International Journal of Advance and Applied Research
www.ijaar.co.in
ISSN – 2347-7075 Impact Factor – 8.141
Peer Reviewed Bi-Monthly
Vol.7 No.22 March –April- 2026

Studies on effect of herbal plant extract against Nosocomial infection

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DOI - 10.5281/zenodo.19327925

Abstract:

Nosocomial Infection, commonly referred to as Hospital–Acquired Infection (HAIs), pose a significant challenge to healthcare facilities worldwide, leading to increased morbidity, mortality, and health care costs. Traditional antibiotic therapies often encounter limitation due to the emergence of multidrug resistant pathogens. As a result, researches have turned their attention to alternative therapeutics strategies, such as the utilization of herbal plant extract with potential antimicrobial properties. Several herbal plant extracts have shown promising antimicrobial activity against a wide range of nosocomial pathogens, including methicillin–resistant Staphylococcus aureus (MRSA), vancomycin–resistant Enterococcus faecium (VRE), and multidrug–resistant Gram-Negative bacteria. The extract exhibited inhibitory effects on bacterial growth and virulence factor expression, thereby reducing pathogenicity. Moreover, synergistic effects were observed with herbal extract suggesting potential therapies to enhance treatment efficacy.

Keywords: ***Nosocomial infections, Multidrug-resistant (MDR) pathogens, antimicrobial resistance activity***

Introduction:

The recent focus on infections acquired in healthcare facilities in both developing and developed countries has highlighted the importance of improving infection control. Healthcare professionals, patients and the general public are regularly being exposed to a multitude of bacterial, viral and fungal pathogens in outpatient and inpatient settings. These infections are referred to as nosocomial infections, hospital-acquired infections or health care-associated infections. The World Health Organization (WHO) raises the following points regarding nosocomial infections.

It is one that occurs in a patient in a hospital or other healthcare facility in whom the infection was not present or incubating at the time of admission. Infections that occur more than 48 hours after admission are usually considered nosocomial. This includes infections acquired in the hospital but appearing after discharge as well as occupational infections among staffs of the facility. It may also be considered either endemic or epidemic. In-patient facilities and in particular intensive care units (ICUs) have been frequently identified as the centers of hospital-acquired infections. The risks however have extended to outpatient facilities such as waiting areas, clinics and pharmacies (Voidarou 2014). Furthermore, nosocomial infections can appear after the infected individuals have left the facilities. Hospitals have sanitation protocols regarding uniforms, equipment sterilization, washing, and other protective measures. More careful use of antibacterial agents, like antibiotics, is also considered vital. As variety hospital acquired infections like MRSA, Clostridium difficile, and MSSA, are produced via a breach of these

protocols, it is known that affected patient makes a medical negligence claim compared to the hospital in question. Universal standard (infection control) measures, such as handwashing with soap and water or using alcohol-based disinfectant before and after each patient visit, are vital in reducing rates of transmission.

For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief. It is estimated that local communities have used about 10% of all flowering plants on Earth to treat various infections, although only 1% have gained recognition by modern scientists. Owing to their popular use as remedies for many infectious diseases, searches for plants containing antimicrobial substances are frequent. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties. A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases due to their availability, fewer side effects and reduced toxicity. There are several reports on the antimicrobial activity of different herbal extracts. Many plants have been found to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections. Cytotoxic compounds have been isolated from the species of Vismia. Antibacterial activity of the essential oil as well as eugenic purified from Ocimum gratissimum to treat pneumonia, diarrhea and conjunctivitis has also been reported earlier. According to the WHO, medicinal plants would be the best source for obtaining variety of drugs. These evidences contribute to support and quantify the importance of screening natural products. The aim of the present study was to investigate the antibacterial and antifungal activity of ethanol extracts of Acacia nilotica, Terminalia arjuna, Eucalyptus globulus, Syzygium aromaticum and Cinnamomum zeylanicum against multi-drug-resistant strains isolated from nosocomial and community acquired infections (Rosina Khan).

In a study, the use of gloves and gowns did not prevent contamination and conclusively did not seem enough to prevent the spread of infections.

Gram-positive bacteria are the commonest cause of nosocomial infections with Staphylococcus aureus being the predominant pathogen. Gram negative bacteria are a common cause of nosocomial infections. These bacteria have a unique cell wall structure that makes them more resistant to certain antibiotics and disinfectants. Some of the most notable Gram-Negative bacteria associated with nosocomial infection includes:

Escherichia coli: Escherichia is a normal resident of the gastrointestinal tract but can cause infections if it enters other parts of the body. In hospital, it is a leading cause urinary tract infection, surgical site infections.

Klebsiella pneumoniae: It is known to cause pneumonia, urinary tract infection, surgical site infection. It is particular concern due to its ability to develop resistance to multiple herbs.

Pseudomonas aeruginosa: Pseudomonas is a versatile bacterium that can cause infections in various body sites, including respiratory tract, urinary tract, wound infection, blood stream infections.

Enterobacter species: Enterobacter can cause a range of infections, including respiratory tract infections, urinary tract infections, bloodstream infections, and surgical site infections.

Material and Method:

Plant Leaves:

Thyme (Thymus vulgaris),Rosemary (Salvia rosmarinus),Green Tea (Camellia sinensis),Cloves (Syzygium aromaticum),Neem (Azadirachta indica)

Labware:

Spreader,Flasks,Funnel,Petri plates,Measuring cylinder,Pipette

Bacterial Cultures:

Escherichia coli,Staphylococcus aureus,Candida spp.

Culture Media (Nutrient Agar),Solvent (Ethanol)

Plant Collection:

The plant materials of Thyme, Rosemary, and Neem were purchased from a nursery in Pune. Leaves of Green Tea and Cloves were collected from a local market.The freshly collected leaves were washed thoroughly under running tap water 2–3 times to remove soil and dust particles. The cleaned leaves were then dried under direct sunlight for 6–7 days. After drying, the plant material was examined to ensure the absence of moisture. If moisture was present, the leaves were dried for additional days or subjected to a hot air oven at 25–35°C for 15 minutes. Once completely dried, the leaves were ground into a fine powder using a grinder or mortar and pestle. The powdered samples were stored in airtight plastic containers at room temperature until further analysis.

Extraction Methods:

Extraction is the initial and essential process used to separate bioactive compounds from raw plant materials. In pharmaceutical and phytochemical studies, extraction involves removing the medicinally active constituents from the inert plant components using selective solvents known as menstruum. Standard extraction methods are aimed at obtaining the desired therapeutic components while eliminating non-useful plant material.

Several extraction techniques are commonly used:

Maceration: In maceration, the whole or coarsely powdered plant material is soaked in a closed container with a suitable solvent at room temperature for at least 3 days, with frequent agitation. After extraction, the mixture is strained, the marc (solid residue) is pressed, and the combined liquid extract is filtered or allowed to settle for clarification.

Infusion: This method is similar to maceration but carried out for a shorter duration. Powdered plant material is placed in a clean container, and hot or cold solvent is poured over it. The mixture is kept for a limited time to allow the extraction of readily soluble bioactive compounds.

Digestion: Digestion involves extraction using gentle heat. Powdered plant material is mixed with solvent and kept in a water bath or oven at approximately 50°C. The applied heat reduces solvent viscosity and enhances the extraction of secondary metabolites. This method is suitable for plant materials with compounds that dissolve easily under mild heating.

Decoction: Decoction is a hot extraction technique carried out using water as the solvent. Powdered plant material is boiled with water for about 15 minutes, allowing extraction of water-soluble and heat-stable constituents. This method is generally used for roots, barks, and tough plant materials.

Percolation: Percolation is performed using a percolator—a cone-shaped glass vessel with openings at both ends. Finely powdered plant material is moistened with solvent and allowed to swell. Solvent is then added from the top until saturation is achieved. The lower opening is opened to allow extract to drip slowly while additional solvent is added. Extraction continues until 75% of the total solvent volume is collected. The extract is then filtered, decanted, and adjusted to the required volume.

Cold Press Extraction: This method is primarily used for obtaining oils from seeds and nuts. Mechanical pressure is applied to the plant material to extract oil without using heat. Cold pressing preserves the natural chemical composition and bioactivity of the oils.

Solvents Used for Extraction: The choice of solvent depends on plant type, plant part, polarity of bioactive compounds, and solvent availability. Polar solvents (e.g., water, ethanol, methanol) are used to extract polar compounds. Non-polar solvents (e.g., hexane, dichloromethane) are used for non-polar phytochemicals. The principle of solid-liquid extraction relies on the migration of soluble components from solid plant material into the solvent upon contact.

Solvent Extraction Method: The dried plant powder was taken in a clean beaker and ethanol was added according to the required powder-to-solvent ratio.The mixture was left undisturbed overnight or for 3–4 days to allow maximum extraction of the constituents.After the extraction period, the mixture was checked to ensure that the ethanol was fully absorbed and had dissolved the active components.The extract was then filtered separately using Whatman's filter paper, or alternatively, centrifuged at 3000–4000 rpm for 3–4 minutes to separate the solid residues. The clear filtrate obtained represented the ethanolic extract, containing only the compounds dissolved in ethanol.The pure supernatant was carefully collected.

The extract was then allowed to evaporate at room temperature until the solvent completely evaporated, leaving behind the concentrated plant extract.

Procedure for Antimicrobial Activity (Agar Well Diffusion Method):

Preparation of Media:

Nutrient Agar medium was prepared according to standard laboratory protocol.The prepared medium was sterilized and poured into sterile Petri plates under aseptic conditions.The plates were allowed to solidify completely.

Inoculation of Test Organisms

Once the medium solidified, a loopful of the following organisms was taken:

Escherichia coli

Staphylococcus aureus

Candida albicans

Each culture was swabbed uniformly over the surface of the Nutrient Agar plates in all directions to obtain a lawn culture.

Assignment of Extracts to Bacterial Cultures

Well Preparation and Loading of Extracts

Observation and Measurement

Results and Discussion:

A total of 5 ethanol extracts were tested against 3 bacterial isolates. Ethanol was selected as extraction solvent, used for the extraction of antimicrobial activity.Results of antimicrobial test, performed by the well diffusion method, were quite variable between each plant extract and the zone of inhibition ranging from 0 to 3 cm.

Table 1:

Plants	**Inhibition zone(cm)**		
	E.coli	**S. aureus**	**Candida**
Thymus vulgaris	NI	2.6+_0.5	NI
Camellia sinensis	2.4 +_0.3	NI	NI
Syzygium aromaticm	NI	NI	1.7+_0.3
Azadirachta indica	NI	NI	1.4+_0.1
Salvia rosmarinus	NI	NI	NI

Values are means +_ standard deviations. NI: No Inhibition

Conclusion:

The study provides strong evidence that herbal plant extracts have the potential to be used as alternative therapeutic options for treating

nosocomial infections. More research is required to confirm these findings, improve extraction methods, identify the active compounds responsible for antimicrobial activity, examine possible drug interactions, and evaluate the practicality of using herbal extracts in clinical practice.

Finally, utilizing the antimicrobial properties of herbal plant extracts may help in developing effective strategies for controlling nosocomial infections and reducing the emergence of multidrug-resistant pathogens in healthcare settings.

Acknowledgement:

The authors gratefully acknowledge the research facility provided by Dr. D. Y. Patil ACS College, Akurdi, Pune, Maharashtra.

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