JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

STUDY THE EFFECT OF CONSORTIUM OF *WITHANIA SOMNIFERA, OCIMUM SANCTUM AND CALENDULA OFFICINALIS* IN MANAGEMENT OF BURN WOUND INFECTIONS

[1]Neha Ailani, [2]Dr Bela Nabar
Student, Professor
[1]Department of Microbiology
[1]Smt CHM College, Ulhasnagar, Mumbai, India

***Abstract*:** Burn wound infections are of global healthcare concern due to the increased incidence of emergence of antibiotic-resistant strains in the burn wound niche. In lieu of this critical challenge, current research work explores a nature-inspired arsenal in the battle against these resistant species causing burn wound infections. Plant-based products to control infection and help in faster wound healing. The study investigates the anti-bacterial properties of alcoholic and aqueous extracts prepared in consortia using Ashwagandha (*Withania somnifera*), Tulsi (*Ocimum sanctum* or Holy Basil) and Pot marigold (*Calendula officinalis*) at its effective Inhibitory Concentration. Inhibition of bacteria causing burn wound infection was observed at 1.5 % (15 mg/ml) concentration of polyherbal consortia. The consortia were then studied to assess their cytotoxicity and wound healing potential in vitro. The Percentage cellular viability and proliferation of 3T3 murine fibroblast cell lines by the consortium was studied at the concentration range of 25 µg/mL to 500 µg/mL. Percentage viability assay performed by colorimetric MTT assay showed negligible effect on viability & proliferation of fibroblast cells even at higher concentration of 500 µg/mL. Thus, to conclude, the consortium of extract prepared using Ashwagandha, Tulsi, and *Calendula* exhibits promising antibacterial activity with no adverse effects on fibroblast viability and migration, emphasizing its therapeutic potential in burn wound treatment.

Keywords: Burn wound infections, Drug resistance, polyherbal extracts, in vitro cytotoxicity assessment, Scratch assay, MIC

I. Introduction

Burns are recognized as one of the traumatic forms of injury, encompassing a vast spectrum of consequences [1,2]. Burns are often associated with susceptibility to bacterial infections owing to various factors such as damaged skin barrier, easy access to nutrients in the wound environment, lack of epithelialization of the basal epidermal tissue, or systemic disorders leading to immunosuppression which can facilitate the entry of pathogens [3]. Prolonged healing time not only increases economic and social costs for patients [4] but also presents a central challenge in the healthcare system for effective and economical management of burn wounds.

The persistence of invasive bacterial pathogens and indiscriminate use of antibiotics for treating burn wound infections has led to the emergence of drug-resistant strains. The gold standard in topical burn treatment is Silver sulfadiazine which has remained a useful antibacterial agent for burn wound treatment for a long time [5]. But the challenge to combat these infections caused by drug resistant species can be leveraged by ancient knowledge that relies on the elucidation of therapeutic properties of medicinal plants. Herbal products are largely preferred to synthetic drugs worldwide considering their widespread and ease of availability [6].

WHO suggests adopting traditional practices as a tool for maintaining health. Medicinal Plants offer a traditional yet novel approach, drawing upon an herbal paradigm, to provide a natural arsenal for controlling infections and optimizing the healing environment. Nevertheless, the validation of these herbal remedies through modern scientific methods is essential to establish their therapeutic effects.

The present research represents a comprehensive evaluation of a polyherbal formulation, assessing various parameters to elucidate its potential in promoting wound healing. Ancient herbal medicine system emphasizes use of polyherbal formulations due to their multifaceted pharmacological actions [7].

Polyherbalism utilizes the concept of synergies which indicates a positive herb–herb interaction. Current research work deals with the development of a polyherbal formulation combining Ashwagandha (*Withania somnifera*), Tulsi (*Ocimum sanctum* or Holy Basil) and Calendula (*Calendula officinalis*) that could potentially offer a synergistic blend of medicinal properties.

Ashwagandha is known as a magic herb and stands as a key ingredient in numerous medicinal formulations [8]. Topical application of Ashwagandha is primarily known for its anti-inflammatory properties during inflammatory responses. Pastes and decoctions derived from Ashwagandha find application in the management of wounds, bed sores, and various skin lesions [9]. Another herb used for polyherbal formulation is Tulsi, i.e., *Ocimum sanctum*, which is very well known for its antioxidant antimicrobial, anti-inflammatory and wound healing properties [10]. It has been reported that Tulsi treated wounds exhibited greater degree of neovascularization and fibroblast proliferation, better granulation tissue formation and collagenization and epithelialization was early and complete [11]. Mixture of polyherbal also uses *Calendula officinalis*, member of Asteraceae family which is very well known for its Antibacterial, anti-inflammatory and antioxidant properties. All the parameters mentioned play a key role in wound healing [12]. Each of the herbs used in consortial extracts is known for its unique therapeutic effects, and their combination may result in a comprehensive formulation.

II. Materials and Methods

1. Collection and Processing of plant material for Extract preparation

Fresh plant materials, namely Ashwagandha roots, Tulsi leaves, and Pot marigold flowers, were obtained from local nurseries and authenticated. After authentication, plant materials were cleaned by gently washing under running tap water and were shade dried followed by grinding it to fine powder. Equal portions of each powdered material were combined in equal proportions and extracted using water and alcohol as solvent. The resulting extracts were concentrated using a rotary vacuum evaporator under reduced pressure and at a temperature maintained below 40 °C [13].

2. Bacterial pathogens from burn wound infections

2.1 Standard Cultures

Standard ATCC strains- *E. coli 25922, S. aureus 25923, P. aeruginosa 27853, K. pneumoniae 700603* were used for comparative studies.

2.2 Clinical isolates

Clinical isolates were obtained by collecting swab samples from burn wound patients. The samples were processed for further isolation, identification for further antibacterial studies. The isolates were first subjected to antibiotic susceptibility testing for screening resistant strains

3. Antibiotic susceptibility testing of clinical isolates

Antibiotic susceptibility to commercially available antibiotic disc was tested according to Clinical and Laboratory Standard Institute guidelines [15] by the agar disk diffusion test using Mueller–Hinton (MH) agar against the following antibiotics: Imipenem, Meropenem, Doripenem, Ertapenem, Cefepime, Ampicillin, Kanamycin, Nitrofurantoin, Gentamicin, Vancomycin, Oxacillin, Cefoxitin, Methicillin, Penicillin G, Amoxicillin, Piperacillin-Tazobactam, Amikacin, Nalidixic acid and Tetracycline.

4. Evaluation of Synergistic Antibacterial activity against sepsis causing organisms

4.1 Antibacterial activity by Agar ditch method

The antibacterial efficacy was assessed by the agar ditch method using both standard and clinical isolates. A concentration range of 10 mg/mL to 50 mg/mL of extract combinations was individually mixed with molten agar butt. Subsequently, the mixture of a particular concentration of polyherbal extract and molten agar butt was poured into a ditch created on the nutrient agar plate, and isolates were streaked perpendicularly to the ditch [16].

4.2 Determination of Minimum Inhibitory Concentration (MIC) of polyherbal extract

The lowest concentration of the antibacterial agent at which the organism is completely inhibited is called its *Minimum Inhibitory Concentration*. Minimum Inhibitory Concentration of the test compounds were determined by plate dilution method. The different concentration of extracts 5 mg/ml to 20 mg/ml were mixed with agar and poured in the sterile empty Petri plates. Isolates were spot inoculated onto the plates and were incubated at 37°C for 24 hours [17].

5. *In vitro* studies using polyherbal extract

5.1 Cytotoxicity assessment by MTT Assay

The cytotoxicity of the Polyherbal formulation was assessed on mouse 3T3 fibroblast cells using the MTT assay. It is a colorimetric assay that monitors and records reduction of yellow 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), functioning on the principle of assessing cell viability. The reduction of MTT served as an indicator of cytotoxicity. The

experiment was carried out over a predefined incubation period and under meticulously controlled experimental conditions [18,19].

5.2 Scratch assay

In vitro Scratch assay mimics wound contracture in vivo. In this assay, an artificial gap, a so- called "scratch" is typically created in a cell monolayer with a sharp object such as a pipette tip or syringe needle. The monolayers recover and wound healing occurs in a process that can be observed over time. The wound heals in a patterned fashion – cells polarize toward the wound, initiate protrusion, migrate, and close the wound. Progression of these events can be monitored by manually imaging samples fixed at different time points, or by time-lapse microscopy [20].

III. Results and Discussion

1. Collection and Processing of plant material for Extract preparation

Individual plant material was collected from the botanical garden followed by washing and grinding to fine powder for further extract preparation. The extraction process involved combining the individual herbal powders in a 1:1 ratio and using water and alcohol as solvents. The hot aqueous extract of the herbal consortium was prepared by soaking and boiling the herbal powders (mixed in a 1:1 ratio) in distilled water until the volume was reduced to 1/4th of the original. On the other hand, the hot alcoholic extract was obtained using a Soxhlet apparatus.

2. Bacterial pathogens from burn wound infections

Systematic approach was employed, with samples collected at weekly intervals to check the presence of predominant isolates from the burn wound niche. A total of 136 isolates were obtained which were identified by biochemical testing. Identification of isolates revealed the isolates belonging to genus *Pseudomonas*, *Staphylococcus*, *Klebsiella* and *Escherichia*. *Pseudomonas* spp (36%) was the commonest pathogen isolated from wound of burnt patients followed by *Staphylococcus aureus* (35%) *Escherichia coli* (15%) *Klebsiella pneumoniae* (14 %).

3. Antibiotic susceptibility testing of clinical isolates

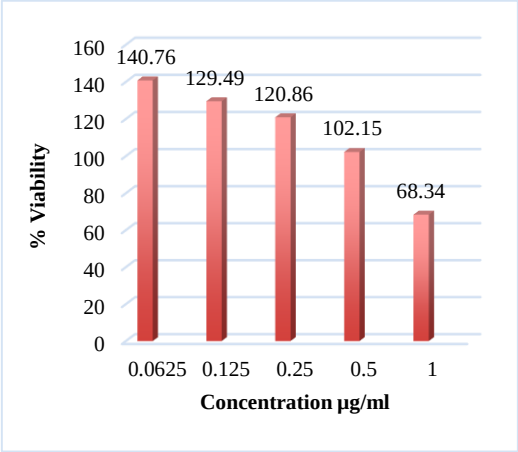
The antibiotic susceptibility testing was carried out to check the resistance patterns of the obtained isolates against antibiotics. Out of the total 136 isolates, 46% exhibited resistance, while 54% showed sensitivity to the tested antibiotics. Notably, 65% of the Gram-positive isolates belonging to genus *Staphylococcus* demonstrated resistance, with the remaining 35% displaying sensitivity. Among the Gram-negative isolates from three genera- *Escherichia, Pseudomonas,* and *Klebsiella*, 41% exhibited antibiotic resistance, while 59% were found to be sensitive to the antibiotics being studied.

4. Antibacterial activity & Minimum Inhibitory Concentration (MIC) Determination

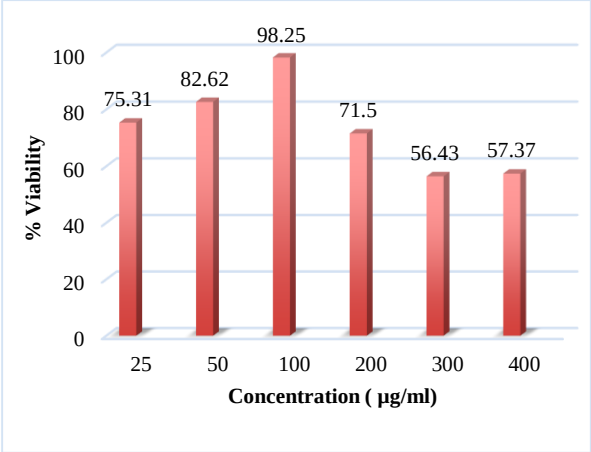
Hot aqueous and Hot alcoholic polyherbal extracts demonstrated equal efficacy in inhibiting the growth of both sensitive and resistant strains, highlighting their broad-spectrum antibacterial activity.Determination of Minimum inhibitory Concentration was carried outat the concentration range of 0.5% to 2% i.e., 5 mg/ml to 20 mg/ml, based on preliminary antibacterial efficacy screening. Lower concentration of the extracts (1%, i.e., 10 mg/ml) exhibited potential antibacterial activity againststandard as well as sensitive strains, while higher concentrations of 1.5 to 2 %were required for resistant strains. These results are of prime importancein deciding an effective concentration range, especially for combating infections caused by prevalent in burn wound pathogens.

5.1 Cytotoxicity assessment by MTT Assay

The MTT (3- [4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide) assay relies on the principle of conversion of MTT into formazan crystals and the determination of mitochondrial activity and cell proliferation by living cells [23]. This *in vitro* method was used to determine percentage cell viability, which is a relative measure of cytotoxicity of test compounds. The effect of increasing concentrations of polyherbal extracts is graphically presented in Graphs **1a** and **1b**, illustrating the relationship between concentration of the extracts and percentage cellular viability.



Graph 1a



Graph 1b

Graph 1a &b: Effect of different concentrations of Polyherbal Hot Aqueous and Hot alcoholic Extract of Ashwagandha Tulsi Calendulaon % Viability of 3T3 fibroblast cell lines performed by MTT Assay

The results of the MTT assay that represent the cell viability of 3T3 fibroblast cells after 24 hrs of incubation as the concentration goes on increasing, viability of cells tends to decrease. At the highest concentration, the polyherbal extracts exhibited a cell viability percentage of more than 50%, while at the lowest concentration, the viability percentage exceeded 80%. Across all concentrations, the extract consortium displayed minimal cytotoxicity, as evidenced by the consistently high percent cell viability values. Cytotoxicity of the sample is rated based on the percentage viability of cells under the influence of increasing concentrations of extract consortium.

5.2 Scratch assay

Fibroblast cells demonstrated significant migratory response under the influence of polyherbal extracts, effectively closing artificially created gaps. In a scratch assay, this migration is monitored at regular time intervals as the cells move to fill the wound or scratch in a monolayer. *In vitro* scratch assay highlights the potential of polyherbal extracts to enhance fibroblast activity and promote wound healing.Both alcoholic and aqueous polyherbal extracts were examined for their effect on scratch closure.

Microscopic analysis of fibroblast migration was recorded every 24 hours over a 72-hour period. Enhanced proliferative activity of fibroblasts was observed in response to polyherbal extracts suggesting potential of these extracts in facilitating woundrepair.Pictorial representation of the wound healing activity is represented in the table 1.

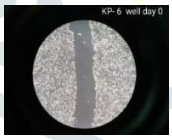
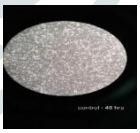
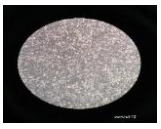
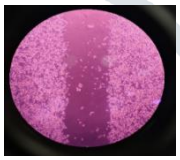
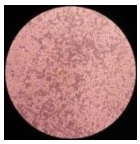
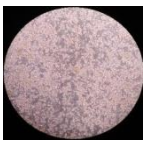
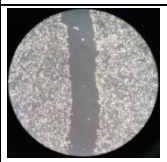
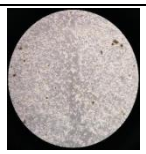
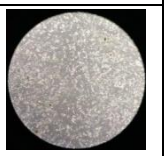
Sr No.	Sample Name	0 hr	24 hrs	48 hrs	72 hrs
1	*Control*				
2	*Hot Aqueous extract of Ashwagandha Tulsi Pot marigold*				
3	*Hot alcoholic extract of Ashwagandha Tulsi Pot marigold*				

Table 1: It shows the effect of polyherbal extracts on 3T3 murine fibroblast cells by in vitro scratch assay at 0, 24, 48 and 72 hrs

It is evident from the results that predominant Burn wound sepsis organisms belong to genus *Pseudomonas*, *Staphylococcus*, *Escherichia*, *Klebsiella*. Furthermore, there is an alarmingincrease in emergence of resistant strains which increases severity of infection. So, control of infection, limited or use of alternative antimicrobial agents.Use of complementary and alternative medicine practices can pave a way to efficient management of infections in burn wound patients.

IV. Conclusion:

The current research establishes a foundational framework for alternative therapeutic approaches, emphasizing the critical need to address antibiotic resistance in clinical settings. A systematic screening approach involving hot aqueous and hot alcoholic polyherbal extracts for antibacterial activity, Minimum Inhibitory Concentration (MIC) determination, and cell viability and proliferative assays has been employed. The outcomes of each screening phase play a pivotal role in guiding the selection of treatment agents for effective burn wound management.

References

1. Ezzati A, Bayat M, Taheri S, Mohsenifar Z. 2009. Low-level laser therapy with pulsed infrared laser accelerates third-degree burn healing process in rats. *J Rehabil Res Dev.*,Vol. 46**,**pp. 543–54.
2. Upadhyay NK, Kumar R, Siddiqui MS, Gupta A. 2011. Mechanism of Wound-Healing Activity of Hippophaerhamnoides L. Leaf Extract in Experimental Burns. *Evid Based Complement Alternat Med.*, vol.2011**.**
3. Dai, T.; Kharkwal, G.B.; Tanaka, M.; Huang, Y.-Y.; Bil de Arce, V.J.; Hamblin, M.R. 2011. Animal Models of External Traumatic Wound Infections. *Virulence*, vol. *2*,pp. 296–315.
4. Young, A.; McNaught, C.-E. 2011. The Physiology of Wound Healing. *Surg. Oxf., vol. 29***,**pp. 475–479.
5. Atiyeh BS, Costagliola M, Hayek SN, Dibo SA. 2007. Effect of silver on burn wound infection control and healing: Review Of The literature.Burns., vol. 33, issue 2, pp. 139-48
6. Chaudhari M, Mengi S. 2006. Evaluation of phytoconstituents of Terminalia arjuna for wound healing activity in rats. *Phytother Res.*, vol. 20**,** pp. 799–805
7. M. Spinella. 2002. The importance of pharmacological synergy in psychoactive herbal medicines. Alternative Med Rev, vol. 7, pp. 130-137
8. Mishra LC, Singh BB, Dagenais S. 2000. Scientific basis for the therapeutic use of Withania somnifera (Ashwagandha): a review. Altern Med Rev., vol. 5,pp. 334-46.
9. Husain N, Trak TH, Panday B. 2018. Ethnomedicinal significance of two important shrubs, viz *Withania somnifera* (L.) and *Datura metal* (L.) (Family Solanaceae) of Chhattisgarh, India. Indian J Sci Res., vol. 9, pp.37-39.
10. Goel A, Kumar S, Singh DK, Bhatia AK. 2010. Wound healing potential of *Ocimum sanctum* Linn. with induction of tumor necrosis factor-α. Indian J. Exp. Biol., vol. 48, pp. 402-6.
11. Asha B, Nagabhushan A, Shashikala GH. 2011. Study of wound healing activity of topical *Ocimum sanctum* Linn in Albino Rats. J. Chem. Pharm. Res., vol.3, pp. 122-26.
12. Preethi K. Chandran &RamadasanKuttan, 2008. Effect of *Calendula officinalis* extract on Acute phase proteins, Antioxidant Defense Mechanism and Granuloma formation during thermal burn. Journal of clin. Biochem. Nutr., vol. 43,58-64.
13. KRC Reedy.2005. KRC Reedy Bhaishjya Kalpana, Vigyan Edition, 235**.**
14. Clinical and Laboratory Standards Institute PS for ASTestingCMSCMAC and LSIPPU 27th edition, 2017. Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing. CLSI-M100-S27, CLSI M02-A12, Clinical and Laboratory Standards Institute, Pennsylvania, PA, USA, 27th edition**.**
15. Spooner SD and SG.1972. Laboratory assessment of antibacterial activity. Academic press, London 7B, 216- 217**.**
16. Edberg SC and BSA. 1983. Antibiotics and Infection. Churchill Livingstone Ltd, 213**.**
17. Savaroglu F Icvaksisur. 2011. Determination of antimicrobial and antiproliferative activities of the aquatic moss Fontinalis antipyretica Hedw. Turk J Biol, vol. 35, issue 3, pp. 361–369.
18. Fernandes A. 2014. Development of experimental in vitro burn model, Acta Cirúrgica Brasileira, vol. 29, issue 2**,** pp. 2015-20.
19. Liang, C.C., Park, A.Y., Guan, J.L. 2007. *In vitro* scratch assay: a convenientand inexpensive method foranalysis of cell migration in vitro. Nature Protocols 2, pp. 329– 333.