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Anti-microbial activity and Phytochemical analysis of Polyherbal Siddha Formulation "*Vallarai Mathirai*"

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ABSTRACT

The main objective is to evaluate the efficacy of polyherbal siddha formulation '*Vallarai Mathirai'*(VM) through phytochemical analysis and anti-microbial studies.*Vallarai Mathirai* . To overcome the antibiotic resistance, reinfection and reversion, the novel herbal antibiotic resistance, the authors have tried Vallarai Mathirai having its reference in Siddha Literature Gunapadam Mooligai. Vallarai Mathirai is indicated for Suram(fever) as per Siddha Literature Gunapadam Mooligai. *Suram* is an elevation of the body temperature accompanying all infectious diseases and occurring in many other conditions. *Suram* is classified into 64 types among which 52 types are caused due to intrinsic factors and 12 types are caused due to extrinsic factors. The ingredients of *Vallarai Mathirai* are *Centella asiatica* (Indian Pennywort), *Ocimum sanctum* (Holy basil) and *Piper nigrum* (Black pepper). These ingredients were collected, authenticated, purified and prepared as per SOP. The antipyretic activity of *Vallarai Mathirai* was proved by anti-microbial activity, phytochemical analysis. The anti-microbial activity of *Vallarai Mathirai* has been tested by Agar Well Diffusion method and evaluated the sensitivity against Staphylococcus aureus, Streptococcus mutans, Klebsiella pneumonia, Bacillus subtilis, Proteus vulgaris, and Escherichia coli. The result revealed significant anti-microbial activity against all the six microorganisms.

KEYWORDS

Polyherbal siddha formulation, *Centella asiatica*, *Vallarai Mathirai*, *Suram*, *fever.*

INTRODUCTION

Vallarai Mathirai is a Polyherbal Siddha formulation for the treatment of all types of Suram which consists of *Centella asiatica* (Indian pennywort), *Ocimum sanctum* (Holy basil), *Piper nigrum* (Black pepper) referenced from *Gunapadam Mooligai*. In Siddha system, *Suram* is documented as a separate disease as per Traditional Siddha Indian Medicine. *Suram* is an elevation of the body temperature accompanying all infectious diseases and occurring in many other conditions such as shock, sunstroke, derangement of digestive system, hysteria and presence of some poison in the blood. *Suram* is classified into 64 types among which 52 types are caused due to intrinsic factors and 12 types are caused due to extrinsic factors. The Intrinsic factors are those which affect the equilibrium of *Vatham, Pitham, Kapam*. The extrinsic factors are environment, diet, climatic conditions, physical activities, and stress. According to the Siddha system, diet and lifestyle play a major role in health and in curing of diseases. This concept of the Siddha medicine is termed as *patthiyam* and *apatthiyam* which is essentially a rule based system with a list of "do's and don'ts".

PATHOLOGY OF SURAM

In stomach, there is *Aamam* which is also known as *Seetham* (chillness). When *Seetham* increases, it increases the *Kapam*, so *Kukkianal* (gastric fire) changing away from it self nature and increase the temperature which spread into the whole body through the nerves.

"There is no fever without indigestion"

"There is no fever without chillness in the intestine"

"There is no fever without stillness in the stomach"

-Theraiyar.

MATERIALS AND METHODS

COLLECTION AND AUTHENTICATION OF FRESH PLANTS AND RAW DRUG

The preparation contains fresh leaves which were collected from wet ground and seeds were collected from Traditional raw drug store, Nagercoil, TamilNadu. The fresh leaves of *Centella asiatica* and *Ocimum sanctum* and seeds of *Piper nigrum* were collected and authenticated by

faculties of Department of Botany and Department of *Gunapadam*, Government Siddha Medical College, Palayamkottai, Tirunelveli, Tamilnadu, India.

METHOD OF PREPARATION

The ingredients of Poly herbal Siddha formulation *Vallarai Mathirai* were purified and it was prepared as per an Standard Operative Procedure (SOP).

The Ingredients list of the test drug *Vallarai Mathirai:*

Table No: 1

NAME OF THE PLANT	PART USED	QUANTITY
FRESH PLANT		
Vallarai (Indian pennywort) *(Centella asiatica)*	Fresh leaves	8 grams
Thulasi (Holy basil) *(Ocimum sanctum)*	Fresh leaves	8 grams
DRIED RAW DRUG		
Milagu (Black pepper) *(Piper nigrum)*	Dried seeds	8 grams

CENTELLA ASIATICA

OCIMUM SANCTUM

PIPER NIGRUM

Classification of fresh herbs and raw drug based on *suvai* (taste) and *Panchabootha thathuvam* theory (five elements theory)

Table no: 2

INGREDIENTS	SUVAI	THANMAI	PIRIVU
Centella asiatica	*Thuvarppu* *Kaippu* *Inippu*	*Thatpam*	*Inippu*
Ocimum sanctum	*Kaarppu*	*Veppam*	*Kaarppu*
Piper nigrum	*Kaippu* *Kaarppu*	*Veppam*	*Kaarppu*

The leaves of *Centella asiatica* and *Ocimum sanctum* were cleaned with fresh water and unwanted ripened leaves were removed. The leaves are grinded softly and the raw drug is powdered finely. These two products are mixed and grinded and made into *65mg* tablet.

Antimicrobial results of *Vallarai Mathirai:*

Samples	Strains					
	Staphylococcus aureus (G+) MTCC 916	***Streptococcus mutans*** (G+) MTCC 497	***Bacillus subtilis*****(**G+) MTCC 1134	***E- coli*** (G-) MTCC 1671	***Klebsiella pneumonia*** (G-) MTCC 503	***Proteus vulgaris***(G-) MTCC 426
VM.Aq 25 µl	9	7	8	9	12	-
VM. Aq 50 µl	10	9	10	10	14	7
VM .Aq 75 µl	11	11	12	13	16	9
VM .Aq 100 µl	13	12	12	15	17	11
PC (Strepto mycin)	20	14	13	28	22	19
NC	-	-	-	-	-	-

Table No: 3

NOTE: VM-*Vallarai Mathirai*, **Aq**-Aqueous, **PC**-Positive Control, **NC**-Negative Control, **MTCC**-Microbial Type Culture Collection, (**G+)**-Gram Positive, (**G-**)-Gram Negative.

Antibacterial Activity Procedure

Dilution : 0.1g in 1ml

Test Organism:

The test microorganisms used for antimicrobial analysis *(Staphylococcus aureus, Streptococcus mutans, Klebsiella pneumonia, Bacillus subtilis, Proteus vulgaris and Escherichia*

coli) were purchased from Microbial Type Culture Collection and Gene Bank (***MTCC***) Chandigarh. The bacterial strains were maintained on Nutrient Agar (NA).

Nutrient Broth Preparation

Pure culture from the plate were inoculated into Nutrient Agar plate and sub cultured at 37ºC for 24 h. Inoculum was prepared by aseptically adding the fresh culture into 2 ml of sterile 0.145 mol/L saline tube and the cell density was adjusted to 0.5 McFarland turbidity standard to yield a bacterial suspension of 1.5×108 cfu/ml. Standardized inoculum Used for Antimicrobial test.

Antimicrobial 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. *(Staphylococcus aureus, Streptococcus mutans, Klebsiella pneumonia, Bacillus subtilis, Proteus vulgaris and Escherichia coli)*. Finally, The Sample or Sample loaded Disc was 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 millimeters. 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)

Staphylococcus aureus

Staphylocoocus aureus is an important pyogenic organism and lesions are localized in nature. Staphylococcal diseases are classified as cutaneous infection(boils, impetigo, cellulitis, scaled skin syndrome) food poisoning, nosocomial infections, skin exfoliative diseases and *toxic shock syndrome* (**High Fever**, nausea, vomiting, diarrhoea, rash on the palm and sole, muscle ache, abdominal pain, confusion).

Streptococcus mutans

Streptococcus mutans is commonly found in the human oral cavity and is a significant contributor to the tooth decay with mild **fever**. It is part of the streptococci, an informal general name for all species in the genus streptococcus.

Streptococcus mutans, a pathogen of dental caries, is known to be associated with bacteremia of infective endocarditis (**Fever**, chills, fatigue, chest pain when breathing, shortness of breath, night sweat, muscle and joint pain).

Bacillus subtilis

Bacillus subtilis, is commonly found in soil and the gastrointestinal tract of ruminants and humans. *Bacillus subtilis* are periodically associated with bacteremia, endocarditis, meningitis, and infection of wounds, the ears, eyes, respiratory tract, urinary tract and gastrointestinal tract. All of these induce mild **fever**.

Escherichia coli

Escherichia coli forms a part of normal intestinal flora of man and animal. There are four major types of clinical syndromes

1.Urinary tract infections.

2.Diarrhoea.

3.Pyogenic infections.

4.Septicemia.

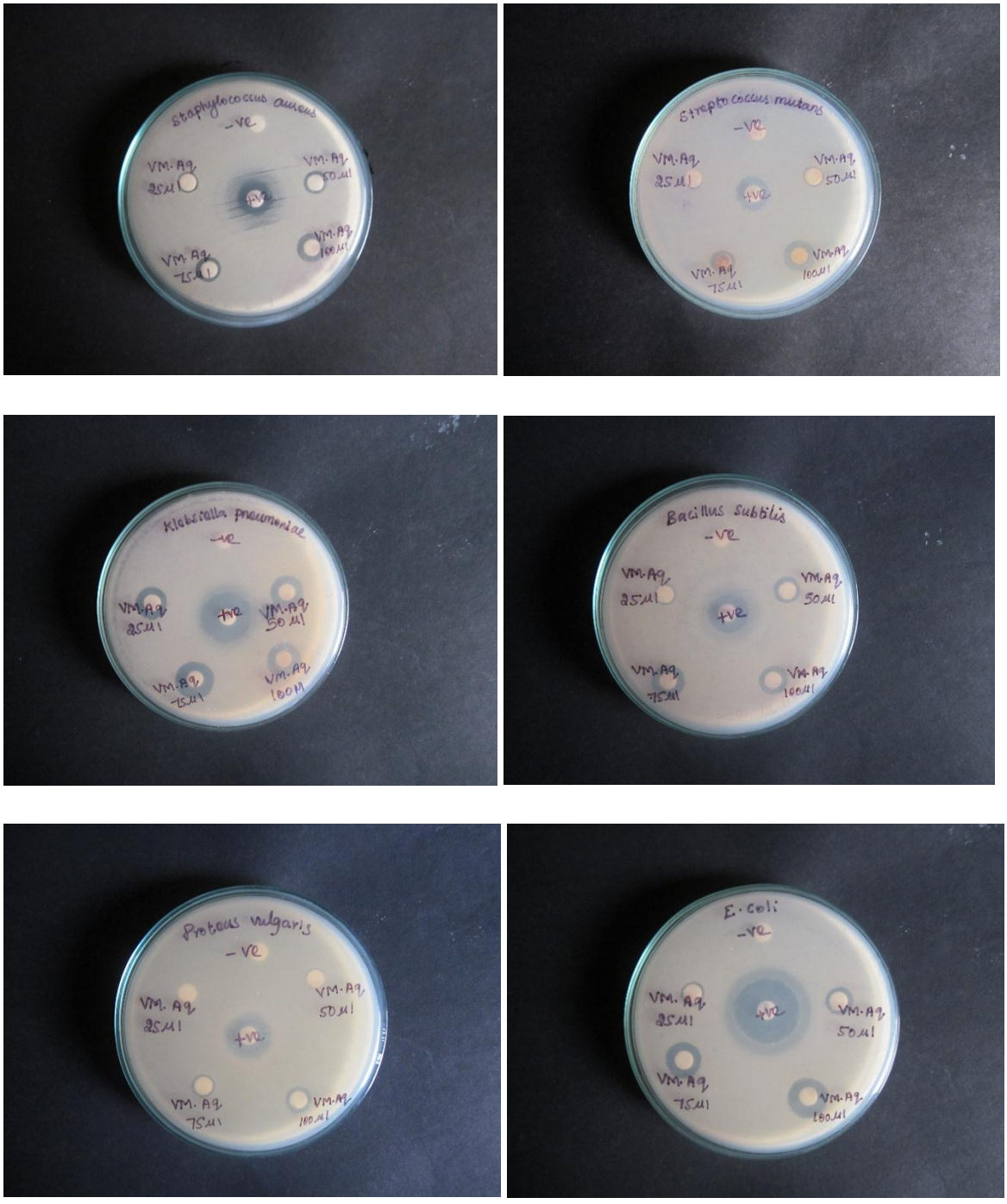
Klebsiella pneumoniae

Klebsiella are widely distributed in nature, occurring as commensals in human and animal intestine and also as saprophytes in soil *Klebsiella pneumoniae* subspecies pneumonia is the second most popular member next to Escherichia coli of aerobic bacterial flora of intestine of humans. It is responsible for severe bronchopneumonia, Urinary tract infection, nosocomial infection, wound infection, septicaemia, meningitis and rarely diarrhoea. Individuals with Klebsiella pneumonia tend to cough up characteristic sputum as well as having **fever**, nausa, tachycardia, and vomiting.

Proteus vulgaris

Proteus vulgaris is a rod-shaped, nitrate- reducing, indole positive and catalase positive, hydrogen sulfide producing, Gram-negative bacterium that inhibits the intestinal tracts of humans and animals. It can be found in soil, water and fecal matter. It commonly causes UTI and it also causes cystitis, pyelonephritis, prostatitis, wound infection and burn infections.

Zone of inhibition of *Staphylococcus aureus, Streptococcus mutans, Klebsiella pneumonia, Bacillus subtilis, Proteus vulgaris and Escherichia coli* against Streptomycin.



Phytochemical Analysis:

Alkaloids:

Mayer's test: (Ansari, 2006)

The extract was evaporated in a test tube. To the residue dilute HCL was added, shaken well and filtered.

Mayer's Test: To the 2-3 ml of filtrate Mayer's reagent was added. Formation of yellow precipitate showed the presence of alkaloids.

Ansari, S. H. 2006. Essentials of pharmacognosy, 1st edition, Birla publications, New Delhi. pp. 357-359, 588-590.

GLYCOSIDES:

Keller-Killani Test: (Ansari, 2006)

To 2 ml of the extract, glacial acetic acid, one drop 5% $FeCl_3$ and conc. H_2SO_4 was added. Reddish brown colour appeared at junction of two liquid layers and upper layer turned bluish green indicating the presence of glycosides.

Ansari, S. H. 2006. Essentials of pharmacognosy, 1st edition, Birla publications, New Delhi. pp. 357-359, 588-590.

TANNINS:

Lead Acetate Test: (Mukherjee, 2002)

On addition of 5% lead acetate solution to the extract white precipitate appeared.

Mukherjee, P. K. 2002. Quality control of herbal drugs, business horizons pharmaceutical publishers, New Delhi. 356 - 358.

STEROIDS:

Salkowski Test: (IP, 1996)

To 2 ml of extract, 2 ml of chloroform and 2 ml of conc. H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Indian Pharmacopoeia (IP). 1996. Govt. of India, Ministry of Health and Family Welfare Published by the Controller of Publications, New Delhi, A-47, A-53, A-54.

SAPONINS:

Foam Test: (Ansari, 2006)

Sample extract was added with distilled water and shaken vigorously. Observe the stable foam formation. **Ansari, S. H. 2006.** Essentials of pharmacognosy, 1st edition, Birla publications, New Delhi. pp. 357-359, 588-590.

PHYTOCHEMICAL RESULTS

Table:4 Qualitative Phytochemical analysis of VM

TEST NAME	Aqueous VM
Alkaloid	**Absent**
Glycoside	**Present**
Tannin	**Present**
Steroids	**Absent**
Saponin	**Present**

The Glycoside, Tannin and Saponin are present in the *Vallarai Mathirai.*

Figures: Qualitative Phytochemical analysis of VM

Quantitative Estimation of Glycosides:

10ml of solution the extract and 10ml of Baljet's reagent are taken and allowed to stand for one hour. Then dilute the with 20ml distilled water and mix. Read the intensity of the colour obtained against blank at 495nm using a spectrophotometer. The difference between test and control is taken for calculation. Standard graph can be prepared using standard digitoxin.

Ref: Solich P, Sedliakova V, Karlicek R. Spectrophotometric determination of cardiac glycosides by flow-injection analysis. Anal Chim Acta. 1992; 269(2): 199-203.

Quantitative Estimation of Saponins: (Evans, 1996)

Aqueous extract was dissolved in 80% methanol, 2ml of Vanilin in ethanol was added, mixed well and the 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 60 ^{o}C for 10min. Absorbance was measured at 544nm against reagent blank. Diosgeninis used as a standard material and compared the assay with Diosgenin equivalents.

Ref: Devanaboyina N et al., "Preliminary Phytochemical Screening, Quantitative Estimation And Evaluation Of Antimicrobial Activity Of Alstoniamacrophylla Stem Bark" **IJSIT, 2013, 2(1), 31-39**

QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS

Table: 5 Qualitative estimation of Phytochemical constituents

SAMPLE : VM	
Test	**Aqueous**
Saponins µg / ml	**95 ± 0.1**
Glycosides µg / ml	**12 ± 0.2**

THIN LAYER CHROMATOGRAPHY ANALYSIS

Mobile phase was prepared by dissolving the Chloroform and water at 6:4 ratio. And about 10µl of Aqueous extract was dropped on TLC sheet 2cm above from the bottom. Incubated the content for 10-15minutes. Then chromatogram was developed by 1% $FeCl_3$. After developed the Rf (Retention Factor) was calculated by using the formula,

$$Rf = \frac{\text{Distance travelled by solvent}}{\text{Distamce travelled by solute}}$$

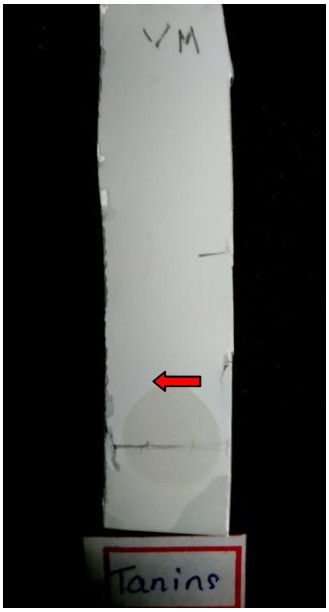
Biradar RS, Rachetti DB (2013). Extraction of some secondary metabolites & thin layer chromatography from different parts of *Centella asiatica* L. *American Journal of Life Sciences*. 1(6): 243-247.

RESULT:

Based on the Rf value, test sample showed the presence of **Tanins.**

$Rf = \frac{2.4}{0.9}$ =2.7

Figure: Thin layer Chromatography



RESULT AND DISCUSSION

The research work was carried out on the poly herbal siddha formulation "*Vallarai Mathirai*" which shows that phytochemical constituents Glycosides, Tanins and Saponins are present whereas Alkaloids, Steroids were found to be absent. In our studies, we found that the presence of Tanins have direct relevance in relieving **fever.** Our medicine *Vallarai Mathirai* effectively acts on *Klebsiella pneumoniae*, *Escherichia coli*, *Streptococcus mutans*, *Staphylococcus aureus* and reduce the fever caused by these microorganisms.

CONCLUSION

To conclude, *Vallarai Mathirai* is efficient and not tolerated by majority of the bacteria. The Table no 3 sums up the results of antimicrobial activity of *Vallarai Mathirai*. It revealed that even lesser dose of *Vallarai Mathirai* has considerable inhibitory effect on pyrogenic microorganisms. So it can be used to treat all types of fever as evident by *Gunapadam Mooligai*. Based on the above

results, it is the appropriate time to move for experimental Randomized Control Trials (RCTs) to prove its clinical evidence to the modern scientific world. An effective polyherbal drug for fever is the need of the hour. We, the team is yet to carry out further studies in this drug to uphold the drug on scientific platform.

REFERENCES

1. *K.S. Murugesa Mudhaliyar, Gunapadam Mooligai* Part-II, 3rd edition, Directorate of Indian Medicine and Homeopathy, Chennai.
2. *T.V. Sambasivampillai*, Siddha Medical Dictionary, Vol-IV (Part-I), Department of Indian Medicine and Homeopathy, Chennai.
3. *Dr.C.P. Baveja*, Textbook of Microbiology, 5th edition, Arya publication.
4. *M. Shanmugavelu, Noinaadal Noimudhal Naadal Thirattu*, Part-II, 3rd edition (Publication of Department of Indian Medicine and Homeopathy,Chennai), 2003, 262-264.
5. *Iwalewa EO, Iwaleva OJ, Adeboye JO*, Analgesic, antipyretic, anti-inflammatory effects of methanol, chloroform and ether extract of *Vernonia cinerea* less leaf, J Ethnopharmacol,86(3) (2003) 229-234.