(suppuration), *amanonja gandha* (unpleasant odour), shotha (inflammation), puyasrava **[2]** (pus discharge). *Kampillaka* is mentioned as both *Vrana nashana* and *Krimihara* in various *Nighantu* **[3]**. An ulcer is a discontinuity of an epithelial surface (skin or mucous membrane). It may follow molecular death of surface epithelium or its traumatic removal, there is usually progressive destruction of surface tissue, cell by cell, as distinct from death of macroscopic portions (eg. Gangrene/necrosis) **[4]**. Pus culture and sensitivity is identified as a tool to identify pathogenic organisms responsible for the infection and through sensitivity evaluation appropriate drug is selected for the management. Even though many drugs are attributed with *krimighna* action in Ayurveda, there are only a few works done on establishing the effectiveness of specific drug activity on specific micro-organisms. Hence such drugs need to be analysed for action against specific micro-organisms so that an *upashaya* effect of such drugs can be generated on micro-organisms invitro.

AIMS AND OBJECTIVES

To evaluate the sensitivity of alcoholic 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

MATERILAS AND METHODS

A minimum of 30 subjects, aged between 18-70 years, of either gender, irrespective of the caste and religion, presented with non-healing ulcer of at least more than six weeks duration with *Puyasrava* (pus discharge) 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,* from out-patients and in-patients departments of a Tertiary Ayurvedic Hospital in Hassan was included in the study. Subjects with any other complications that may interfere with the

study like varicose vein ulcer, tubercular and malignant ulcers were excluded from the study.

RESEARCH DESIGN

An observational experimental study

METHODOLOGY

The extracts of *Kampillaka* were prepared by hot extraction method (Soxhlet extraction) using *Shodhita Kampillaka 50 grams* and 250 ml ethanol. *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 the extraction solvent (Ethanol). The Soxhlet then equipped with a condenser. The solvent was heated and the solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensure that any solvent vapour cools, drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet extractor is almost full, the chamber automatically emptied by a siphon side arm, and the solvent run back down to the distillation flask. This cycle repeated for 2 days. The filtrate was then kept over water bath in a China dish at 70 ^{0}C. 7.00gms of *Kampillaka* extract were obtained from this process.

The early morning pus sample from the patients fulfilling the diagnostic and inclusion criteria with *Dushtavrana* was collected. Further culturing was done on McCkonkey 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 ^{0} C. After 24-48 hours of incubation, the cultural characteristics like colony, morphology were studied. From results showing positive cultures, a loop full of inoculum was transferred to MHA plates and culturing was done by streak culture method. The sample was also subjected to microscopical examination for the identification of various pathogenic bacteria. Then the sensitivity with *Kampillaka* was performed by Agar well diffusion method.

Different concentrations of alcoholic extracts were prepared by dissolving 3 grams of alcoholic extract in 900ml of ethanol, that gave a stock solution carrying 3000µl of drug concentration. From the stock solution, different concentrations like 2000µl, 1000µl, 500µl and 100µl of the alcoholic extracts were prepared. MHA plates were uniformly swabbed with sterile non- toxic cotton swab (lawn culture). The different concentrations of drug were then subjected to anti-bacterial sensitivity test by agar well diffusion method. Six equidistant wells were made on the plates with the help of a sterile

cork baurer. Different concentrations of the extract were poured into labelled wells, including ethanol as a control in the sixth well. All the plates were incubated at 37 ^{0}C for 24 hours and then zone of inhibition was measured with a ruler in mm.

Table 1: Organisms identified

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

Table 2: Colony characteristics of *Staphylococcus spp.* [5]

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 3: Colony characteristics of *E. coli* [6]

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

Table 4: Colony characteristics of *Pseudomonas spp*[7].

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 circular 'halo' (zone of inhibition) appears around the well that denotes the absence of bacteria which indicates the drug is effective against the bacterium.
- Based on the extend of sensitivity it can be of three zones
  - ✓ Sensitive zone
  - ✓ Intermediate/ Moderately sensitive zone
  - ✓ Resistant zone

OBSERVATION AND RESULTS

Invitro anti- bacterial activity of alcoholic extract of *Kampillaka* was evaluated by agar well diffusion method and zone of inhibition was measured as shown in **Table 5**.

Table 5: Mean values of zone of inhibition at different concentrations of alcoholic extract of *Kampillaka*

Different concentrations of alcoholic extract of *Kampillaka*	3000µg/ml	2000µg/ml	1000µg/ml	500µg/ml	100µg/ml	Control
N	30	30	30	30	30	30
Mean (mm)	14.43	13.10	12.23	16.23	19.90	8.23

The present study shows that the susceptibility of *Staphylococcus spp., Pseudomonas spp. and E.coli* against the alcoholic extract of *Kampillaka* is fairly evident between 20- 18mm. hence it is considered as sensitive. 16 to 12 mm is considered as moderately sensitive. Less than 12 mm is considered as resistant. Therefore, with the current study it is evident that *Staphylococcus spp., Pseudomonas spp. and E.*coli are sensitive to 100 µg/ml and moderately sensitive to 500 µg/ml, 1000 µg/ml, 2000 µg/ml and 3000 µg/ml. On comparison with the mean resistance of control (ethanol) used, it become evident that the antimicrobial activity is further enhanced by the active biomolecules from the drug.

DISCUSSION

In this present study 44 subjects were screened. Among them 30 subjects fulfilled the diagnostic and inclusion criteria and the remaining 14 subjects were excluded. Among excluded 14 subjects, 5 pus samples had contamination during the incubation and remaining samples does not show any growth during culture.

Acharya Charaka enlist *Kampillaka* as one of *Phalini Dravya* **[8]**. *Acharya Susruta* quoted *Kampillaka in Shyamadi varga* with special indication in *Dushtavrana* **[9]**. The drug *Kampillaka* possess *Katu rasa, Ushna virya, Katu vipaka* and it is *Krimighna* and *Vranapaha* **[10]**. In the present study Soxhlet extraction was used because *Kampillaka* has limited solubility in the solvents. By assessing the mean values of the zone of inhibition shown by the alcoholic extract of *Kampillaka,* against *Staphylococcus spp., Pseudomonas spp. and E.coli,* it was observed that the organism is sensitive to 100 µg/ml and moderately sensitive to 500 µg/ml, 1000 µg/ml, 2000 µg/ml and 3000 µg/ml. Alcohol provides an efficient way of maximising the bioavailability of the active principles from the plant. Ethanol is a molecule with both polar and non-polar ends. Plant extracts generally exist as a combination of different types of bioactive compounds or phytochemicals with varying polarities. Ethanol extract both polar and non-polar compounds from the plant. So that the saturation level of these phytochemical compounds is maximum in the alcohol extracts.

Tannins have been reported to have bacteriostatic or bactericidal activity against

pathogenic bacteria. The astringent property of tannin may induce combining with the enzyme or substrates to form complexes. Several microbial enzymes in raw culture filtrates or in purified forms are inhibited by combination with tannins. Tannins hamper with the plasma coagulating property of pathogenic bacteria preventing the formation of fibrin rich membranous structures by these organisms. 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. Different concentrations of alcoholic extracts of *Kampillaka* showed different zones of inhibition. This is because different components diffuse at different rates that produce varying zone of inhibition against various pathogenic bacteria.

It was evident that when the concentration of alcoholic extract reduced there was subsequent increase in the zone of inhibition. The maximum zone of inhibition was obtained in 100 µg/ml of alcoholic extract. This may be because on diluting the concentrations, the active components completely dissolve into the solution. Also in the lower concentration, the molecular size of the active components will be too small and by complete dissolution easily penetrates through the cell membrane. In higher concentration the molecular size may be large and hence may not be potent enough to act at capsular and cell membrane levels.

CONCLUSION

From this study it is evident that mean zone of inhibition of alcoholic extract of *Kampillaka* showed *Krimighna* (antibacterial) action against pathogenic bacteria like *Staphylococcus spp., Pseudomonas spp. and E.coli* obtained from subjects diagnosed with *dushtavrana* (non-healing ulcer). It is also evident that as the concentration of alcoholic extract of *Kampillaka* decreases, the zone of inhibition increases.

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