EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 10
Issue: 7
201-205
Year: 2023

IN-VITRO ANTI-MICROBIAL ACTIVITY AND QUALITATIVE BIOCHEMICAL ANALYSIS OF PADIGA NELLI CHOORANAM- A SIDDHA HERBOMINERAL FORMULATION

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Article Received on 08/05/2023 **Article Revised on 29/05/2023** **Article Accepted on 18/06/2023**

ABSTRACT
Siddha system is one the traditional system of medicine practised in south India. Saint Yugi mentioned the signs and symptoms of Metchu Vatham in Yugi vaithiya chinthamani may be correlated with that of Urinary Tract Infection in Bio medicine. This paper scientifically reviews the herbomineral formulation Padiganelli Choornam that have been mentioned in the ancient Siddha literature siddhar tharum seeriya maruthuvam for the treatment of Metchu Vatham. **Aim**: The main aim of this study is to analyse the in-vitro anti microbial activity and bio chemical analysis of Padiganelli Chooranam. **Methodology:** Padiganelli Chooranam has its reference in siddhar tharum seeriya maruthuvam page no 254. It is tested against some gram positive, gram negative bacteria and fungus. This study also analyzed the qualitative analysis of biochemicals in herbomineral drug of Padiganelli Chooranam. **Result:** The study result was concluded that Padiganelli Chooranam has significant Anti-microbial activity.

KEYWORDS: *Siddha medicine, Metchu Vatham, Padiganelli Chooranam, Urinary Tract Infection, Anti-microbial activity.*

1. INTRODUCTION
Urinary tract infections (UTIs) are a severe public health problem and are caused by a range of pathogens, but most commonly by Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis and Staphylococcus saprophyticus. High recurrence rates and increasing antimicrobial resistance among uropathogen threatens to greatly increase the economic burden of these infections. While UTIs are typically considered extracellular infections, it has been demonstrated that uropathogenic E. coli can invade and replicate within the bladder cells to form intracellular bacterial communities (IBCs). Antibiotics are essential for the control and treatment of E. coli infections in humans and animals. However, it is generally accepted that antimicrobial resistance is associated with the quantity of antibiotic consumption. The worldwide emergence of multidrug-resistant bacteria has dramatically limited the number of antibiotics that retain activity against these pathogens. This problem has been further amplified by the dearth of novel classes of antibiotics. Therefore, development of novel therapeutic strategies for infectious diseases is high demand. Siddha system of medicine has excellent antimicrobial drugs with combination of bioactive components thats acts synergistically in multiple way thereby chance of resistance is greatly reduced and also several siddha traditional drugs are been prescribed for treating UTI infection one such drug is Padiganelli Chooranam. Hence this current study was carried out to prove the antimicrobial activity of "Padiganelli Chooranam" by invitro assays and biochemical analysis.

2. MATERIALS AND METHODS
2.1 Drug Selection
The siddha heromineral formulation has taken from the classical siddha literature "siddhar tharum seeriya maruthuvam" for treatment of metchu Vatham.

2.2 Ingredients of Padiganelli Chooranam
1.Padigaram (Potassium Aluminum Sulfate)
2.Vaal Milagu (Piper Cubeba)
3.Maasikkai (Quercus Infectoria)
4.Nelli Vatral (Phyllanthus Emblica)
5.Vellai Kunthiriyam (Boswellia Serrata)

2.3 Authentication of raw drugs

The identification of herbomineral drugs are authenticated by faculties of Department of Gunapadam, government siddha medical college hospital, palayamkottai.

2.4 Methods of purification

All the raw drugs purified as per the purification method mentioned in siddha literature.

2.5 Method of preparation

Above purified drugs are made in to fine powder separately. Mix all the powders and take equal amount of karkandu and mix it also. Finally it is stored in air tight container.

2.6 Antibacterial Activity

Determination of Minimum inhibitory concentration (MIC) of the siddha formulation *P.N.Chooranam* using Resazurin Microtitre Assay

Procedure

Test was carried out in a 96 well Plates 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 to determine the sensitivity of the fungal species tested.

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**

Anti-Bacterial Activity- PNC

Growth of inhibition Chart for the Sample and Standard Drug

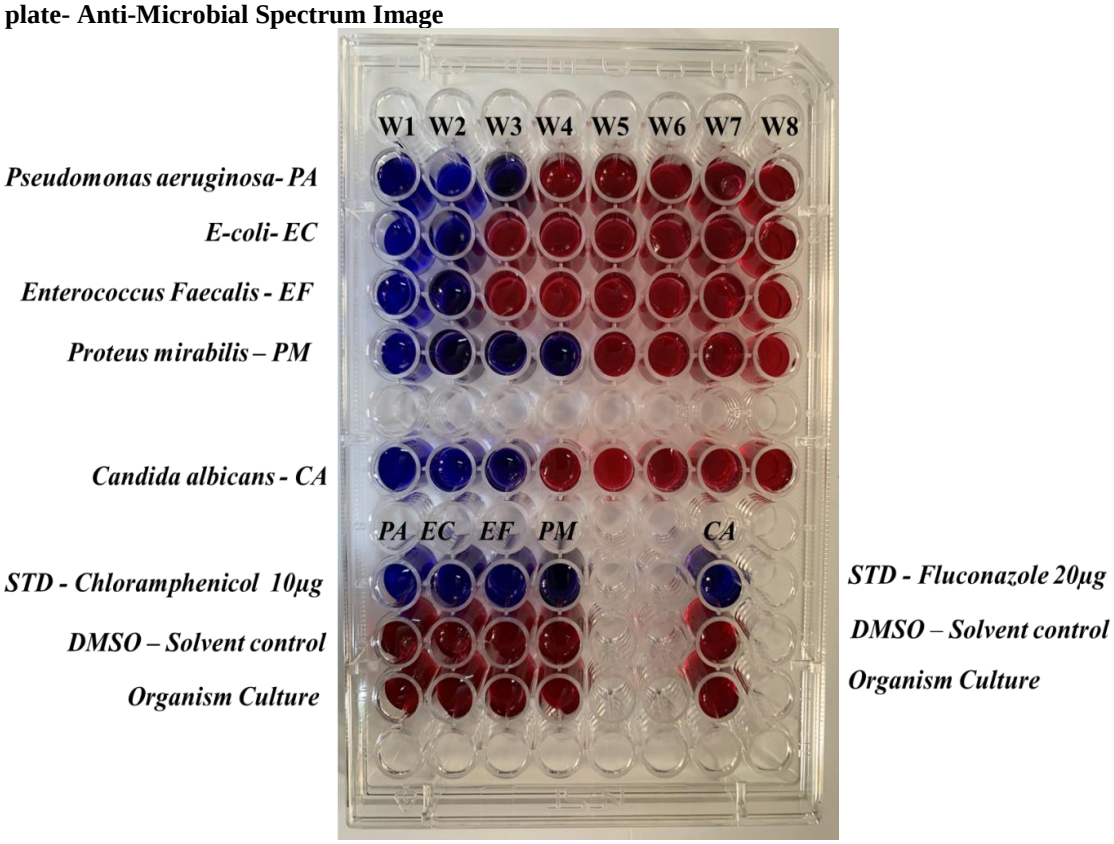
S.No	Sample/ Microorganisms	Growth of inhibition										
		W-1 1000 µg	**W-2 500 µg**	**W-3 250 µg**	**W-4 125 µg**	**W-5 62.5 µg**	**W-6 31.2 µg**	**W-7 15.6 µg**	**W-8 7.8 µg**	**STD Chloramphenicol (10µg)**	**DMSO**	**Culture**
1	*Pseudomonas aeruginosa– PA*	-	-	-	+	+	+	+	+	-	+	+
2	*E-coli - EC*	-	-	+	+	+	+	+	+	-	+	+
3	*Enterococcus Faecalis - EF*	-	-	+	+	+	+	+	+	-	+	+
4	*Proteus mirabilis– PM*	-	-	-	-	+	+	+	+	-	+	+

Anti-Fungal Activity

Growth of inhibition Chart for the Sample and Standard Drug

S.No	**Sample/ Microorganisms**	Growth of inhibition										
		W-1 1000 µg	**W-2** 500 µg	**W-3** 250 µg	**W-4** 125 µg	**W-5** 62.5 µg	**W-6** 31.2 µg	**W-7** 15.6 µg	**W-8** 7.8 µg	STD Fluconazole (20µg)	DMSO	Culture
1	***Candida albicans - CA***	-	-	-	+	+	+	+	+	-	+	+

96 Well plate- Anti-Microbial Spectrum Image



Report on MIC (Minimum inhibitory concentration) value of the Sample - PNC.

S.No	Name of the Organism	MIC Value (µg)
1	***Pseudomonas aeruginosa – PA***	250 µg
2	***E-coli - EC***	500 µg
3	***Enterococcus Faecalis - EF***	500 µg
4	***Proteus mirabilis – PM***	125 µg
5	***Candida albicans - CA***	250 µg

OBSERVATION AND CONCLUSION

It was observed from the results of the present investigation that the sample reveals convincing anti-microbial activity among all tested organisms. It was observed that the sample reveals significant activity against the pathogen*Proteus mirabilis (MIC 125* µg*)* and *Pseudomonas aeruginosa (MIC 250* µg*)* with the lowest MIC values when compared to that of *E-coli(MIC 500* µg*)and Enterococcus Faecalis*with MIC value of 500 µg. Similarly, the sample demonstrate consistent anti-fungal activity against *Candida albicans*with the MIC value of 250 µg.

2.7. Biochemical Analysis of Padiganelli Chooranam

Preparation of the extract

5 gms of the PADIGANELLI CHOORANAM was weighted accurately and placed in a 250 ml clean beaker then 50 ml of distilled water is added and dissolved well. Then it is boiled well for about 10 minutes. It is cooled and filtered in a 100 ml volumetric flask and it is made to 100 ml with distilled water. This fluid is taken for analysis.

QUALITATIVE ANALYSIS

S. No	Procedure	Observation	Inference
1.	**Test for calcium:** To 2 ml of the above prepared extract taken in a clean testtube. To this add 2 ml of 4% ammonium oxalate solution.	White precipitate is formed	Presence of calcium
2.	**Test for sulphate:** To 2ml of the extract is added to 5% barium chloridesolution.	White precipitate is formed	Presence of sulphate
3.	**Test for chloride:** The extract is treated with silver nitrate solution.	White precipitate is formed	Presence of chloride

4.	**Test for carbonate:** The substance is treated with concentrated HCL.	No brisk effervescence is formed	Absence of carbonate
5.	**Test for Starch:** The extract is added with weak iodine solution.	Blue colour is formed	Presence of starch
6.	**Test for ferric iron:** The extract is acidified with glacial acetic acid andpotassium ferro cyanide.	No blue colour is formed	Absence of ferric iron
7.	**Test for ferrous iron:** The extract is treated with concentrated nitric acidammonium thiocyanate solution.	Blood red colour is formed	Presence of ferrous iron
8.	**Test for phosphate**: The extract is treated with ammonium molybdate andconcentrated nitric acid.	Yellow precipitate is formed	Presence of phosphate
9.	**Test for albumin:** The extract is treated with esbach's reagent.	No yellow precipitate is formed	Absence of albumin
10.	**Test for tannic acid:** The extract is treated with ferric chloride.	blueblack precipitate is formed	Presence of tannic acid
11.	**Test for unsaturation:** Potassium permanganate solution is added to the extract.	It gets decolourised	Presence of unsaturatedcompound
12.	**Test for the reducing sugar:** To 5 ml of benedict's qualitative solution is taken in a test tube and allowed to boil for 2 minutes and add 8 to 10 drops of the extract and again boil it for 2 minutes.	No Colour changes occurs	Absence of reducing sugar
13.	**Test for amino acid:** One or two drops of the extract is placed on a filter paper and dried well. After drying, 1% ninhydrin is sprayedover the same and dried it well.	Violet colour is formed	Presence of amino acid
14.	**Test for zinc:** The extract is treated with Potassium Ferro cyanide.	No white precipitate is formed	Absence of zinc

The qualitative bio chemical analysis of *Padiganelli chooranam* reveals the presence of Calcium, Sulphate, Chloride, Starch, Ferrous iron, Phosphate, Tannic acid, Unsaturation compound, Amino acid.

This Bio chemical qualitative analysis of Padiganelli chooranam study was done in PG – Biochemsitry laboratory in Government Siddha medical college and Hospital, Palayamkottai.

3. DISCUSSION

The antimicrobial studies showed that there is a well sensitive against the pathogen proteus mirabilis, pseudomonas aeruginosa, fungus candida sps, and intermediate effect against the E-coli, Enterococus faecalis. It can be recommended for Urinary Tract Infection disease.

4. CONCLUSION

From this study, we can conclude that the herbomineral formulation of *Padiganelli Chooranam* possess significant anti-microbial property, so it is the most promising drug for Metchu Vatham. In future in-vivo study will be done on *Padiganelli Chooranam* for further extensive research.

ACKNOWLEDGEMENT

I wish to express my sincere thanks to Dr. A. Balamurugan, Lecturer Grade – II, Dr. S. Sundarrajan, Head of the Department, Department of Noi Naadal, GSMC, Palayamkottai for their valuable support.

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