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EVALUATION OF IN-VITRO ANTI-MICROBIAL POTENTIAL OF THE NOVEL SIDDHA FORMULATION POOVARASU NEI AGAINST STAPHYLOCOCCUS AUREUS, STREPTOCOCCUS PYOGENES, CANDIDA ALBICANS AND ASPERGILLUS NIGER

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Abstract

Siddha medicines benefit the society in managing dreadful diseases since several centuries. People rely of Indian system of traditional medicines as it has wide range of therapeutic activity in management of several infectious diseases. Most prevailing problem in treating infectious diseases is resistance. Recently scientist had found that evolutionary pathway on microbes made them to adopt and become resistant to currently available marketed antibiotics. Till date in allopathic system of medicine there are very limited number of antibiotics are available for clinical management of several infectious disease. Still limitation prevails in using such antibiotics because of resistance, side effects and allergy etc. Hence the exploration of alternative therapy for the infectious disease grabs the attention of the siddha researchers and medicinal microbiologist as well. The main objective of the present study is to screen the antimicrobial potency of the siddha formulation *PoovarasuNei* (PN). The result of antimicrobial screening study clearly projects that the drug PN did not shown anti-microbial activity at the concentration of 100 to 300 µg against *Staphylococcus aureus*, *Candida albicans* and *Aspergillus Niger*. Whereas the formulation PN shown significant activity against *Streptococcus pyogenes* with the inhibition zone ranges from 2mm to 10mm at the concentration of 100 to 300 µg. From the study it was concluded that the drug PN was effective against *Streptococcus pyogenes* and has no anti-microbial property against *Staphylococcus aureus*, *Candida albicans* and *Aspergillus Niger* and hence further study has to be carried out in higher concentration and also against other gram negative , positive and fungal species other than the tested ones

Introduction

According to the global survey it has been identified that nearly 80% of individuals from developed and developing countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to understand their properties, safety and efficacy [1].In ASU systems plants, minerals, and animal products are used as main drugs to cure various ailments [2]. Herbal medicine also called botanical medicine or phytomedicine refers to the use of plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Herbalism has a long tradition of use outside of conventional medicine. It is becoming more mainstream as

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improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in treating and preventing disease [3]. Knowledge of herbs using from generation to generation for hundreds of years as part of traditional. It is used for medicine because it is easily available and inexpensive. Phytochemicals useful as drugs in terms of broad spectrum activity for many microorganisms that leads for treating microbial diseases [4].

Traditional medicine is the main source of medical care for a great proportion of the population of the developing world. In Africa, indigenous plants play an important role in the treatment of a variety of diseases [5]. WHO (1996) has listed 21,000 plants that have medicinal uses around the world [6].Use of plant products for the control of human diseases has certain advantages besides being cheap to produce; they are biodegradable and readily available. Effective plant extracts can combat human pathogenic bacteria without toxic side effects and environmental hazards [7].

It is justifiable to search for alternative therapy in natural products, as plants have been known for many years as a source of therapeutic agents. Few researches have correlated *in vitro* activity, safety studies and mode of action besides isolating the bioactive compound(s). Different kinds of studies on the mechanisms of action should be given high priority [8].The main goal of the present research work is to investigate the possible anti-microbial potential of the siddha formulation *PoovarasuNei* (PN) a well-known classical drug against selective bacterial and fungal species using the disc diffusion method.

Materials And Methods

Source of raw drugs

The *Poovarasampattai* and *Nunaver* is collected from southern zone of Tamil Nadu, Tiruvarur and other required raw drug is procured from a well reputed indigenous drug shop from Parryscorner , Chennai, Tamil Nadu, India .All raw drugs were authenticated by the Pharmacognosist, SCRI Chennai., Tamil Nadu, India

Purification of raw drugs

Raw drugs are purified as mentioned in *Sikicharathna deepam SarakkuSuthi Muraigal* [9].

Ingredients

The siddha formulation *PoovarasuNei* comprises of the following herbs as phyto ingredients

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1.PoovarasampattaiSaaru(*Thespesiapopulnea*)	-1.3 liters
2.Sittramanakuennai(*Ricinuscommunis*)	-1.3 liters
3.Kasakasapaal(*PapaverSomniferum*)	-1.3 liters
4.Seeragam(*cuminumcyminum*)	-10gms
5.Karunjeeragam(*Nigella sativa*)	
6.Karpogarisi(*psoraleacorylifolia*)	
7.Nunaver(*Morindatinctoria*)	- 35gms
8.Elarici(*Elettariacardamomum*)	
9.Sadamanjil(*Nardostachysgrandiflora*)	
10.ManjalKarisalaiSamulam(*wideliacalendulaceae*)	
11.Kirambu(*syzygiumaromaticum*)	
12.Sarkarai(*Saccharumofficinarum*)	- 200gms

Formulation of *PoovarasuNei* (PN)

All the drugs were purified. Milk of kasakasa and the juice of poovarasampattai were taken. Both are mixed with castor oil. All other Ingredients are made into fine powder. Boil the mixture of castor oil until the kadukuthiralpadham occurs and then add the powdered drugs to the above oil till it becomes nei form and filter it.

Collection of Microorganism

To evaluate the anti-microbial property of the formulation PN, the cultures were procured from various accredited pathology laboratories in and around Chennai. The organisms used were s*taphylococcuss aureus*, *Streptococcus pyogenes*, *Candida albicans* and *Aspergillus Niger*. All the organisms were confirmed using specific biochemical tests.

Anti-microbial activity [10-13].

The antibacterial activities of the sample PN were carried out by disc diffusion method. The test compounds were used at the concentration of 100, 200, 300 µg. The target microorganisms were cultured in Mueller–Hinton broth (MHB). After 24 h the suspensions were adjusted to standard sub culture dilution. The Petri dishes containing Mueller Hinton Agar (MHA) medium were cultured with diluted bacterial strain. Disc made of Whatman No.1, diameter 6 mm was pre-sterilized and was maintained in aseptic chamber. Each concentration was injected to the sterile disc papers. Then the prepared discs were placed on the culture medium. Standard drug Ciprofloxacin (5µg) for anti-bacterial and Fluconazole (25µg) was used as a positive reference standard to determine the sensitivity of each microbial species tested. Then the inoculated plates were incubated at 37^{o} C for 24 h (Bacterial) - 72 hr (Fungal). The diameter of the clear zone around the disc was measured and expressed in millimeters as its anti-microbial property.

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Results

Effect on PN on Antimicrobial activity

The test drug PN did not exhibited significant zone of inhibition against *Staphylococcus aureus*, *Candida albicans* and *Aspergillus Niger*. Further PN has shown significant activity against *Streptococcus pyogenes* with the inhibition zone ranges from 2mm to 10mm at the concentration of 100 to 300 µg. The maximum inhibitory zone diameter (IZD) of 25mm was observed in Ciprofloxacin (5µg) against *Streptococcus pyogenes* and 22 mm against *Staphylococcus aureus*. Similarly IZD of 21 mm was observed in Fluconazole (25µg) against *Candida albicans* and 18 mm against *Aspergillus niger*. All IZD corresponding to test organisms are tabulated in Table 1 and represented in figure 1-4.

Table 1: Zone of Inhibition data of Anti-bacterial activity of the formulation PN

Sample Code	*Streptococcus pyogenes*			*Staphylococcus aureus*			*Candida albicans*			*Aspergillusniger*		
Concentraion	100 µg	200 µg	300 µg	100 µg	200 µg	300 µg	100 µg	200 µg	300 µg	100 µg	200 µg	300 µg
PN	2	2	10	-	-	-	-	-	-	-	-	-
Ciprofloxacin (10µg)	25			22			NA			NA		
Fluconazole (25µg)	NA			NA			21			18		

- = Not active and NA = Not Applicable

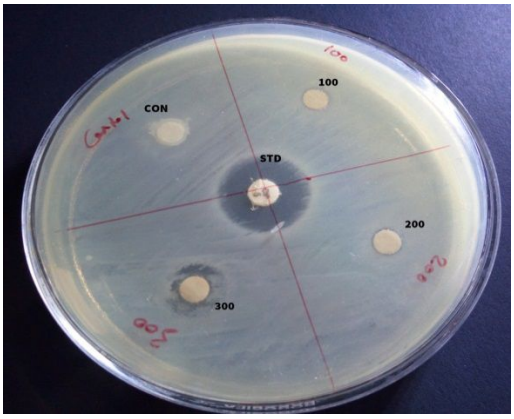

Figure 1: Anti- Microbial Effect of PN against Streptococcus pyogenes

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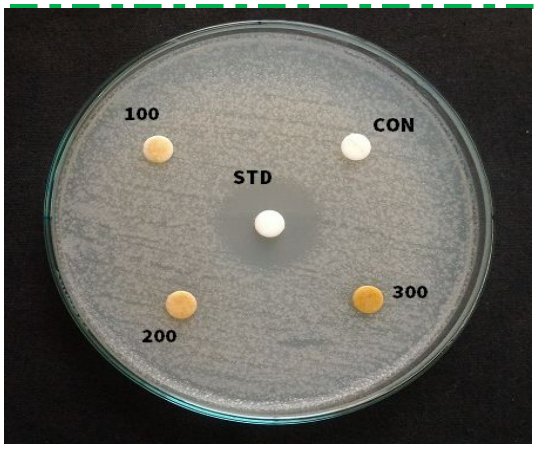

Figure 2: Anti- Microbial Effect of PN against Staphylococcus aureus

Figure 3: Anti- Microbial Effect of PN against Candida albicans

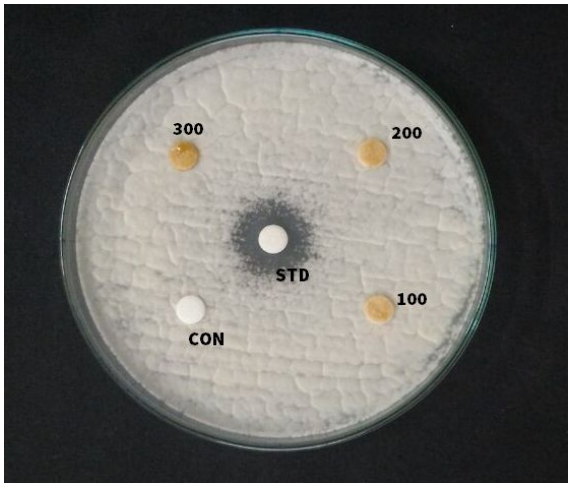

Figure 4: Anti- Microbial Effect of PN against Aspergillusniger

Discussion

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Antibiotics have saved the lives of millions of people and have contributed to the major gains in life expectancy over the last century. However, the clinical efficacy of many existing antibiotics is being threatened by the emergence of multi-drug resistant (MDR) pathogens [14] the recent appearance of strains with reduced susceptibility as well as, undesirable side effects of certain antibiotics [15,16]. Infectious diseases caused by resistant microorganisms are associated with prolonged hospitalizations, increased cost, and greater risk for morbidity and mortality. Resistance is an especially vexing problem for people with impaired immune systems, such as AIDS, cancer patients and recipients of organ transplants.

The resistance problem demands that a renewed effort be made to screen various medicinal plants for their potential antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, phenolic compounds, steroids, resins, fatty acids and gums which are capable of producing definite physiological action on body. Another driving factor that encouraged scientists to search for new antimicrobial substances from various sources including medicinal plants has been the rapid rate of plant species extinction. Medicinal plants are relied upon by 80% of the world's population and in India there is a rich tradition of using herbal medicine for the treatment of various infectious diseases, inflammations, injuries and other diseases. Many of the plant materials used in traditional medicine are generally proved more effective and relatively cheaper than modern medicine [17] against certain ailments while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [18].

Streptococci are Gram-positive, non-motile, non-sporeforming, catalase-negative cocci that occur in pairs or chains. In recent years, increasing attention has been given to other streptococcal species, partly because innovations in serogrouping methods have led to advances in understanding the pathogenetic and epidemiologic significance of these species. In humans, diseases associated with the streptococci occur chiefly in the respiratory tract, bloodstream, or as skin infections. Human disease is most commonly associated with Group A streptococci. Acute group A streptococcal disease is most often a respiratory infection (pharyngitis or tonsillitis) or a skin infection (pyoderma) [19].

Penicillin remains the drug of choice for the empirical treatment of *S. pyogenes* infections, despite over sixty years of use. *S. pyogenes* has also remained uniformly susceptible and resistance to penicillin type antibiotics. Hence it is highly essential to find an alternate drug for choice for clinical management of *S. pyogenes* infections.Siddha medicine being a polyherbal preparation's the chances of occurrence of resistance is very minimal. In the present investigation it was observed that the siddha formulation PN has shown significant anti-microbial activity against *Streptococcus pyogenes* with the inhibition zone ranges from 2mm to 10mm at the concentration of 100 to 300 µg.

S. aureus has evolved a comprehensive strategy to address the challenges posed by the human immune system.*S. aureus* has an extraordinary repertoire of virulence factors that allows it to survive extreme conditions within the human host. Such an elaborate armamentarium might prompt one to speculate that human kind would be no match for this pathogen and could be highly vulnerable to severe *S. aureus* infection [20].

Methicillin-resistant *Staphylococcus aureus*(MRSA) is responsible for the largest outbreak of hospital-acquired infection (HAI) that the world has ever seen. Siddha system of medicine has several formulations which are effective against *S. aureus*infection [21]. *C. albicans* and to a lesser extent other *Candida* species are present in the oral cavity of up to 75% of the population [22]. In healthy individuals this colonization generally remains benign. However, mildly immunocompromised individuals can frequently suffer from recalcitrant infections of the oral cavity. These oral infections with *Candida* species are termed "oral candidiasis" (OC). Such infections are predominantly caused by *C. albicans* and can affect the oropharynx and esophagus of persons with dysfunctions of the adaptive immune system. Indeed, HIV is a major risk factor for developing OC. Further risk factors for developing OC include the wearing of dentures and extremes of age [23].

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It is estimated that approximately 75% of all women suffer at least once in their lifetime from vulvovaginal candidiasis (VVC), with 40–50% experiencing at least one additional episode of infection [24, 25]. A small percentage of women (5–8%) suffer from at least four recurrent VVC per year [26].

The incidence of opportunistic fungal infections has undergone an alarming increase over the past two decades, generally attributed to the improved survival of cancer patients through the use of immunosuppressive and cytotoxic drugs. Infections due to Aspergillus species result in significant morbidity and mortality [27,28].

Aspergillosis is a spectrum of diseases cause by the *Aspergillus* spp. that are ubiquitous saprophytic fungi. The clinical spectrum of aspergillosis varies from the colonisation of the organism to the presence of fungus ball (aspergilloma) or an allergic response known as allergic bronchopulmonaryaspergillosis (ABPA) to subacute invasive aspergillosis (SAIA) [29-31].

According to the WHO report in 2011, around 1.2 million people in the world have been estimated to have chronic pulmonary aspergillosis (CPA) as a sequel to tuberculosis (TB) and most cases occur in South-East Asia, Western Pacific and African regions. Scarce data is available on CPA as a post-TB sequel and in structural lung diseases from developing countries [32]. Siddha system of medicine being a pioneer in formulations comprises of potential anti-microbial agents shall be considered as a source for identification of next generation antibiotics which is effective against wide range of microbes with less or no side effects.

Conclusion

From the results of the present investigation it was clear that the formulation PN was effective against *Streptococcus pyogenes* and further PN did not reveal significant anti-microbial activity against *Staphylococcus aureus*, *Candida albicans* and *Aspergillus Niger*. Hence further study has to be investigated at increased concentration of the same formulation at concentration higher than the 300µg to explore the possible anti-microbial activity of the study drug against such organism.

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