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Original Article

Revealing Anti-Microbial Potential of Traditional Siddha Formulation *Pancha Karpa Chooranam* against Infectious Pathogens

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ABSTRACT

Background: Infectious diseases are considered to be the most significant threat to human health due to the fact that they are responsible for one-half of all deaths in tropical countries. The majority of the currently available antibiotics have significant drawbacks in terms of antimicrobial spectrum and side effects. Over usage of such antibiotics has contributed to the development of clinical resistance in formerly sensitive microorganisms. Siddha system of medicine offers interesting possibilities to combat drug–resistant pathogens. Medicinal herbs constitute an essential component of Siddha preparations are known for its diverse range of biological activity. One such potential formulation that exists in the Siddha system is *Pancha Karpa Chooranam* (PKC) which comprises a unique blend of five (pancha) potential herbal ingredients. **Materials and Methods:** The main objective of the present investigation is to evaluate the anti-microbial efficacy of the formulation PKC using the disc diffusion method. **Results:** Results of the study clearly emphasise that the drug PKC exhibits significant antimicrobial activity against all the tested organisms. Potency was measured in terms of zone of inhibition against *Escherichia coli* (15–23 mm), *Staphylococcus aureus* (13–19 mm), *B. subtilis* (12–19 mm), *Salmonella typhi* (16–23 mm), and *Candida albicans* (17–21 mm). It was concluded from the results of the present investigation that the Siddha formulation PKC reveals broad spectral anti-bacterial activity against both gramme-positive (*Staphylococcus aureus, B. subtilis*) and gram-negative (*Escherichia coli, Salmonella typhi*) pathogens. **Conclusion:** Further, it was evident that the formulation advocates potential anti-fungal activity against *Candida albicans* represented by the maximal zone of inhibition which attributes to the existence of structurally diverse phytochemicals present in the preparation. Hence traditional formulations like PKC shall be recommended for clinical management of drug–resistant pathogens in near future.

Keywords: Infectious diseases, Siddha formulation, *Pancha Karpa Chooranam*, Medicinal herbs, Phytochemicals, Anti-bacterial, Anti-fungal.

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INTRODUCTION

Infectious diseases are still considered a serious health problem accounting for 41 percent of the worldwide healthcare burden.[1] One of the primary factors contributing to this issue is the widespread development of bacterial resistance to existing antibiotics.[2] As a result, the world is currently dealing with a severe danger to global public health, which manifests itself not only in the form of epidemics but also in pandemics of antibiotic resistance.[3]

Emerging antibiotic resistance is now refocusing the research interest on herbal bioactive components. This is because these phytotherapeutics has the potential to generate a viable source of antibacterial and antifungal moieties.[4] The antibacterial activity of herbs has a direct correlation with their capacity to synthesize their structurally diverse secondary metabolites.[5]

In the present scenario clinicians are left with a limited number of antibiotics to manage a wide range of microbial infestations, despite of this limitations some of the potential side effects caused by existing therapeutics further narrow down the scope of utilising the same.[6] This serves as an alarming signal to the researchers in the pharmaceutical industry to design a new spectrum of antibiotics to combat multi drug resistant (MDR) pathogens.[7] Natural products have the potential to serve as a source of unique

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biologically active chemicals, which might pave the way for the development of brand new medicines.[8]

Antibiotics of herbal origin are considered to be "Ideal antibiotics". The term ideal not only to emphasise its potential to kill infectious pathogens but also for the novel mechanism by which these agents mediate antimicrobial activity.[9] Therefore, bacteria, fungi, and viruses are unable to become resistant to majority of botanicals due to the lack of a counteracting strategy.[10] From the perspective of drug discovery, the phytochemicals found in herbs have a tendency to reach the drug target at precise locations more effectively than synthetic substances will.[11]

Bioactive components derived from plants may consider one of the richest and most fruitful sources for the discovery of major novel drugs. In recent years, there has been a rise in interest regarding herbal medicines as a subject that is relevant to both economic, and scientific interests.[12] Siddha is an age-old traditional practise that aims to restore both the physical and mental health of individuals. Even though it has a long history of use as a natural remedy, recent advances in technology have allowed researchers to investigate the true mechanism by which the formulation works.[13] Herbal therapies contain treatments that are physiologically active and are known as secondary metabolites. These treatments have the potential to stop the progression of a number of different diseases.[14] Each Siddha formulation, in terms of its innovative potential, is essentially a mixture of many different medicinal components.[15] One such potential formulation that exists in Siddha system of medicine is *Pancha Karpa Chooranam* (PKC) which comprises a unique blend of five (pancha) potential herbal ingredients.

As per the documented research, it was evident that the herbal ingredients such as *Curcuma aromatica, Piper nigrum, Azadirachta indica, Terminalia chebula*, and *Phyllanthus emblica* present in the Siddha formulation PKC possess diverse pharmacological activities. It is well known that *curcuma aromatica* possesses anti-radical, anti-cancer, anti-infective, and anti-oxidant properties.[16] The herb *Azadirachta indica* has been utilised as a treatment for a wide variety of conditions such as febrifuge, anti-cancer, anti-microbial, anti-inflammatory etc.[17] *Terminalia chebula* becomes an integral part of traditional therapy for the management of liver disorders, cancer, dental caries, diabetes, rheumatoid arthritis, gastric, and respiratory diseases.[18] *Phyllanthus emblica* is another potential herb that has been shown to have a variety of medicinal and pharmacological properties. These properties include anti-infective, hypoglycaemic, anti-ulcer, anti-hyperlipidemic, anti-ulcer, and anti-inflammatory properties.[19] *Piper nigrum* is a well–known bio–enhancer according to Indian traditional practise and it is reported to have anti-inflammatory, anti-diabetic, anti-microbial, and anti-cancer activities.[20] Considering the anti-infective therapeutic potential of all five herbal ingredients the present work aimed at evaluating the anti-microbial activity of the formulation PKC using the disc diffusion method.

MATERIALS AND METHODS

Anti-microbial profiling of the siddha formulation PKC was evaluated by disc diffusion method. The test sample was used at the concentration of 500, 1000, 2000, and 4000 µg/ml. The microorganisms of interest (*Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 29213), *B. subtilis* (ATCC 6633), *Salmonella typhi* (ATCC6539), and *Candida albicans* (ATCC 10231)) were grown using Mueller–Hinton culture broth in the laboratory condition (MHB). After a period of twenty-four hours, the suspensions were brought to the level of standard sub culture dilution. Petri dishes that contained Muller Hinton Agar (MHA) media were used to cultivate a diluted version of the bacterial strain.[21] The development of fungal strains was facilitated by the addition of sabouraud dextrose (SDA).[22] A disc with a Whatman No. 1 diameter of 6 millimetres was pre-sterilised and kept in an aseptic condition throughout the process.[23] The sterile disc sheets were injected with each concentration individually. After that, the discs that had been made were put on top of the culture media. Standard antibiotic streptomycin (10 µg) and ketoconazole (20 µg) procured from sigma–aldrich were used to assess the sensitivity of each microbial species that were examined, and 20 µl of double distilled (DD) water was utilised as the vehicle control. Then the inoculated plates were incubated at 37 degrees Celsius for 24 hr for the bacteria and 72 hr for the fungus. The anti-microbial property of the test drug PKC was measured by calculating the diameter of the clean zone that surrounded the disc and the result was stated in millimetres.

RESULTS

Anti-microbial Effect PKC against Infectious Pathogens

In the present investigation five microbial cultures namely *Escherichia coli, Staphylococcus aureus, B. subtilis, Salmonella typhi*, and *Candida albicans* were used to screen the anti-microbial potential of the siddha formulation PKC by measuring the extent of zone of inhibition rendered against each pathogen. It was observed from the study that the test drug PKC exhibit significant antimicrobial activity against all the tested organisms. *Escherichia coli* (15–23 mm), *Staphylococcus aureus* (13–19 mm), *B. subtilis* (12–19 mm), *Salmonella typhi* (16–23 mm), and *Candida albicans* (17–21 mm). It is surprising that according to our documented results the efficacy of the test formulation PKC was considerably higher when compared to that of the respective standards (Streptomycin and Ketacanazole). The results were tabulated in Table 1 and represented in Figure 1.

Documented results suggested that the test formulation PKC reveals significantly higher spectrum of anti-microbial activity

Table 1: Diameter of the inhibition zone (DIZ in mm) exhibited by siddha formulation PKC against pathogenic microorganisms.

Test Drug PKC	*Escherichia coli*	*Staphylococcus aureus*	*B. subtilis*	*Salmonella typhi*	*Candida albicans*
500 µg/ml	15	13	12	16	17
1000 µg/ml	17	13	14	20	18
2000 µg/ml	21	14	17	21	20
4000 µg/ml	23	19	19	23	21
Streptomycin (10 µg) -Bact Ketacanazole (20 µg) – Fung	20	14	16	14	12

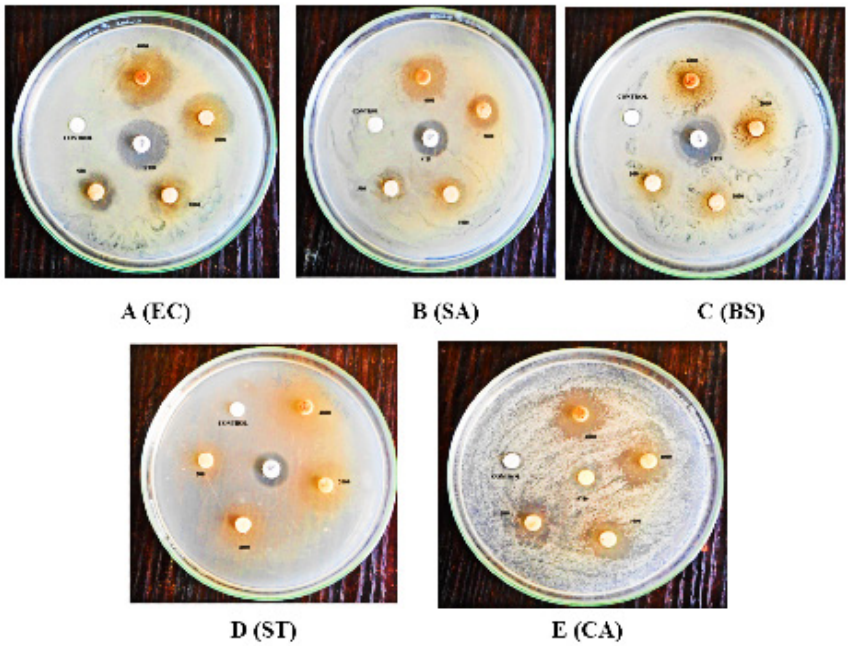

Figure 1: Illustration of culture plates representing anti-microbial efficacy of the test drug PKC.

when compared to that of the respective standards (Streptomycin and Ketacanazole).

Sample PKC reveals broad spectral anti-microbial activity against both gram positive (*Staphylococcus aureus, B. subtilis*) and gram negative (*Escherichia coli, Salmonella typhi*) pathogens along with fungal strain *candida albicans* represented by maximal zone of inhibition against specific pathogen.

DISCUSSION

The worldwide epidemic of infectious diseases caused by bacterial and fungal pathogens poses a significant risk to the general population's health.[24] When it comes to treating bacterial infections, antibiotic therapy is the method of choice; nevertheless, the development of resistance to antimicrobial agents, and concerns regarding toxicity have reduced the utilisation of antibacterial agents.[25,26] Because of their equivalent toxicity and efficacy, biological research on the antibacterial role of medicinal herbs is made more relevant as a result of the safety, and efficacy–related constraints of antibiotics.[27]

Despite the advancement of modern medicine, the World Health Organisation reports that 80 percent of the people in underdeveloped nations rely on the usage of plant–based medicines for their healthcare needs.[28] It has been estimated that the yearly value of the global trade in medicinal plants is greater than one hundred billion US dollars, with an annual growth rate of between ten, and fifteen percent.[29,30]

Isolating and identifying physiologically active chemicals and molecules from herbs have led to the creation of novel therapies.[31] Phytochemicals are a source of new compounds for pharmaceutical R&D.[32] In oncology, plants directly, or indirectly supplied 60% of anti-cancer medications.[33] Medicinal plants are used to treat ailments across the world. Ancient literature mentions the widespread use of medicinal plants for herbal medicine and healing. Plants have a huge range of pharmacologically active chemicals, making them a rich source of drugs.

Even today, *Escherichia coli* is one of the most prevalent pathogens responsible for a variety of bacterial illnesses that can affect both humans, and animals. Enteritis, urinary tract infections, septicemia, infant meningitis, and other clinical illnesses are frequently caused by *E. coli*. Additionally, *E. coli* is resistant to a number of different types of antibiotics, each of which has a unique mode of action.[34,35] *Staphylococcus aureus* is a commensal human pathogen and the colonisation with *S. aureus* occurs in around thirty percent of the world's population of humans.[36] It is also a prominent cause of bacteremia and infective endocarditis in addition to being a leading cause of osteoarticular, skin, soft tissue, pleuropulmonary, and implant–related infections.[37] It was observed from the study that the test drug PKC exhibit significant antimicrobial activity against *Escherichia coli* (15–23 mm) and *Staphylococcus aureus* (13–19 mm) with the higher spectrum of efficacy compared to Streptomycin (14–20 mm).

Salmonella spp. is the most common cause of hospitalisation in the United States and the second most prevalent cause of recorded zoonoses in European countries.[38] This infectious agent has more than 2500 different serotypes, making it a significant threat to health security globally.[39] *S. typhi* has rapidly developed resistance to medicines that were previously effective, such as ciprofloxacin.[40] As a result, there is a requirement for the development of novel anti-typhoid agents. Data of our study show that PKC justifies maximal zone of inhibition (16–23 mm) against *Salmonella typhi* when compared to standard drug streptomycin (10 µg) with a maximal zone of inhibition of 14 mm.

Bacillus species are rod–shaped aerobic bacteria as a result it has a tendency to produce spores that remains unaffected by heat, cold, and most disinfectants.[41] *Bacillus subtilis* can cause septicemia in immunocompromised persons.[42] Studies further confirm that *Bacillus subtilis* has developed a resistance to antibiotics and creates heat–stable toxins called amylosins.[43] Data of the current study advocate that the formulation PKC reveals a zone of inhibition that ranges from 12–19 mm against *Bacillus subtilis* in comparison with standard drug streptomycin (10 µg) with a maximal zone of inhibition of 16 mm.

Candidiasis is an opportunistic fungal infection of the oral cavity that is caused by yeast species such as *Candida albicans*. It is one of the most common and significant illnesses of its kind. For the purpose of treating candidiasis, antifungal medications are applied topically (such as nystatin and clotrimazole) and taken orally (such as azoles, and amphotericin B) in a variety of formulations.[44] However, during the past several years, a large number of studies have revealed that the aforementioned anti-fungal agents were found to be ineffective for individuals due to drug resistance, which can restrict the therapeutic applications of these drugs.[45] The outcome of our present investigation clearly signifies that the formulation PKC demonstrates promising anti-fungal activity against *Candida albicans* with a zone of inhibition ranging from 17-12 mm in comparison with standard drug ketacanazole with a maximal zone of inhibition of 12 mm.

CONCLUSION

Microbes often cause prevalent illnesses that pose a substantial public health burden in developing countries. Antimicrobials are an effective way to prevent or treat infections. Rapid and widespread drug resistance among harmful microbes requires the continual development of new antibiotics with novel modes of action. The Siddha system of medicine is an age-old medical practise that has persisted and even flourished over the course of several millennia. Medicinal herbs are still the major source of traditional medicines in several regions of the world. These formulations have been used for the management of several dreadful infectious diseases. It was concluded from the results of the present investigation that the Siddha formulation PKC reveals broad spectral anti-bacterial activity against both gram-positive (*Staphylococcus aureus, B. subtilis*) and gram-negative (*Escherichia coli, Salmonella typhi*) pathogens along with anti-fungal activity against *candida albicans* represented by a maximal zone of inhibition against specific pathogens.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PKC: *Pancha Karpa Chooranam*; ***B. subtilis:*** *Bacilus subtilis*; ***E. coli:*** *Escherichia coli*; **MHA:** Muller Hinton Agar; **MHB:** Mueller–Hinton culture broth; **SDA:** Sabouraud dextrose; **DD Water:** Double Distilled Water.

SUMMARY

It was observed from the study that the test drug PKC exhibit significant antimicrobial activity against *Escherichia coli* (15–23 mm) and *Staphylococcus aureus* (13–19 mm) with the higher spectrum of efficacy compared to Streptomycin (14–20 mm). PKC justifies maximal zone of inhibition (16–23 mm) against *Salmonella typhi* when compared to standard drug streptomycin (10 µg) with a maximal zone of inhibition of 14 mm. PKC reveals a zone of inhibition that ranges from 12–19 mm against *Bacillus subtilis* in comparison with standard drug streptomycin (10 µg)

with a maximal zone of inhibition of 16 mm. PKC demonstrates promising anti-fungal activity against *Candida albicans* with a zone of inhibition ranging from 17–12 mm in comparison with standard drug ketacanazole with a maximal zone of inhibition of 12 mm. It was concluded from the results of the present investigation that the Siddha formulation PKC reveals broad spectral anti-bacterial activity against both gram-positive (*Staphylococcus aureus*, *B. subtilis*) and gram-negative (*Escherichia coli*, *Salmonella typhi*) pathogens along with anti-fungal activity against *candida albicans* represented by a maximal zone of inhibition against specific pathogens.

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