

In vitro and in Vivo Antimicrobial Activity of Padigara parpam Against Staphylococcus Petrasii Subsp. Pragensis, Recently Identified Pathogen

K. Saravanasingh^a, E. David^b*, P. Parthiban^c, M. Ramamurthy^d

^aDepartment of Biotechnology, Research Scholar, Thiruvalluvar University, Vellore, Tamilnadu, India

^bDepartment of Biotechnology, Head of the Department, Thiruvalluvar University, Vellore, Tamilnadu, India

^cJoint Director, Department of Indian Medicine and Homeopathy, Chennai, Tamilnadu, India

^dAssistant Professor, National Institute of Siddha, Chennai, Tamilnadu, India

*Corresponding author. *E.mail address:* ernestdavid2009@gmail.com

ABSTRACT

The Siddha treatment is among India's oldest healthcare systems. The Siddha medication is the oldest medical system. This scheme was devised and developed by the Chinese over a millennium ago. *Padikaram* is one among the kaarasaram that is utilized in both the domestic and therapeutic purposes. Histomorphological examination of wound tissue infection, negative control group showed erosion in epidermis and infiltration of inflammatory cells in the dermis extending into the adjacent subcutaneous connective tissue. Granulation tissue and capillary formation was found to be minimal when compared to treated groups.

Keywords: *wound, Histomorphological, In vivo, Padikara parpam, Traditional siddha medicine*

1. INTRODUCTION

The term "siddha" was kept a pharmacology Herbal medicine is a part of Siddha medicine in animal products, natural molecules, and inorganic materials resulting in a variety of preparations varying from plant-based to animal-based herbo mineral medications include parpam [1]. All in nature, as per siddha doctrines, has two qualities: good and bad. They have two levels when used for any reason, particularly in medicine. As a consequence, when any natural matter is used for medication, the properties that could cause negative effects must be neutralised or removed [2]. The sea is nature's principal constituent of minerals such as calcium, phosphorus, iron, and a few other trace elements. Pharmaceutical companies and cosmeceuticals both benefit from the biologically active products produced by marine species [3]. According to siddha literature, Palagarai is one of the marine tools (kadalpadu draviyantal) [4].

Medication is an art of profound significance to humankind's stable existence. Siddha, scientific research, as ancient as historical events, is very old in origin. One of its kaarasaram that is used for both residential and therapeutic reasons is the padikaram. Padikara parpam is commonly employed in siddha medication for the therapy of urinary tract infections, menorrhagia, haematuria, and urinary frequency. It is primarily employed for water treatment in domestic applications. It is being employed as styptic, disinfectant, and astringent for therapeutic reasons. Lingam (Mercury Red Sulphide) is a padanam. The siddhars were conscious of the occurrence of metallic substances, ores and were so developed in their experience that they would be ready to organize them with simpler ingredients. In Siddha medicine, Mercury maintains a very higher role. Parpam (mineral / metallic oxides) is the most widely utilized preparations in association with nutrients in this drug method [5]. Parpam types of medicinal products are commonly used, with possible therapeutic values. For 100 years, Parpam maintains its strength (shelf life).

The philosophy of Siddha, that envisages the cosmos, is the identical inside and outside the bodies, meaning that the anatomy of the brain craves to be controlled as an effective season even by climate and the dominant season safety precaution for one's excellently-being. Climate (Nilam): Habitat Habitat Nilam defines the location of one's presence. Season

(Pozhuthu): seasonal effect the occurrence of disease and experiencing significant were established. These disorders have been recommended to be prevented. (NaalOzhukkam) Daily Regimen: NaalOzhukkam investigates the sequential series of each individual's regular exercises to eliminate lifestyle problems and associated illnesses. It elucidates the system for our body and mind to actually take care of [6].

India is renowned for its ancient Ayurveda, Siddha, and Unani medicine systems. Though in the ancient Vedas as well as other scripture, methods are also described. Among 2500 and 500 BC in India [7], the ayurvedic definition emerged and evolved. The exact implication of ayurvedic medicine is "science of life," since the earliest Indian medical scheme centred on man's perceptions and his disease. It's been figured out that physiologically well-balanced living things reflect positive health. The "science of immortality" is also named Ayurveda as it provides a complete structure for a long, safe existence. It provides programmed via food and health to rejuvenate the organ. It includes treatment types to treat several serious ailments, like food allergies, that have few conventional therapies.

While human medications have made considerable progress, viral infections produced by microbes, fungi, viruses, and pests are also a serious public health problem. Since 1926, an experimental study has been undertaken in laboratories on the properties of plants that suppress microbes but are essential for public health [8]. The production of microbial resistance to antibiotics presently offered has necessitated the hunt for novel antibacterial agents. Alternative sources have been examined, mainly naturally occurring products, since traditional medicines have been commonly used throughout Asia to manage many forms of acute and chronic conditions, and several plants of antibacterial activities were identified [9]. The antibiotic risk to humans and animals is usually regarded low.

Nevertheless, prolonged usage of such antibiotics could reduce the number of intestinal plants that could has a detrimental effect on health. Today, Siddha method is utilized to mitigate these negative impacts [10]. Siddha medication is among the most traditional medical practice systems known to humankind. Thus, scenery is human, and a person is nature and, consequently, fundamental-based. Man is told to a microcosm and a world is macrocosm, since what exists in a world exists in man. Siddha's basic concepts never separated man from the universe. Agasthiyar was the first siddhar and thousands of Siddha documents like medication were created by his disclosures and siddhar from several other schools and formed the system's proponents to the globe [11].

Few of Antibacterial Siddha Medicines are *Acalypha indica*, *Padikara parpam*, *Andrographis paniculata*, *Lawsonia inermis*, *Curcuma longa*, *Butea monosperma*, *Embelia ribes* and *Corallocarpus epigaeus*. For instance, *Acalypha indica* is a yearly herb that belong to a group of Euphorbiaceae. Leaves, roots, entire plants are essential pieces. It has behaviors which are anthelmintic, anodyne, cathartic, analgesic, and diuretic. Dental carriers, burns, digestive problems, hemorrhoids, rhinitis are healed [12]. As a replacement for senega, the flora is utilized as expectorant, it has diuretic activity. Valuable medication for bronchitis, pneumoniae, tuberculosis, and rheumatic treatments. Moreover, historically used as a stimulant and anthelmintic agent [13]. The methanolic extracts of *acalypha indica* demonstrated optimum solubilization towards *B. cereus* and *subtilis*, *E. coli*, *K. pneumoniae* and *V. cholerae* while examining the antibacterial activity. Complete inhibition towards *S. aureus*, *K. pneumoniae* and *S. flexeri* was shown in ethanol extract of *Acalypha indica*, *Vibrio cholera* and *Bacillus cereus* is immune to ethyl acetate. *P. aeruginosa* was resist to ethyl acetate for the extraction of *Acalypha indica*. C_2H_5OH and H_2O extracts from *Acalypha Indica* leaves, stems, seeds, and roots were efficient towards bacterial strains *E. coli*, and

Aspergillus fumigates, and *C. albicans*. All *Acalypha indica* are immune to both water and ethanol extracts [14].

Padikaram is one among the kaarasaram that is utilized in both the domestic and therapeutic purposes [15]. One of its ten types of organic salts is padikaram. In the process is specifically noted in Thathu Jeeva Vahuppu Padikaram of organic salt. *Andrographis paniculata* is an upright, spaced annual shrub belongs to the -Acanthaceae group. The stem and leaves were useful pieces. It has an abdominal, tonic, alternative available, relaxing action. All kinds of fever, sinusitis, giddiness is cured [16,17].

The current research was assumed to find the antimicrobial action of the formulations of polyherbal siddha. *Syzygium aromaticum*, and so on the latex are part of the recipe. The medicines used throughout the preparation were exposed both in isolation and in isolation to antibacterial activities.

2. MATERIALS AND METHODS

Purified herbo mineral drug Padikara parpam stock solution prepared by dissolving 2 gm of each drugs in 10 ml of distilled water. Then keep on rotary shaker at 190-220 rpm for 24 hrs. The extracts were filtered using Whatman filter paper (125 mm) and stored at 4°C in air tight bottles until further use. The *in vivo* antimicrobial efficacy of Padikara parpam for gram positive (*Staphylococcus petrasii subsp. Pragensis*) bacteria.

Male BALB/C mice six week of age (18-20 gm) were obtained from the animal house of *in vivo* Bio sciences, Bengaluru. Mice were kept under specific pathogenic free condition, housed, fed and treated in accordance with the CPCSEA guidelines. They were maintained on standard pellet diet and water *ad libitum* for 2 weeks prior to the investigation. *Staphylococcus petrasii subsp. Pragensis* was isolated from human pus samples and identified by biochemical and molecular (16s ribosomal rna sequencing) methods and submitted to NCBI (ACCESSION MG970131). Bacterial culture was routinely grown in nutrient broth at 37°C for 24hr. Active culture was harvested by centrifugation, washed twice and resuspended in sterile saline. Infection of BALB/c mice with 100 ml of *Staphylococcus petrasii subsp. Pragensis* suspension at a dose of 1×10^8 colony forming unit (CFU)/mouse was conducted into shaved mid dorsal skin.

Table 1. Animal groups and treatment

Group	No. of animals	Details of treatment
G1	6 M	No induction and No treatment
G2	6M	Induction but No treatment
G3	6M	Induction+ treatment with reference item(ciprofloxacin) for 5 days
G4	6M	Induction+ treatment with test item (Low dose 1) for 5 days
G5	6M	Induction+ treatment with test item (Mid dose 2) for 5 days
G6	6M	Induction+ treatment with test item (High dose 3) for 5 days

Mice were randomly divided into 6 groups of 6 mice each. Various doses of Padigara parpam were prepared and preliminary tested for their tolerance in BALB/c mice to select the optimum dose intended for the treatment of experimental animals as shown in Table 1. 100, 300, and 1000 mg/kg were used as low, mid and high doses respectively. Mice

were housed and maintained on normal food and water *ad libitum* during the whole period of experiment.

3. RESULTS AND DISCUSSION

Agar Well diffusion method

The Padikara parpam exhibited the maximum inhibitory activity towards *Staphylococcus Petrasii Subsp. Pragensis* as shown in Figure 1.

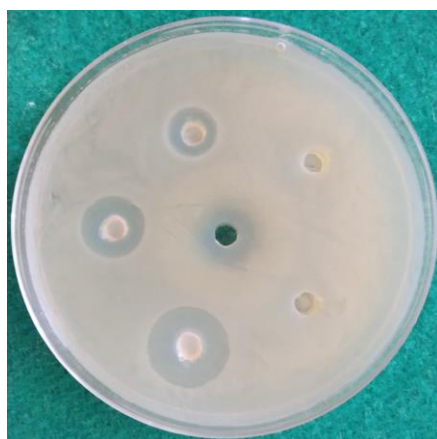
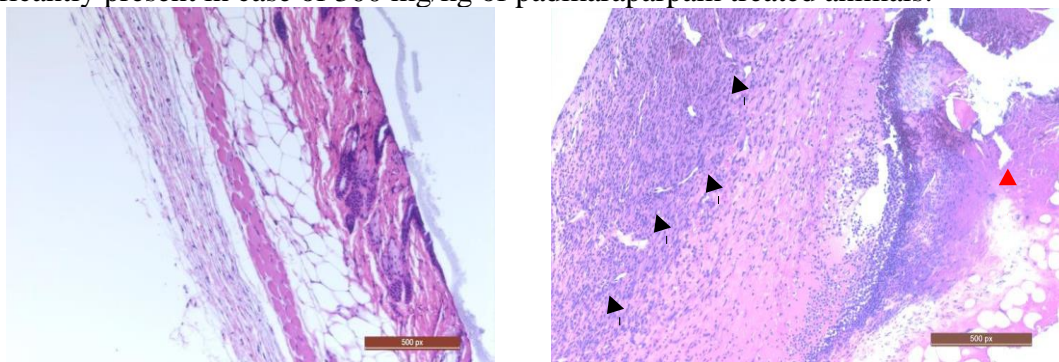


Figure 1. Antimicrobial activity of aqueous extract of Padikara parpam

Because of its better anti-microbial activity of Padikara parpam against *Staphylococcus Petrasii Subsp. Pragensis*, we have taken these extracts for further in vivo study.

Histopathological profile

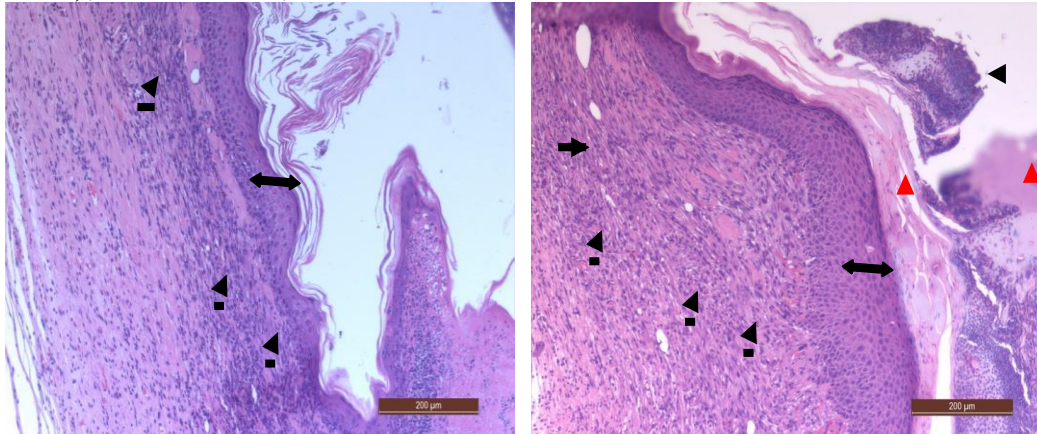
Histomorphological examination of wound tissue on the 5th day of infection, negative control group showed erosion in epidermis and infiltration of inflammatory cells in the dermis extending into the adjacent subcutaneous connective tissue, shown in Figure 2. Granulation tissue and capillary formation was found to be minimal when compared to treated groups. The aqueous extract of Padikaraparpam treated animals showed almost the same degree of re-epithelization. However, 1000 mg/kg of padikaraparpam treated animals exhibited much reduced inflammation with more number of capillary and granulation tissue formation in comparison to 100 and 300 mg/kg of padikaraparpam treated animals. Mild edema could still be seen in 100 mg/kg of padikaraparpam treated animals. These were significantly present in case of 300 mg/kg of padikaraparpam treated animals.



G1 Sham control: Skin showing normal architecture; H&Estain; 10x

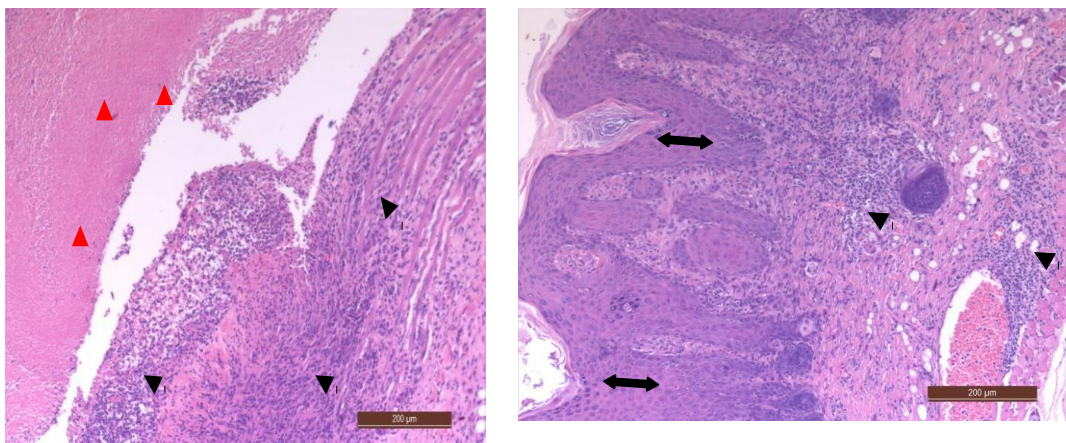
G2 Infection control; Skin showing necrosis (red arrow), inflammation (arrow)

head), H & E stain; 10x



G3, Positive Control, Skin showing epidermal hyperplasia (two headed arrow) and inflammation (arrowhead), H & E stain; 10x

G4 Treatment group 1; Skin showing epidermal hyperplasia (two headed arrow), inflammation (arrow head), necrosis (red arrow) and Parakeratosis (black arrow) H & E stain; 10x



G5 Treatment group 2 Skin showing necrosis (red arrow), inflammation (arrow head), H & E stain; 10x

G6 Treatment group 3 Skin showing epidermal hyperplasia (two headed arrow), and inflammation (arrow head), H & E stain; 10x

Figure 2. Histomorphological examination of burn wound tissue

4. CONCLUSION

The results of the findings are presented conveniently in the following manner. Aqueous extract of padikara parpam was screened for antimicrobial activity against *Staphylococcus petrasii subsp. Pragensis* bacteria. It shows very excellent inhibition. *In vivo* study Histomorphological examination of wound tissue infection, negative control group showed erosion in epidermis and infiltration of inflammatory cells in the dermis extending into the adjacent subcutaneous connective tissue. Granulation tissue and capillary formation was found to be minimal when compared to treated groups. The aqueous extract of Padikara parpam treated animals showed almost the same degree of re-epithelization. The overall results of the present work provide baseline information for the possible of Padikara parpam

in the treatment against Staphylococcal, especially wound infection. These observations can justify the traditional use of the Padikara parpam in the treatment of wound.

ACKNOWLEDGEMENT

This study was supported by a grant in aid of The Ministry of AYUSH, AYUSH BHAWAN, New Delhi-110023

CONFLICT OF INTEREST

The authors have declared that there is no Conflict of Interest.

REFERENCES

1. Dr. Chidambarathanu Pillai. S, Siddha System of Toxicology, Siddha Medical Literature Research Centre, Madras -102, 1st Edition 1993, 1.
2. Pandey, M. M., Rastogi, S., & Rawat, A. K. S. (2013). Indian traditional ayurvedic system of medicine and nutritional supplementation. Evidence-Based Complementary and Alternative Medicine, 2013.
3. Kavitha, D., & Nirmaladevi, R. (2009). Assessment of Aristolochiabracteolata leaf extracts for its biotherapeutic potential. African Journal of Biotechnology, 8(17).
4. Xu, Y., Marshall, R. L., & Mukkur, T. K. (2006). An investigation on the antimicrobial activity of Andrographis paniculata extracts and andrographolide in vitro. Asian journal of plant sciences.
5. Sieler, R. (2015). Lethal spots, vital secrets: Medicine and martial arts in South India. Oxford University Press, USA.
6. Malarvizhi, E. (2019). A Prospective Open Labelled Phase-II Non Randomized Clinical Trial Drug on Herbal Formulation of NannariVerOoralKudineer for the treatment of Vali Azhal Keel Vayu (Rheumatoid Arthritis) (Doctoral dissertation, Government Siddha Medical College, Palayamkottai).
7. Kirtikar, K. R., & Basu, B. D. (1991). Indian Medicinal plants second edition, B Singh and MP Singh publications.
8. Murali, P. M., Rajasekaran, S., Paramesh, P., Krishnarajasekar, O. R., Vasudevan, S., Nalini, K., & Deivanayagam, C. N. (2006). Plant-based formulation in the management of chronic obstructive pulmonary disease: a randomized double-blind study. Respiratory medicine, 100(1), 39-45.
9. Rajalakshmi, S., Sathiyarajeswaran, P., Samraj, K., & Kanagavalli, K. (2020). A Review On Scopes, Methods And Rationale Of Integrative Approach In Siddha Medicine With Biomedicine.
10. Rajalakshmi, K. (2016). Screening Of Common Siddha Formulations For Antimicrobial Activity Against Respiratory Pathogens. Screening, 9(2).
11. Gopalakrishnan, T., Seetharaman, T., Ganesan, S., Raja, B. T., Ganapathi, T., Sampathkumar, B., ... & Shreedevi, M. S. (2015). Evaluation of antimicrobial activity of siddha formulation against dental pathogens. Malaya Journal of Biosciences (MJB), 2(2), 139-142.
12. Gopal, M., Thinakaran, S. R., & Venkat, P. P. (2014). Quality Assessment of a Traditional Siddha Drug "Mupoorachendurum".

13. Kabilan, N., Murugesan, M., Balasubramanian, T., & Geethalakshmi, S. (2017) Physico-Chemical Analysis of Siddha Drug Poora parpam-A Comparative Evaluation between Natural and Synthetic Source 5(2).
14. Vignesh, K., Nivesh, S. J., Saravanasingh, K., & Uma, A. P. (2019) Padikaram (Alum)-A Unique drug and its Utilization in Siddha Medicine: A Pharmacological review. 14(2), 1-12.
15. Kuo CF, Chen CC, Luo YH, Huang RY, Chuang WJ, Sheu CC, Lin YS. Cordyceps sinensis mycelium protects mice from group A streptococcal infection. Journal of Medical Microbiology. 2005 Aug 1;54(8):795-802.
16. Godin B, Touitou E, Rubinstein E, Athamna A, Athamna M. A new approach for treatment of deep skin infections by an ethosomal antibiotic preparation: an in vivo study. Journal of Antimicrobial chemotherapy. 2005 Jun 1;55(6):989-94.
17. Kugelberg E, Norström T, Petersen TK, Duvold T, Andersson DI, Hughes D. Establishment of a superficial skin infection model in mice by using Staphylococcus aureus and Streptococcus pyogenes. Antimicrobial agents and chemotherapy. 2005 Aug 1;49(8):3435-41.