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Invitro Study of Anti-Microbial Activity and Qualitative Bio-Chemical Analysis of Herbomineral Siddha Formulation - Vellaikku Chooranam

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Abstract: ***Background: The Vellaikku Chooranam is a herbomineral drug used for the treatment of vellai noi (Leucorrhoea), Neerchchurukku (Dysuria), neererichal (Burning micturition). Objective: The main objective of the study is to evaluate the antimicrobial activity and the Bio-chemical analysis of vellaikku chooranam. Methodology: Vellaikku Chooranam has its reference in Kannusamiyam ennum vaithiya sekaram, page no: 33. It is tested against some gram positive, gram negative bacteria and fungus. The mean inhibitory concentration (MIC) against the Candida sps, which shows the maximum zone of inhibition, is determined. This study also analysed the Qualitative analysis of bio-chemicals in herbomineral drug of Vellaikku chooranam. Result: Vellaikku Chooranam is well effective against candida sps, and intermediate effective against staphylococcus sps. Conclusion: Vellaikku Chooranam has a significant role against the leucorrhoea.***

Keywords: Vellaikku chooranam, leucorrhoea, antimicrobial, somarogam

1. Introduction

The ancient Siddha medical system based mostly on plants, metals and minerals. Somarogam is one among the 4448 diseases, as classified in the ancient siddha medical texts. Signs and symptoms of the somarogam disease is closely correlated with leucorrhoea as a modern medical terminology. It is more common in reproductive age group women and in older age women. More number of medicines mentioned in siddha literature for soma rogam disease. This study aims at the efficacy of Vellaikku chooranam.

Somarogam in Siddha

Synonyms: vellai noi, vettai noi, piramiyam, ozhukku noi, piramegam.

It is characterized by body pain, burning sensation present in the body, aversion of food, burning sensation present in urethral orifice and excessive white discharge.

Pathogenesis: Due to inappropriate dietary habits the pitham (Azhal kutram) is increased which affect the Abhana vaayu and viyana vaayu. Affected abhana vaayu alters the theyu pootham which leads to burning sensation in urethra and excessive white discharge. Affected viyanan alters the akaya pootham which leads to aversion of food, loss of appetite and pain all over the body.

Leucorrhoea: Lecorrhoea means white discharge. It includes conditions when the vaginal discharge is excessive and associated with or without an obvious local pathology, in practices all excessive vaginal discharge, white purulent, yellowish or water, but not blood stained, is labeled leucorrhoea. It is caused by physiological or pathological. The physiological causes are puberty, ovulation, the premenstrual period and pregnancy. The pathological causes are pelvic tumours such as fibroids, ovarian neoplasms, PID, cervical lesions, cervical erosion, vaginitis which may be specific due to infections caused by STD such as gonorrhea, trichomonas vaginalis and candida albicans or non specific due to infections through mixed organisms such as streptococcus, staphylococcus, e.coli or anaerobes.

2. Materials and Methods

Drug Selection:

The siddha formulation *Vellaikku Chooranam* has taken from the Kannusamiyam ennum vaithiya sekaram and it is indicated for vellai noi (leucorrhoea).

Ingredients of vellaikku chooranam:

1) Padikaram (Potassium aluminum sulfate)
2) Nelli vatral (Phyllanthus emblica)
3) Karkandu (Saccharum officinarum)

Collection of Raw Drugs:

The drugs are purchased from ASN herbal drug shop, Melapalayam, Tirunelveli.

Authentication of Raw Drugs:

The identification of herbomineral drugs are authenticated by faculties of Department of Gunapadam, Government siddha medical college and Hospital, Palayamkottai.

Purification of Raw Drugs:

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

1) Padikaram (Potassium aluminum sulfate)
   Dissolved it in water, strained it and then boiled upto form jelly like consistency then cool it.
2) Nelli vatral (Phyllanthus emblica)
   Remove the dust particles and dry in the sunlight.

Methods of Drug Preparation

Above purified drugs are made into fine powder separately and sieved in fine cotton cloth. Mix all the powders and take equal amount of sugar and mix it also. Finally it is stored in airtight container.

Shelf Life:
3 Months

Dosage:
10 gram Bds – 3 Days

Adjuvant:
Butter milk

Antimicrobial Activity Procedure

Antimicrobial activity of Vellaikku chooranam study was done in INBIOTICS RESEARCH INSTITUTE in Nagercoil, Tamilnadu, India.

Sample preparartion:

1 mg (1000 µg) sample was dissolved in 1ml (1000 µl) solvent to get 100 µg/100 µl

Agar Well Diffusion Test

The antibacterial screening of the ***Vellaikku Chooranam*** **(VC)** was carried out by determining the zone of inhibition using agar well diffusion method (Bauer, 1996). The drug extracts were tested against pathogenic bacteria *(Escherichia coli, Staphylococcus sps* and *Streptococcus sps).*

Bacterial Inoculums Preparation

Inoculum of *Escherichia coli, Staphylococcus sps* and *Streptococcus sps* were prepared individually in a respective broth and kept for incubation at suitable temperature.

Antibacterial Test:

The medium was prepared by dissolving 38 g of Muller Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 Lbs pressure at 121^0C for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured petriplates (25 ml/plate) the plates were swabbed with Pathogenic Bacteria culture viz. analysis *Escherichia coli, Staphylococcus sps and Streptococcus sps.* Finally, About 10 µL of sample (Aqueous extract of VC) was loaded onto the disc then placed on the surface of Mullar-Hinton medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimetres. The size of the zone of inhibition (including disc) was measured in millimeters. The absence of zone inhibition was interpreted as the absence of activity (Kohner *et al*., 1994; Mathabe *et al*., 2006). The activities are expressed as resistant, if the zone of inhibition was less than 7 mm, intermediate (8-10 mm) and sensitive if more than 11 mm (Assam *et al*., 2010).

Antifungal Activity

Test Organism

The test Fungi used for antifungal analysis *Candida sps* were isolated from the environment.

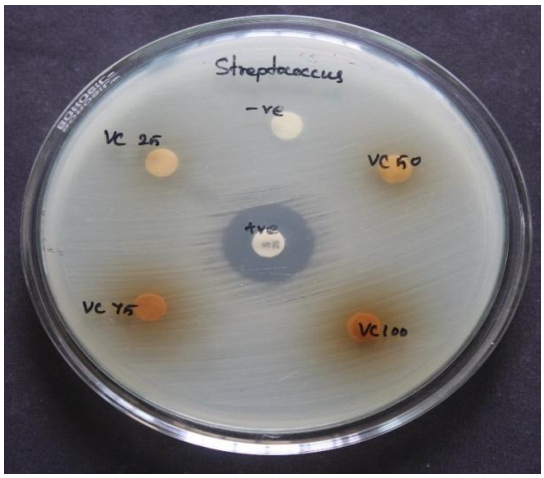
Antifungi Assay by Disc Diffusion Method (Bauer et al., 1966)

Antibiotic susceptibility tests were determined by agar disc diffusion (Kirby–Bauer) method. Fungi strains *Candida* sps were swabbed using sterile cotton swabs in SDA agar plate. 10 µL of each sample was respectively introduced in the sterile discs using sterile pipettes. The disc was then placed on the surface of SDA medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 22°C for 48 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimeters.

Table 1: Anti-bacterial potential of hydro alcoholic extract of *Vellaikku Chooranam* (VC)

Sample Code and Conc. (VC)	Bacteria Strains Name and Zone of inhibition (mm in diameter)		
	E.coli (G-)	*Staphylococcus Sps (G+)*	*Streptococcus Sps (G+)*
25 µg	-	-	-
50 µg	-	-	-
75 µg	-	7	-
100 µg	-	8	-
Positive Control (Streptomycin 25mg)	13	16	16
Negative Control	-	-	-

Keywords: ***PC*** *Positive control (Streptomycin),* ***NC*** *Negative control, "-" No Zone,* ***mm*** *(Millimetre),* ***G+*** *(Gram Positive Organism),****G-*** *(Gram Negative Organism),*



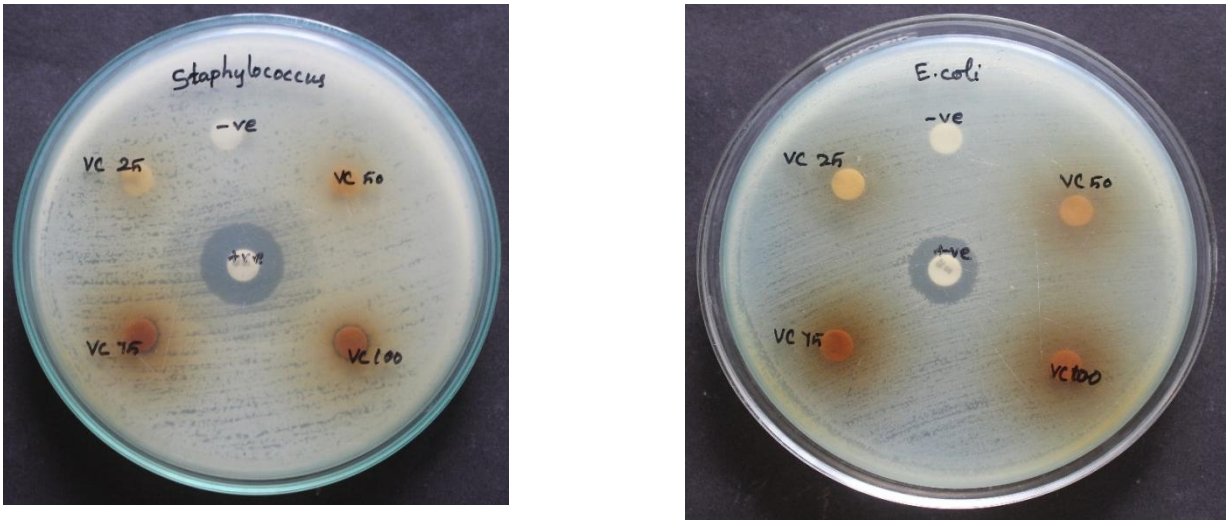


Figure 1: Anti-bacterial potential of hydro alcoholic extract of *Vellaikku Chooranam* (VC)

Table 2: Anti-Fungal potential hydro alcoholic extract of *Vellaikku Chooranam* (VC)

Fungai Name	Samples Zone of inhibition (mm in diameter)					
	S1 25µg	S1 50µg	S1 75µg	S1 100µg	Positive Control (Fluconazole)	Negative control
Candida sps	12	14	18	20	22	-

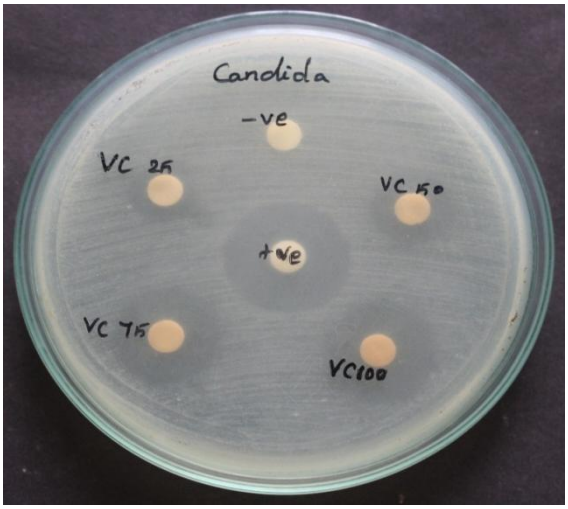


Figure 2: Anti-bacterial potential of hydro alcoholic extract of *Vellaikku Chooranam* (VC)

Biochemical Analysis of Vellaikku Chooranam

Preparation of the extract:
5gms of the drug was weighed accurately and placed in a 250ml clean beaker then 50ml of distilled water is added and dissolved well. Then it is boiled well for about 10 minutes. It is cooled and filtered in a 100ml volumetric flask and then it is made to 100ml 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 test tube. To this add 2 ml of 4% ammonium oxalate solution.	No white precipitate is formed	Indicates the absence of calcium
2.	**Test for sulphate:** To 2ml of the extract is added to 5% barium chloride solution.	A White precipitate is formed	**Indicates the Presence of sulphate**
3.	**Test for chloride:** The extract is treated with silver nitrate solution.	No white precipitate is formed	Absence 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:**	No blue colour is	Absence of starch

	The extract is added with weak iodine solution.	formed	
6.	**Test for ferric iron:** The extract is acidified with glacial acetic acid and potassium ferro cyanide.	No blue colour is formed	Absence of ferric iron
7.	**Test for ferrous iron:** The extract is treated with concentrated nitric acid ammonium thiocyanate solution.	Blood red colour is formed	**Indicates the presence of ferrous iron**
8.	**Test for phosphate**: The extract is treated with ammonium molybdate and concentrated nitric acid.	No yellow precipitate is formed	Absence 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.	Blue black precipitate is formed	**Indicates the presence of Tannic acid**
11.	**Test for unsaturation:** Potassium permanganate solution is added to the extract.	It gets decolourised	**Indicates the Presence of unsaturated compound**
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.	Colour changes occurs	**Presence 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 sprayed over 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 *Vellaikku chooranam* reveals the presence of Sulphate, Ferrous iron, Tannic acid, Unsaturation compound, Reducing sugar, Amino acid.

This Bio chemical qualitative analysis of Vellaikku 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 fungus candida sps, and intermediate effect against the gram positive bacteria staphylococcus sps. It can be recommended for Leucorrhoea disease.

4. Conclusion

From this study, we can conclude that the herbomineral formulation of *Vellaikku Chooranam* possess significant anti-fungal property, so it is the most promising drug for leucorrhoea due to the candida sps,. In future in-vivo study will be done on *Vellaikku Chooranam* for further extensive research.

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