# STUDY OF ANTIBACTERIAL ACTIVITY OF LEAF EXTRACTS FOR CONTROL OF SEPSIS CAUSING ORGANISMS

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**Abstract**: The ethanolic and aqueous extracts of leaves of *Ocimum sanctum* (Tulsi), *Eucalyptus globulus* (Nilgiri) & *Vitex negundo* (Nirgudi) were tested for its anti-bacterial activity and Minimum Inhibitory Concentrations against clinical isolates .Extracts were found to be antibacterial against *S. aureus ,Pseudomonas aeruginosa, Klebsiella pneumonia* & *Escherichia coli*. The cold extracts were effective compared to hot extracts. The extracts were compounded with bases viz. Petroleum jelly and honey .The antibacterial activity and toxicity of the compounded extract was carried out .Honey as base was effective and it was found to be bactericidal in 3 h against *Pseudomonas aeruginosa.* The herbal extract with honey was found to be non-toxic on the skin surface of male albino rats. The study conducted shows promising results in control of sepsis causing organisms and a topical agent can be formulated.

**Keywords:** *Ocimum sanctum* (Tulsi), *Eucalyptus globules* (Nilgiri) & *Vitex negundo* (Nirgudi) leaves, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae* & *Escherichia coli*.

**Introduction:** Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties [1]. The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. In present decade, antimicrobial viz; antibacterial, anti-fungal potential of medicinal plants are being studied and reported globally [2].Conventional drugs usually provide effective antibiotic therapy for bacterial infections but there is an increasing problem of antibiotic resistance and a continuing need for new solutions.

Mainstream medicine is now increasingly becoming receptive to the use of antimicrobial and other drugs derived from plants, as traditional antibiotics (products of microorganisms or their synthesized derivatives) become ineffective and as new, particularly viral, diseases remain intractable to this type of drug [3].

Chemotherapy has always been the first line of treatment for curing and controlling infections. Antimicrobial agents have transformed the modern world. Diseases that were responsible for high morbidity and mortality on a large scale were brought under control with the aid of antimicrobial agents. Chemotherapy consists of the use of broad spectrum antibiotics like β-Lactams, Glycopeptides, Tetracycline, Aminoglycosides, Macrolides and others [4]. Along with the power of controlling infections, these chemotherapeutic agents are also capable of developing various side effects such as allergic reactions, nephrotoxicity, ototoxicity, nausea, pseudo membranous colitis and many more.

The majority of sepsis causing microorganisms is found in the most superficial layers of the epidermis and the upper parts of the hair follicles. They consist largely of Micrococci (*Staphylococcus epidermidis* and Micrococcus

sp.) and Corynebacteria. These are generally nonpathogenic and considered to be commensal, but can also become mutualistic and parasitic in the patient. Nasal carriers were found to bear potentially pathogenic *S. aureus* on face as well as on hands.

The above normal flora and some other potential pathogenic microorganisms can cause skin infections if the skin is not intact. *Pseudomonas spp, Klebsiella spp, Escherichia coli, Enterobacter spp*, etc. are some of the other causative agents. Infection arises in a nosocomial context and requires broad-spectrum antibiotic therapy. In addition to this, antimicrobial resistance has been fueled by inappropriate use of antimicrobial agents.

Traditional medicinal plants are being used regularly in many of the developing nations to maintain good health. [5]. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies [6]. In rural sections of the society, herbal remedies are used commonly to treat minor ailments [7].

A great deal of scientific research has been carried out on herbal medicines in the past few decades.

Thus the present study involves study of plant extracts in control of sepsis causing organisms

**Materials & methods:** The leaves of the plants selected for the study were *Ocimum sanctum* (Tulsi), *Eucalyptus globulus* (Nilgiri) & *Vitex negundo* (Nirgudi). The leaves were collected from plants and authenticated, then dried and used for extract preparation.

**Extract preparation:**

Hot alcoholic extract was prepared by continuous hot extraction in the Soxhlet apparatus [8]. For cold ethanolic extract 30 grams of crude dried powder was mixed with 300ml of ethanol in a flask. The flask was kept under shaker conditions for 72 hrs,this was then filtered and the filtrate was evaporated at 50$^{o}$C and stored under refrigeration.

Microorganisms causing sepsis such as *Pseudomonas aeruginosa*, *Staphylococcus aureus, Klebsiella pneumoniae* & *Escherichia coli* selected for the study, were collected from pathological samples.

**Antibiotic Susceptibility testing of the isolates**:

Antibiotic Sensitivity testing was carried out by Kirby Bauer Disc Diffusion method. The method is used to distinguish the isolates as sensitive, resistant and intermediate to broad spectrum antibiotics [9].

**Anti-bacterial screening:**

The antibacterial activity of individual and combination of plant extracts was checked against the isolates by Agar Ditch Method and Minimum Inhibitory concentration (MIC) of plant extracts was determined by Plate dilution method [10].This method allows a single compound to be evaluated against a number of organisms simultaneously [11].

**Formulation:**

Ointments are semisolid preparations for external application of such consistency that they may be readily applied to the skin by inunction. They serve as vehicles for topical application of medicinal substances and also function as protectives and emollients for the skin [12]. To determine the antibacterial activity of supportive base, Agar Cup method was chosen. Honey & Petroleum Jelly, Hydrophilic ointment, aloe gel, petroleum jelly, oil base and emulsifying wax were chosen for the study. [13]**.**Different bases were tried out and according to the results obtained a better base was selected for further studies.

**Determination of the antibacterial activity of the ointments:**

**Agar-cup method/ Agar Well Diffusion assay:** [13]-[14]

The basic principle underlying the test is to determine the antibacterial activity of the herbal gel against the isolates by measuring the zone size of inhibition. The method used for this test is agar well diffusion method in which the activity of the test compound in the test is measured by correlating with the inhibition zone around it. Active extracts of individual plants and also the combinations were formulated in various bases mentioned earlier. Thus the ointments prepared were checked for their antibacterial activity.

**Kill-time studies of extract and base:**

The extract and effective base was subjected to bactericidal time efficacy (Contact time period). This method assesses the bactericidal effect of the combination under study. The organisms are maintained in contact with the combination and its bactericidal effect was monitored at regular time interval ranging from 0 to 8h at an interval of 1 h [15].

**Skin toxicity testing: -**Skin toxicity is designed to measure the potential of herbal combination to cause sensitization and it also provides a measure of irritancy potential [16]. The test material was applied to the depilated dorsal surface of the male albino rat for 72 hrs. 100 mg of dose was applied each day. Then the skin was observed for adverse reactions of erythema and edema.

**Results & discussions**:

A total of 4 isolates (1 Gram positive and 3 Gram negative organisms) responsible for sepsis were obtained from pathological samples. These included *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae & Escherichia coli*. Antibiotic Sensitivity testing of these isolates showed resistance to more than 50% of the antibiotics but were intermediate in nature. *Pseudomonas aeruginosa* was resistant to broad spectrum antibiotics.

**Antibacterial Screening of the Extracts**:

The hot alcoholic extract (HAE), Cold alcoholic extract(CAE) of *Ocimum sanctum* (Tulsi) leaves, *Vitex negundo* (Nirgudi) leaves and *Eucalyptus globulus* (Nilgiri) leaves in combination showed significant anti-bacterial activity.

The hot and cold aqueous extracts did not exhibit any significant activity against any of the test isolates. The hot and cold alcoholic extracts showed activity above 1% and 0.5%respectively.

**Screening of antibacterial activity of supportive base**:

For present work, Honey and petroleum jelly, Hydrophilic ointment, aloe gel, oil base and emulsifying wax were chosen as supportive bases and the anti-bacterial activity of extract with base against test isolates was studied by Agar Cup method. As the extract showed MIC Value of 1%,the study was also carried out at 1% concentration Petroleum jelly ,Hydrophilic ointment, aloe gel, oil base and emulsifying wax exhibited no inhibition zone against the selected strains; this may be due to less drug releasing property of the bases. Significant results were obtained with Honey as base. The combination of extract with honey showed zone of inhibition in the range of 18-25mm.Results are presented in Table no 1.Thus honey was selected for kill time studies of the extract.

**Kill-time studies of extract & base**:-Kill-time studies of Cold alcoholic extracts with Honey as base on both gram positive and gram negative test isolates was carried out for 8 hours. Results are presented in Table 2. Extract with honey started its inhibitory activity against *P. aeruginosa* after a contact time period of 2 hours. *S. aureus* was inhibited after a contact time period of 3 hours. *K. pneumoniae* was inhibited after time period of 3 hours. *E. coli* was

not inhibited even after a contact time period of 8 hours.

Table 1:-Determination of antibacterial activity of extract with base by Agar cup method

| Isolates<br>Types of combination | *S.a* | *P.a* | *K.p* | *E.c* |
|---|---|---|---|---|
| | Zone of inhibition(mm) | | | |
| Extract | 14 | 15 | 13 | 12 |
| Honey control | 14 | 16 | 15 | 15 |
| Extract + Honey | 25 | 20 | 18 | 18 |
| Petroleum Jelly | - | - | - | - |
| Extract + Petroleum Jelly | - | - | - | - |

**Key: - '-----':-No inhibition seen**

*Staphylococcus aureus-S.a Pseudomonas aeruginosa-P.a*

*Klebsiella pneumoniae-K.p Escherichia coli-E.c*

Table 2:- Kill time studies of extract with honey as supportive base

| Isolates<br>Time Interval(h) | *S.a* | *P.a* | *K.p* | *E.c* |
|---|---|---|---|---|
| 0 | + | + | + | + |
| 1 | + | + | + | + |
| 2 | + | + | + | + |
| 3 | + | - | + | + |
| 4 | - | - | - | + |
| 5 | - | - | - | + |
| 6 | - | - | - | + |
| 7 | - | - | - | + |

Key: + indicates growth/no inhibitory effect observed till that time.

- indicates detection of cidal effect at particular time interval.

*Staphylococcus aureus -S.a,Pseudomonas aeruginosa -P.a,*

*Klebsiella pneumoniae -K.p,Escherichia coli- E.c*

**Skin Toxicity Tests (in vivo methods)**:-The combination of plant extracts were checked for its toxicity on topical application by Draize-shelanski method. Toxic effect in the form of Erythema and Edema was not seen. Hence, the extract was confirmed to be non-toxic in nature

& can be used as a topical agent to control infection.

**Conclusion:** The alcoholic extracts of Tulsi, Nilgiri & Nirgudi showed significant anti-bacterial activity against multi-drug resistant test isolates in comparison to aqueous extracts. The extract in combination with honey showed greater inhibitory effect on all test isolates as compared to extract alone and was found to be non-toxic on the skin surface of male albino rats.

Thus, we conclude that the herbal extract under study shows promising results and as a topical agent to control sepsis causing organisms can be formulated.

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