Annals of R.S.C.B., ISSN: 1583-6258, Vol. 24, Issue 1, 2020, Pages. 1145 -1151
Received 15 April 2020; Accepted 23June2020

Antibacterial Activity Of Selected Siddha Preparations Against Clinically Important Standard Reference Bacterial Strains

Anitha Akilan, K. Revathi*,
Meenakshi Academy of Higher Education and Research (CRL – MAHER), Chennai
*Corresponding author –K. Revathi
email: reva63@rediffmail.com

ABSTRACT
One of India's great cultural legacies is the Siddha system of medicine. Most chronic disorders are healed in the Siddha system of medicine by medications made from metal and mineral products, such as Parpam, Chendooram, and Chunnam. In this study, antibacterial activity of some clinically used herbs-mineral Siddha drugs such as **Kungiliya parpam, Vengara parpam, Padikara parpam and Silasaththu parpam** was investigated against clinically important standard reference bacterial strains (**ATCC- American Type Culture Collection**) such as ***Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus***. In Indian medicine, these siddha formulations are used to treat a variety of diseases. The disc diffusion method was used to test the antibacterial activity of siddha formulations. To standardise the antibacterial activity test, **5 mcg of levofloxacin** was used as a control. Levofloxacin is used to treat infections like pneumonia, kidney infections, prostate infections, and skin infections. The findings revealed that Vengara parpam and Padikara parpam have antibacterial activity against all clinically important standard reference bacterial strains. Antibacterial activity of Kungiliya parpam and Silasaththu parpam did not show antimicrobial effects against any of the bacterial strains. These herbo-mineral siddha formulations may be effective as an alternative medicine in the treatment of numerous diseases, according to a study.

Keywords: Siddha preparation - **kungiliya parpam, vengara parpam, padikara parpam and silasaththu parpam**. ATCC (American Type Culture Collection) - ***Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus***. Positive control - **Levofloxacin 5 mcg**

1. INTRODUCTION

Pathogens that are clinically important are considered to be major sources of hospital-acquired infection. They're also common sources of infection in the community. Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumoniae are the four most often isolated bacteria. After decades of major problems with modern medicine, numerous people turned to ancient medical systems such as Siddha, Ayurveda, and Unani. Synthetic medications are causing a slew of issues for people. One of India's ancient traditional medical systems is Siddha medicine **(Shobha et al., 2014).**

All other medical systems in the world compared to the Siddha System of Medicine, which was popular in ancient Tamil land. The Siddha System's originality is demonstrated by its ongoing service to humanity for more than 5000 years in treating illnesses and keeping physical, mental, and moral health, due to the fact that most of its contemporaries had emerged as extinct long ago **(Savarimuthu Michael et al., 2011).**

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 24, Issue 1, 2020, Pages. 1145 -1151
Received 15 April 2020; Accepted 23June2020

Natural goods are still used in primary health care as a source of synthetic and traditional herbal treatment. Antimicrobials based on Siddha provide a great unexplored source of pharmaceuticals, and further research into plant antimicrobials is needed. Organic antimicrobials have significant therapeutic promise. Natural materials have attracted a lot of attention in the last twenty years as potential sources of novel antibacterial agents. Many studies have shown that traditional herbs are efficient against microbes; as a result, medications are one of the foundations for contemporary medicine to achieve new principles. Many antibiotics are employed in the synergism assay because the majority of bacteria are resistant to them **(Purushotham et al., 2010).**

Additionally, scientific research and knowledge on the Siddha material's therapeutic potential are inadequate. Antibiotic susceptible and resistant bacteria were used to test the antimicrobial properties of several medicinal plants and Siddha medicines. Secondary metabolites generated in the secondary metabolism are responsible for the antibacterial properties of Siddha preparations **(Caroline jeba et al., 2013).**

2. MATERIALS AND METHODS

Collection of Siddha drugs

IMPCOPS pharmaceuticals in Chennai provided authenticated Kungiliya parpam, Vengara parpam, Padikara parpam, and Silasaththu parpam products. These commercially available formulations were used in traditional Indian clinical practice to treat a variety of diseases. They are usually prescribed at a dose of 100-200 mg per day, and they are best taken with a good complement. For further processing, the items were shipped to MAHER's Central Laboratory in Chennai.

Collection of Bacterial Isolates

ATCC culture was purchased from KWIK-STIK^{TM} for clinically important standard reference bacterial strains. Standard reference bacterial strains such as *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 29213) were used to isolate for this study. They were CLSI reference strains that were used in antibacterial susceptibility tests. For long-term storage, stock cultures were kept in 20% glycerol at -70^{o}C, while working cultures were kept in Nutrient agar (NA) slants at 4^{o}C. The strains were sent to MAHER's Central Laboratory in Chennai for additional processing.

Identification of Bacterial Isolates

ATCC cultures were inoculated into Blood agar and MacConkey agar for subculture. Blood agar was used for gram-positive bacterial strains (Staphylococcus aureus) and MacConkey agar was used for gram-negative bacterial strains (Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeruginosa). After inoculation, those were incubated at 37^{0}c for 24 hours. Gram's stain, motility testing, Coagulase test, oxidase, catalase, and conventional biochemical

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 24, Issue 1, 2020, Pages. 1145 -1151
Received 15 April 2020; Accepted 23June2020

tests were used to identify the isolates up to the genus and species level using standard microbiological procedures after incubation.

Antibiotic susceptibility testing

The 1% drug solution was made by dissolving 0.1 gm of the drug in 1 mL of distilled water. The antibacterial susceptibility of these medications was tested using a disc diffusion test and the results were interpreted using the zone of inhibition. The disc diffusion assay was carried out following with the Clinical and Laboratory Standards Institute's guidelines (CLSI 2020, 30th edition). The clinically important standard reference bacterial strains (ATCC) were inoculated in peptone water and incubated at 37°C for 4 hours. 0.5 McFarland standards ($1.5x10^8$cfu/ml) were used to adjust the inoculum. The microorganisms were spread onto the Mueller Hinton Agar plates using sterile Swabs. With the use of a sterile borer, wells were punched in the agar plates. Wells were inoculated with 50µl of the prepared 1% drug. As a control, 5 mcg disc levofloxacin was applied. These inoculated culture plates were incubated at 37°C for 1 day. The inhibition zones around the disc were investigated at the end of the incubation period **(Shobha et al., 2014).**

Analysis of zone of inhibition

The zone was measured using a clear ruler. The inhibitory zone's size was measured in mm. The lack of zone inhibition was interpreted as the absence of activity. If the zone of inhibition was less than 7 mm, the activity was classified as resistant; if it was between 8 - 10 mm, it was classified as intermediate; and if it was greater than 11 mm, it was classified as sensitive **(Suvetha et al., 2018).** This study was carried out at a six-month interval.

3. RESULTS

Blood agar and MacConkey agar were used to subculture the ATCC strains. Gram-positive bacteria like Staphylococcus aureus were cultured on blood agar, while gram-negative bacteria like Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa were cultured on MacConkey agar. The bacterial strains were isolated from Blood agar and MacConkey agar. Gram's stain, Coagulase test, oxidase, catalase, and conventional biochemical assays were used to identify the isolates by using standard microbiological procedures.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 24, Issue 1, 2020, Pages. 1145 -1151
Received 15 April 2020; Accepted 23June2020

Fig 1: Isolation of standard reference bacterial strains (ATCC - American Type Culture Collection)

The antibacterial activity of Vengara parpam and Padikara parpam was proven to be effective against all of the standard reference bacterial strains. The antimicrobial properties of Kungiliya parpam and Silasaththu parpam were not shown antimicrobial effects against any of the bacterial strains. As shown in Fig. 1, the zone of inhibition was determined.

ZONE OF INHIBITION	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Kungiliya parpam	**R**	**R**	**R**	**R**
Vengara parpam	**11 mm**	**11 mm**	**17 mm**	**14 mm**
Padikara parpam	**11 mm**	**14 mm**	**14 mm**	**11 mm**
Silasaththu parpam	**R**	**R**	**R**	**R**
Levofloxacin 5 mcg **(control)**	**30 mm**	**25 mm**	**30 mm**	**27 mm**

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 24, Issue 1, 2020, Pages. 1145 -1151
Received 15 April 2020; Accepted 23June2020

Fig 2: The zone of inhibition

A - S.Aureus
B - K.Pneumoniae
C - P.Aeruginosa
D - E.Coli

1 – Kungiliya parpam
2 – Vengara parpam
3 – Padikara parpam
4 – Silasaththu parpam

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 24, Issue 1, 2020, Pages. 1145 -1151
Received 15 April 2020; Accepted 23June2020

4. CONCLUSION

Traditional uses of natural chemicals have attracted great attention in recent years since they have been thoroughly studied for efficacy and are usually thought to be safe for human use. They clearly deserve to be examined along modern scientific lines such as phytochemical research, biological evaluation on experimental animal models, toxicity studies, the molecular mechanism of action research, and clinical trials of isolated phytochemicals. It is the most traditional method for discovering new lead compounds for the treatment of many diseases. A thorough review of the literature on herbo-mineral medications revealed that it is a common therapy for a number of disorders among diverse ethnic groups, Vaidyas, Hakims, and ayurvedic practitioners **(Shobha et al., 2014).**

Our findings indicate that Siddha herbo mineral formulations have significant antibacterial activity against therapeutically relevant standard reference bacterial strains (ATCC-American Type Culture Collection). Modifying the route, the same medicine can be prescribed successfully for a variety of conditions. As a result, these herbo mineral formulations can be used to control or prevent bacterial illness. More research is needed to discover the chemicals that are responsible for the antibacterial activity found **(Savarimuthu Michael et al., 2011).**

The goal of antimicrobial activity was to examine the past, present, and future of medical plants in order to recommend that research on plant extract mechanisms of action, interactions with antibiotics, and interactions with other medicinal plants be focused. Synergism research is restricted, with only a few studies using the Kirby and Bauer approach. Further, Siddha have a wide range of biological activity, including antiviral activity **(Purushotham, 2010).**

The current research examines the antibacterial activity of traditional Indian medicine in the treatment of a variety of diseases. These active Siddha formulations could be submitted to further biological and pharmacological studies in order to isolate medicinal components. Antibacterial activity tests on various Siddha formulations have confirmed their medicinal use **(Pavithra et al., 2010).**

REFERENCES

1.V. Baby Shalini and J. Sriman Narayanan, "Antibacterial activity of Andrographis paniculata Nees against selective human pathogens" African Journal of Microbiology Research 9.16 (2015): 1122-1127.

2.Pavithra P. S., Janani V. S., Charumathi K. H., Indumathy R., Sirisha Potala and Rama S. Verma "Antibacterial activity of plants used in Indian herbal medicine" International Journal of Green Pharmacy (IJGP) 4.1 (2010).

3.Shobha K.L, Akshatha S.J, Revathi Shenoy, Ramachandra L & Bernitis L., "Antibacterial Activity of Selected Siddha herbo-mineral Preparations Against Clinically Isolated Enterococcus Species" International Journal of Pharmacology and Toxicology 2.2 (2014): 38-40.
4.Kannan P. , Ramadevi S.R. and Waheeta Hopper , "Antibacterial activity of Terminalia chebula fruit extract" African Journal of Microbiology Research 3.4 (2009): 180-184.
5.J. Savarimuthu Michael, A. J. A. Ranjit Singh and C. Padmalatha "Antibacterial potential of some herbomineral Siddha preparation An alternative medicine for enteric pathogens" J. Chem. Pharm. Res 3.3 (2011): 572-578.
6.R.Caroline Jeba 1 and G. Rameshkumar 2 “Antimicrobial activity of chosen medicinal herbs and Sidda Drugs" International Journal of Bio-Technology and Research (IJBTR) ISSN 2249-6858 Vol. 3, Issue 3, Aug 2013, 5-18
7.Suvetha C1*, Seetha Lakshmi G2 , Vanitha, A3, “Antimicrobial screening of a Siddha formulation Brahmanantha Bairavam Maathirai” Int. J. Adv. Res. Biol. Sci. (2018). 5(1): 22-24
8.Muzaffer Alam, S. Joy, T. Susan, and A. Saraswathy, "On the antibacterial activity of some Siddha medicines." Ancient science of life 17.3 (1998): 194.
9.Purushotham, K. G. ; Arun, P. ; Jayarani, J. J. ; Vasnthakumari, R. ; Sankar, L. and Reddy, B. R., "Synergistic in vitro antibacterial activity of Tectona grandis leaves with tetracycline" International journal of pharm tech research 2.1 (2010): 519-523.
10.Rajalakshmi, K. "Screening of common Siddha formulations for antimicrobial activity against respiratory pathogens" Screening 9.2 (2016).