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ORIGINAL ARTICLE **OPEN ACCESS**

Study on the Efficacy of Chandanadi Churna as an Antimicrobial Agent Against the Selected Pathogens

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ABSTRACT
The present study evaluates the antibacterial and antifungal properties of Chandanadi churna against various bacterial and fungal strains. Bacillus subtilis, Klebsiella pneumonia, E-coli, and Streptococcus pyogenes were found to be inhibited by different concentrations of Chandanadi churna, with minimum inhibitory concentrations (MIC) of 1000 µg for Bacillus subtilis, 500 µg for Klebsiella pneumonia, 250 µg for E-coli, and 1000 µg for Streptococcus pyogenes. Additionally, Chandanadi churna exhibited significant antifungal activity against Candida albicans (MIC: 1000 µg) and Aspergillus flavus (MIC: 500 µg). The active compounds present in Chandanadi churna, such as flavonoids, alkaloids, carbohydrates, glycosides, terpenoids, saponins, berberine, starch, protein, lignins, and amino acids, were found to interfere with bacterial cell membrane integrity, protein synthesis, and DNA replication, resulting in bacterial cell death. Moreover, bioactive compounds like Azadirachtin and Neemol present in Chandanadi churna disrupted fungal cell membranes and walls, inhibited fungal cell wall synthesis, and hindered fungal growth. Notably, no antibacterial activity was observed against Pseudomonas aeruginosa, Salmonella Typhi, and Staphylococcus aureus. Based on the study findings, Chandanadi churna can be considered as a potential treatment option for urinary tract infections, pneumonia and gastroenteritis infections.
Keywords: Chandanadi churna, *Candida albicans, Bacillus subtilis, Klebsiella pneumonia, Escherichia coli, Streptococcus pyogenes.*

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INTRODUCTION

Traditional medical practices differ widely from nation to nation and area to region due to influences from culture, history, individual attitudes, and philosophy. Their theory and practice frequently diverge significantly from those of mainstream medicine. The safety and effectiveness of traditional medicine have been proven over a long period of time via the use of several techniques, including knowledge passed down from generation to generation. [1]. Due to their extensive biological- therapeutic activity, higher safety margins, and lower cost, herbal medicines are much sought after for primary healthcare in both developed and developing countries. [2]. Plant medicines are used on a worldwide scale to prevent and treat infectious diseases [3]., Microbes have been on Earth for billions of years, and can adapt to new situations than humans. They are constantly challenging human beings with ingenious new survival tactics. Bacterial enteric infections are a major public health concern worldwide, with millions of cases reported annually. The most common bacterial pathogens responsible for these infections are *Bacillus subtilis, Klebsiella pneumonia, E.coli, Pseudomonas aeruginosa, Salmonella Typhi, Staphylococcus aureus, Streptococcus pyogenes, Aspergillus flavus* and *Candida albicans. Escherichia coli* causes urinary tract infections [8], Gastro enteritis which is an inflammation of stomach and intestines that can result in symptoms such as diarrhea, abdominal pain and vomiting [9], *Klebsiella pneumonia* cause various diseases such as pneumonia [12], Urinary tract infections [13] and liver abscess which is localized collections of pus in the liver [14]. Pneumonia [15], Urinary tract infections [16], skin and soft tissue infections [17] and blood stream infections [18] are the diseases caused by the bacteria *Pseudomonas aeruginosa*. *Salmonella Typhi* is responsible for causing typhoid fever which is a systemic and potentially life- threatening disease. It shows symptoms such as high fever, abdominal pain, headache, diarrhea and rash [19]. Diseases caused by *Bacillus subtilis* are bacteremia, endophthalmitis and endocarditis[20]. *Staphylococcus aureus* causes minor skin and soft tissue infections to severe life-threatening infections such as pneumonia, sepsis and endocarditis [21]. *Streptococcus pyogenes* also known as Group A Streptococcus (GAS) can cause a wide range of diseases including pharyngitis (strep throat), skin and soft tissue infections, cellulitis, scarlet fever, necrotizing fasciitis (flesh eating disease), and streptococcal toxic shock syndrome [22]. The fungus such

as *Candida albicans* cause infections like oral thrush and vaginal candidiasis particularly in immunocompromised individuals [23]. Respiratory infections are caused by the fungus *Aspergillus flavus* [24]. The use of antibiotics to treat these infections has become a widespread practice, but the emergence of antibiotic-resistant bacteria has become a significant challenge in the treatment of bacterial enteric infections. As a result, alternative treatment strategies such as natural remedies have gained increasing attention. Ayurveda, a traditional system of medicine originating from India, offers a wide range of natural remedies that have been used for centuries to treat various ailments. The need of treating microorganisms was emphasized in order to improve the internal environment and thwart extrinsic causes of disease. Plant-derived medicines are often safer to use than synthetic ones as these drugs contain a variety of bioactive chemicals and play a key role in the maintenance of health [4] Adding this formulation as co medication may increase the antimicrobial spectrum and action potency. Recently, it has been demonstrated that many human pathogenic bacteria have developed resistance against several synthetic drugs. So, it is time to find alternatives with Ayurveda formulations or with single drugs. Chandanadi churna is an ayurvedic herbal powder formulation that is traditionally used in ayurveda for its various health benefits. It contains a combination of herbs, with sandalwood being a key ingredient. It has ayurvedic formulation of potent ingredients like Lal Chandan, Bilva, Khas, Nagarmotha and several others. It works as a diuretic and helps with burning micturition. It also helps with renal insufficiency, jaundice and ascites. It is believed to possess cooling, anti-inflammatory, digestive, and detoxifying properties. Some of its traditional uses include Respiratory Disorders, Digestive Issues, Urinary Disorders, Skin Conditions, Fever and inflammation. Eg. Useful in genitourinary infection, thirst extreme heat and also works as a blood purifier. It is helpful in conditions of pain in the stomach accompanied by belching and acidity. Hence an attempt was made to screen the antimicrobial potential of Chandanadi Churna, and to estimate its Minimum Inhibitory Concentration (MIC) against *Bacillus subtilis, Klebsiella pneumonia, E.coli, Pseudomonas aeruginosa, Salmonella Typhi, Staphylococcus aureus, Streptococcus pyogenes, Aspergillus flavus* and *Candida albicans*.

MATERIALS AND METHODS

CHANDANADI CHURNA INGREDIENTS

Chandanadi Churna was purchased from Tansukh Herbals Pvt Ltd, Lucknow

Chandana - *Santalum album*, Shalmali- *Salmalia malabarica* ,Twak- *Cinnamon* , Ela- *Cardamom*, Patra-*Cinnamomum tamala*, Haridra-Turmeric , Daruharidra- *Berberis aristata* , Ananta,Sariva- *Hemidesmus indicus*, Musta-*Cyperus rotundus* , Ushira- *Vetiveria zizanioides*, Yashtimadhu- Licorice , Amla-*Emblica officinalis* , Talamuli-*Chlorophytum tuberosum*, Shubha- *Bambusa bambos*, Bharngi- *Clerodendron serratum* , Devadaru-*Cedrus deodara* , Haritaki- *Terminalia chebula*, Loha bhasma- Processed iron -360 grams .

ANTIMICROBIAL ACTIVITY

PROCEDURE

Test was carried out in a 96 well Plate under aseptic conditions. A sterile 96 well plate was labelled. Volume of sample in DMSO comprises of 1000 µg was pipetted into the first well of the plate and transferred to subsequent wells by half of its weight until 8th Well. To all other wells 50 µl of nutrient broth was added and serially diluted it. To each well 10 µl of resazurin indicator solution was added. 10 µl of bacterial/ fungal suspension was added to each well. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. The plate was incubated at 37 °C for 24-48 h. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value.

Standard drug Chloramphenicol (10µg) was used as a positive reference standard to determine the sensitivity of the bacterial species tested.

- Standard drug Fluconazole (20µg) was used as a positive reference standard todetermine the sensitivity of the fungal species tested.

RESULTS AND DISCUSSION

ANTIBACTERIAL ACTIVITY

Chandanadi Churna were tested against bacterial culture to determine and investigate their antibacterial activity. From the results it is evident that Chandanadi Churna has significant antibacterial activity against the tested strains. Chandanadi Churna reveals significant activity against the pathogen, *E-coli*-EC (MIC 250 µg), *Klebsiella pneumonia*-KP (MIC 500 µg), *Bacillus subtilis*-BS (MIC 1000 µg) and *Streptococcus pyogenes*-SP (MIC 1000 µg). The results are recorded in Table 2.

ANTIFUNGAL ACTIVITY

The pathogenic fungi used in antifungal test were *Candida albicans* and *Aspergillus flavus*. The antifungal test results of the extract of Chandanadi churna with multiple plant species demonstrate consistent anti-fungal activity against *Candida albicans* (MIC 1000 µg) and *Aspergillus flavus* (MIC 500 µg). The results were

tabulated in Table 3. It was observed from the results of the present investigation that the Chandanadi Churna reveals convincing anti-microbial activity among all tested organisms. It was observed that the Chandanadi Churna reveals significant activity against the pathogen E-coli-EC (MIC 250 µg) and Klebsiella pneumonia-KP (MIC 500 µg) with the lowest MIC values when compared to that of *Bacillus subtilis*-BS (MIC 1000 µg) and *Streptococcus pyogenes*-SP with MIC value of 1000 µg. Similarly, the Chandanadi Churna demonstrate consistent anti-fungal activity against *Candida albicans* (MIC 1000 µg) and *Aspergillus flavus* (MIC 500 µg). The Chandanadi Churna reveals no anti-microbial activity against *Pseudomonas aeruginosa*, *Salmonella Typhi* and *Staphylococcus aureus*. Chandanadi Churna contains a variety of herbs with Chandana(sandalwood) being the main ingredient. Chandana (Santalum album) had shown zone of inhibition for *Escherichia coli*, *Proteus vulgaris*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Streptococcus pyogens*, and zone of inhibition for Pneumococcal pneumonia [5]. Similarly [6], study have also revealed that Santala album has antibacterial activity in leaf, bark and seed methanol extract against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli.* The most commonly known phytochemical compounds of Chandanadi churna are alkaloids, carbohydrates, tannins, phenols, steroids and flavonoids [7]. Bioactive compounds, ammino acids, proteins, saponins, glycosides, berberine, berbamine, aromoline, starch, terpenoids, lignins and inulins also have been known for antimicrobial properties. Recent increases in morbidity and mortality, particularly in developing nations with low health status indices, are a result of the growth in life-threatening diseases brought on by pathogenic microbes. It is reported that bacteria such as *E.coli* also causes Hemolytic Uremic syndrome (HUS) is a severe condition characterized by kidney failure, anemia, and low platelet count [10] and Neonatal meningitis in new born which is an infection of membranes surrounding brain and spinal cord [11]. Chandanadi Churna reveals significant activity against the pathogen *E.coli*-EC with the minimum inhibitory concentration 250 µg. The inhibition of Escherichia coli (*E.coli*) by Chandanadi churna may be attributed to the antimicrobial properties of its constituent herbs. Chandanadi churna typically contains herbs such as Chandana (sandalwood), Musta (*Cyperus rotundus*), Usheera (*Vetiveria zizanioides*), among others, which possess various medicinal properties including antimicrobial effects. The specific mechanism of action through which Chandanadi churna inhibits *E. coli* may vary and may involve multiple factors, such as disruption of bacterial cell membranes, interference with metabolic processes, or inhibition of bacterial enzyme activity. Chandanadi Churna reveals significant activity against the pathogen *Klebsiella pneumonia* -KP with the minimum inhibitory concentration 500µg. It is noted that the Chandanadi churna with multiple plant species extract exhibit antibacterial effect against both Gram-positive and Gram-negative bacteria. The Chandanadi churna with multiple plant species extract, however, showed greater anti- bacterial activity against Gram-positive bacteria than Gram-negative bacterial strains. Plant extracts have good activity against Gram positive bacteria, because these bacteria contain only peptidoglycan layer, which is easily penetrated by the antimicrobial chemicals in the plant extracts. Gram- negative bacteria contain a single layer of peptidoglycan surrounded by an outer membrane and it is possible that plant extracts are futile because they are unable to penetrate the cell wall of Gram-negative bacteria. In this study, Chandanadi churna have various effects and different minimum inhibitory concentrations on Gram-negative bacteria such as *Klebsiella pneumonia, E-coli, Pseudomonas aeruginosa, Salmonella Typhi* and Gram-positive bacteria such as *Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogenes*. The minimum inhibitory concentration (MIC) is an important laboratory diagnostic tool used to confirm the resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents [25]. MIC is generally regarded as a basic laboratory measurement of the activity of an antimicrobial agent. The Minimum Inhibitory Concentration (MIC) of Chandanadi churna with multiple plant species was found to be effective against *Bacillus subtilis (MIC 1000* µg*) and Streptococcus pyogenes (MIC 1000* µg*).* Similarly, the extract of Chandanadi churna with multiple plant species demonstrate consistent anti-fungal activity against *Candida albicans (MIC 1000* µg*) and Aspergillus flavus (MIC 500* µg*)*. The herbs in Chandanadi churna which may help in arresting bacterial multiplication Inhibiting bacterial cell division: Certain active compounds in these herbs may interfere with the ability of bacteria to divide, ultimately leading to the arrest of bacterial multiplication. Inhibiting bacterial metabolism: Some of the bioactive compounds in these herbs may interfere with bacterial metabolic processes, which can disrupt cellular processes and cause the arrest of bacterial multiplication. Disrupting the bacterial cell membrane: As mentioned earlier, some of the bioactive compounds in these herbs may interfere with the integrity of the bacterial cell membrane, causing leakage of essential nutrients and ions, ultimately leading to the death of the bacterial cell and arresting bacterial multiplication. It's important to note that the antimicrobial activity of these herbs has mostly been studied in vitro, which means that it has been tested in a laboratory setting, and its effectiveness in a living organism is not entirely clear. Additionally, the specific mechanisms by which these herbs inhibit bacterial growth and multiplication are still being studied and may vary depending on the type of bacteria. Overall, while Chandanadi churna may not have a direct antibacterial effect, some of its

ingredients may have indirect antimicrobial effects, which may contribute to arresting bacterial multiplication and its overall effectiveness in treating respiratory disorders. [26].

Table 1: Inference of the Resazurin Test

Color Pale Pink	**(+) Positive Value indicates the pale pink color in the well – Means there is no anti-microbial activity for the sample in that particular well**
Color Dark purple	**(-) Negative Value indicates the pale Dark purple Colour in the well – Means there is good anti-microbial activity for the sample in that particular well**

Table 2: MIC (Minimum Inhibitory Concentration) value of - Chandanadi Churna against the selected bacterial strains

Bacillus subtilis-BS	*1000µg*
Klebsiella pneumonia-KP	*500 µg*
E-coli-EC	*250 µg*
Streptococcus pyogenes-SP	*1000µg*

Table 3: MIC (Minimum Inhibitory Concentration) value of - Chandanadi Churna against the fungal strains

Aspergillus flalavus -AF	***500 µg***
Candida albicans -CA	***1000 µg***

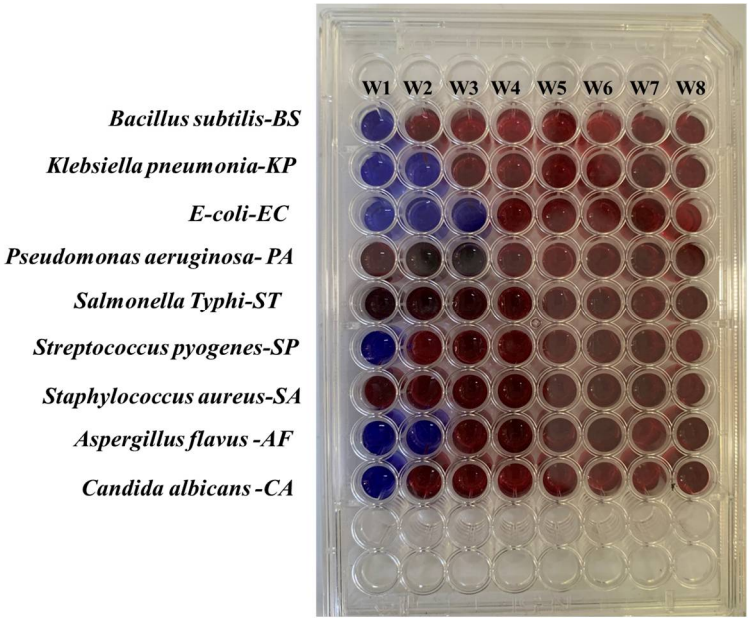

Figure 1: 96 Well Plate - Anti-Microbial Spectrum Image of Chandanadi Churna

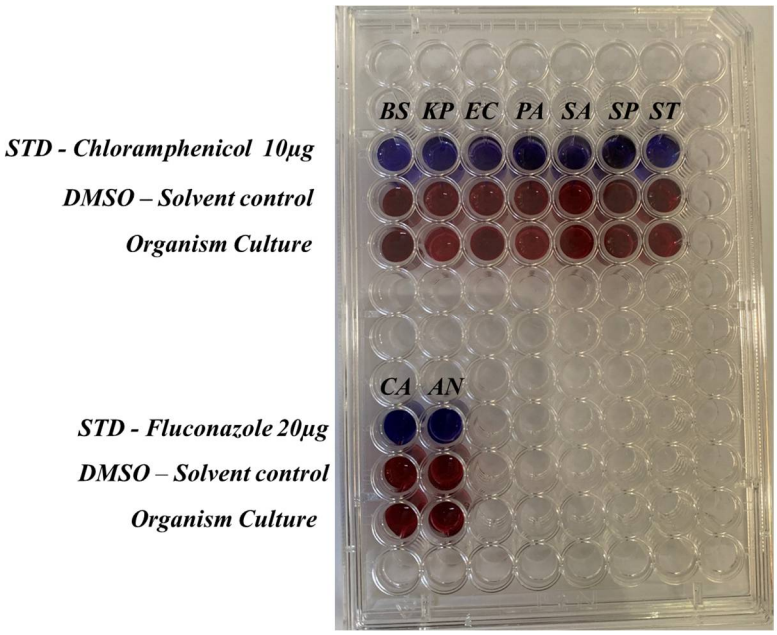

Figure 2: 96 Well Plate - Anti-Microbial Spectrum Image of Standard Drug Chloramphenicol and Fluconazole

CONCLUSION

The present study suggested that, selected ayurvedic herbal drug Chandanadi Churna have greater potential as antimicrobial agent against enteric bacteria and urinary tract pathogens. Hence, they can be used as alternative medicine for treating bacterial and fungal infection.

AUTHOR'S CONTRIBUTION

Manuraj Performed antimicrobial studies and wrote the first draft. Geetha conceived the idea and designed the work and finalized the manuscript. Both authors have read and approved the final manuscript

DATA AVAILABILITY

Data generated were analysed during the study and included in the published article .

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