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ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF SIDDHA FORMULATION – *SEEDHABETHY CHOORANAM*

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ABSTRACT

Purpose:

Dysentery is a type of gastroenteritis that results in diarrhoea with blood. It is caused by bacterial, viral or protozoal infections. Antibiotic resistance develops when the bacteria adapt and grows in the presence of antibiotics. The World Health Organization (WHO), in Global Action Plan on Antimicrobial resistance mentioned that there is a need on effective antimicrobial drugs for curative measures, thus protecting the patients from potentially fatal diseases. In siddha aspect *Seedhabedhy* is the term equivalent to describe dysentery. *Seedhabethy Chooranam, is* a polyherbal formulation mentioned in siddha literature for its antidysenteric activity. The paper aims at the extent of its efficacy against the etiologic agents of *Seedhabethy.*

Objective :

The main objective of the study is to evaluvate the phytochemical analysis and antimicrobial activity of *Seedhabethy Chooranam*.

Methodology :

Seedhabethy Chooranam has its reference in *Kannusamy parambarai vaithiyam.* It is tested against some gram positive and gram negative bacteria. The Mean Inhibitory Concentration (MIC) against the Klebsiella pneumoniae, which shows the maximum zone of inhibition is determined. It is analysed phytochemically and the Quantitative analysis of the phytochemicals, tannin and saponin is also done.

Result :

Seedhabethy Chooranam is effective against *Staphylocooccus aureus, Streptococcus mutans, Enterococcus, E.coli, Klebsiella pneumoniae, Proteus vulgaris.*

Conclusion :

Seedhabedhy Chooranam has a significant role against the dysenteric and diarrhoeal diseases.

KEYWORDS :

Dysentery, *Seedhabedhy Chooranam*, Siddha literature, Antimicrobial.

INTRODUCTION :

Siddha Medicine is an ancient system of medicine predominantly the essence being plants, metals and minerals. Medicinal plants have been used as traditional treatments for human disease for thousands of years. Dysenteric and diarrhoel diseases are a common scenario being witnessed all over the world. There is a lots of herbal formulations mentioned in Siddha literature. This paper aims at the efficacy of *Seedhabedhy Chooranam.*

DYSENTERY IN SIDDHA :

Synonyms : *Seedhakazhichal, Seedha rathabethy, Kaduppu kazhichal.*

It is charcterized by irritation of the stomach with frequent passage of stools in small quantities or the passage of large quantities of stools with blood and mucus without much irritation.

Pathogenesis

Due to inappropriate dietary habits the *pitham (Azhal kutram)* is increased which gradually stimulates the *Abhana vaayu* (One among the Dasavaayu). Generally the *abhana vaayu* governs the elimination of all substances from the body. *Seedhabethy* is caused by the disturbance of *Abhana vaayu.* Increased *pitham* depurifies the blood thus leading to the elevation of *Kabham.* The deranged *pitham* and *Kabham* ulcerates the colon and forms mucus with blood, thus causing the abdominal pain, abdominal cramps which are the main signs of *Vaatham.* As a result, all the three *Kutrams* are disturbed resulting in dysentery.

Seedhakazhichal means the dysentery due to specific inflammation & ulceration of mucus lining of large intestine resulting in evacuation of stools mixed with mucus and blood. .
(TV Sambasivampillai Dictionary)

As per *Gurunaadi nool* the **Pathogenesis of dysentry,**

Due to excessive heat the pathogenic microorganisms multiplies in large number in the intestine. They make the stool dry, decomposed & produce foul smelling gases resulting in *Seedhabethy.*

DYSENTERY :

It is the acute inflammation of large intestine characterized by diarrhoea with blood and mucus in the stools. The bacteria causing dysentery include Escherichia coli, Staphylococcus, Enterococcus, Klebsiella, Proteus vulgaris. Risk factors include contamination of food and water due to poor sanitation.

Pathogenesis :

Infection occurs by ingestion. Incubation period is about 2 – 7 days. The bacilli infect epithelial cells of intestinal villi in the large intestine and multiply inside them. Bacteriemia may occur in severe infections.

Signs and Symptoms :

- Nausea and vomiting
- Change in the colour of the stools
- Mucus, pus, blood, fat in stools
- Dehydration
- Abdominal pain and cramps
- Bloating
- Generalised body weakness and tiredness

Complications : If untreated

- Dehydration
- Convulsions
- Sepsis
- Rectal prolapse

SEEDHABETHY CHOORANAM :

Source : *Kannusamy parambarai vaithiyam.*

Ingredients :

S.No	Name of the herb	Common Tamil and English Name	Suvai	Thanmai	Pirivu
1	*Syzygium aromaticum Myrtaceae*	*Kraambu, Clove*	*Kaarpu*	*Veppam*	*Kaarpu*
2	*Cinnamomum verum Lauracae*	*Lavangappattai, Bark of cinnamon*	*Kaarpu, Inippu*	*Thatpam*	*Inippu*
3	*Terminalia chebula Combretaceae*	*Kadukkai, Chebulic myrobalan*	*Thuvarppu*	*Veppam*	*Inippu*

Kaarpu: Pungent; *Inippu :* Sweet; *Thuvarppu :* Astringent; *Veppam :* Heat; *Thatpam :* Cold

S. No	Name	Parts used	Action	Phytoconstituents	Medicinal uses
1	*Syzygium aromaticum*	Flower bud	Antispasmodic Carminative Stomachic	Eugenol, Eugenyl acetate, Thymol, Biflorin Gallic acid,	Dysentery, Loss of appetite Tooth ache, Vomiting.
2	*Cinnamomum verum*	Bark	Stimulant Carminative Aphrodisiac	Phenols, Tannins, Saponins, Terpenoids	*Iraippu, Rathakaduppu, Vayirukaduppu, Gunmam*
3	*Terminalia chebula*	Flower	Stomachic Tonic	Gallic acid, Chebulic acid, Ellagic acid.	Diarrhoea, Vomiting Hiccups Sore throat

Preparation :

Drugs are purified as per SOP Procedure. This preparation was done based on the evidences on the Siddha literature.

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. 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-Killiani 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.

Table: Qualitative Phytochemical analysis of plant samples

TEST NAME	Aqueous SBC
Alkaloid	Absent
Glycoside	Absent
Tannin	Present
Steroids	Absent
Saponin	Present

Quantitative Estimation of Tannins: (Robert, E.B. 1971. Agro.J.63, p.511)

1ml of the extract was mixed with 5ml of vanillin hydrochloride reagent (mix equal volumes of 8% HCL in methanol and 4% vanillin in methanol). The mixed was allowed to stand for 20mins and measure the absorbance at 500nm. The standard graph was plotted for working standard catechin solution (0 to 250µg/µl).

Ref: *Robert, EB,* "Method for estimation of tannin in grain sorghum ", *Agro J* , vol. *63, 1971,p.511*; 10.

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: Qualitative estimation of Phytochemical constituents

SAMPLE : DYS	
Test	**Aqueous**
Tanin µg / ml	**140 ± 0.2**
Saponin µg / ml	**13 ± 0.3**

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 10-15 minutes. 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.**

$$Rf = \frac{2.8}{1.4} = 2$$

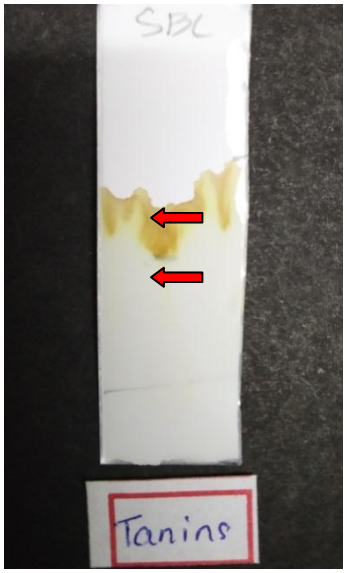


Figure: Thin layer Chromatography

ANTIMICROBIAL ACTIVITY PROCEDURE

Antibacterial Activity Procedure: **Dilution :** 0.1g in 1ml

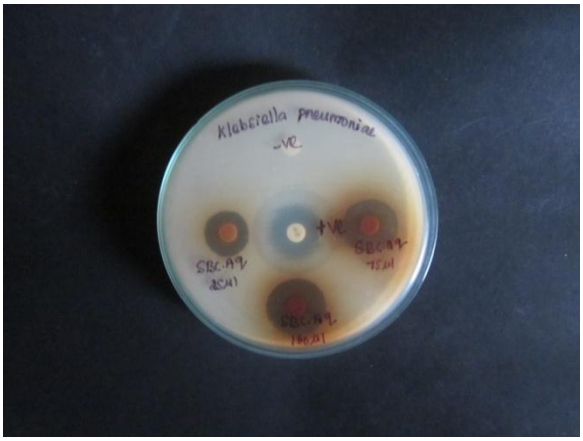
Test Organism: The test microorganisms used for antimicrobial analysis were purchased from Microbial Type Culture Collection and Gene Bank (***MTCC***) Chandigarh. The bacterial strains were maintained on Nutrient Agar (NA).

Nutrient Broth Preparation

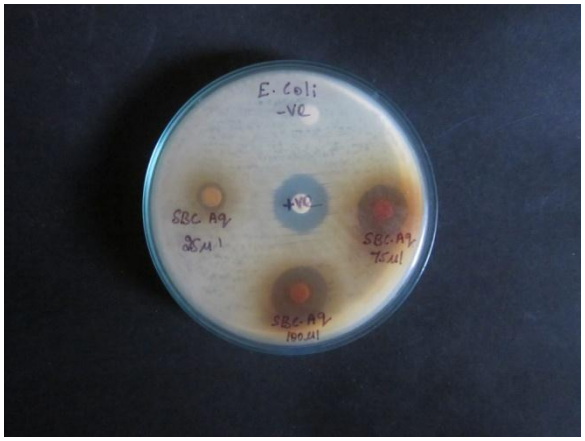
Pure culture from the plate were inoculated into Nutrient Agar plate and sub cultured at 37ºC for 24 h. Inoculum was prepared by aseptically adding the fresh culture into 2 ml of sterile 0.145 mol/L saline tube and the cell density was adjusted to 0.5 McFarland turbidity standard to yield a bacterial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

Antimicrobial Test:

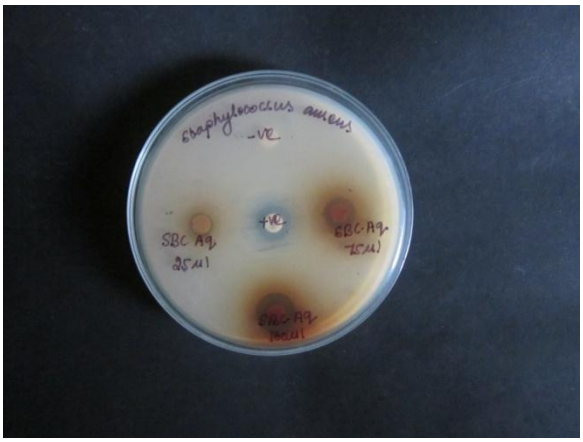
The medium was prepared by dissolving 38 g of Muller Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 Lbs pressure at 121^0C for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured petriplates (25 ml/plate) the plates were swabbed with Pathogenic Bacteria culture viz. 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)



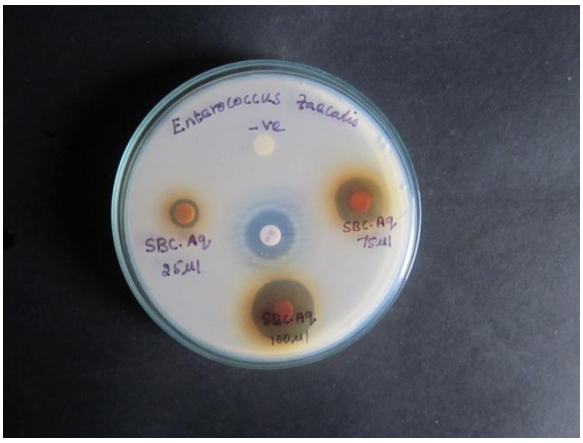
Zone of inhibition for Klebsiella pneumoniae



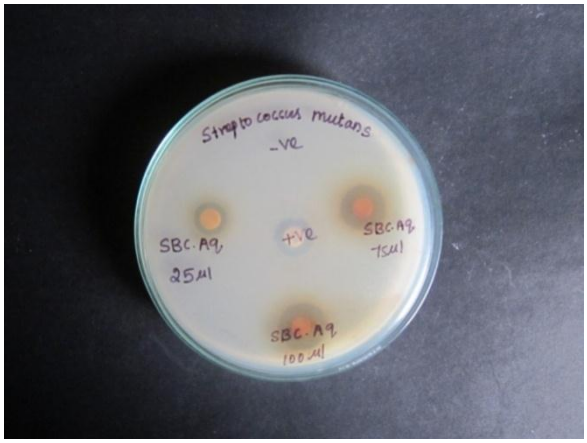
Zone of inhibition for E.coli



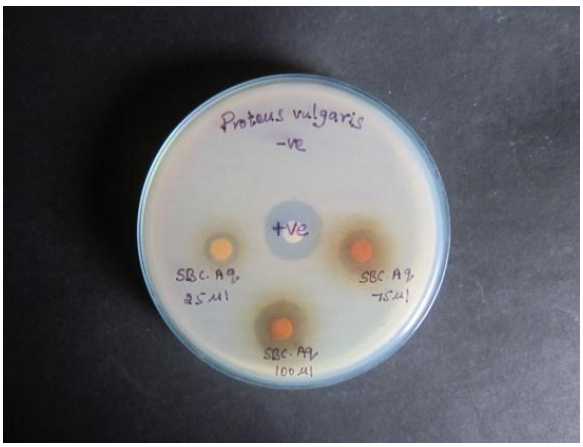
Zone of inhibition for Satphylococcus aureus



Zone of inhibition for Enterococcus



Zoneof inhibition for Staphylococcus



Zone of inbition for Proteus vulgaris

ANTIMICROBIAL RESULTS

Strains	SBC.Aq 25 µl	PC	NC
Staphylococcus aureus (G+) MTCC916	**7**	**13**	**-**
Streptococcus mutans (G+)MTCC 439	**11**	**13**	**-**
Enterococcus (G+) MTCC 439	**10**	**17**	**-**
Escherichia coli (G-) MTCC 1671	**10**	**20**	**-**
Klebsiella pneumonia (G-) MTCC	**17**	**21**	**-**
Proteus vulgaris (G-) MTCC 426	**11**	**20**	**-**

Result : Maximum Zone (17 mm) Observed in Klebsiella pneumoniae

Keys

PC (Bacterria) - ***Positive control (Streptomycin- S 25)***

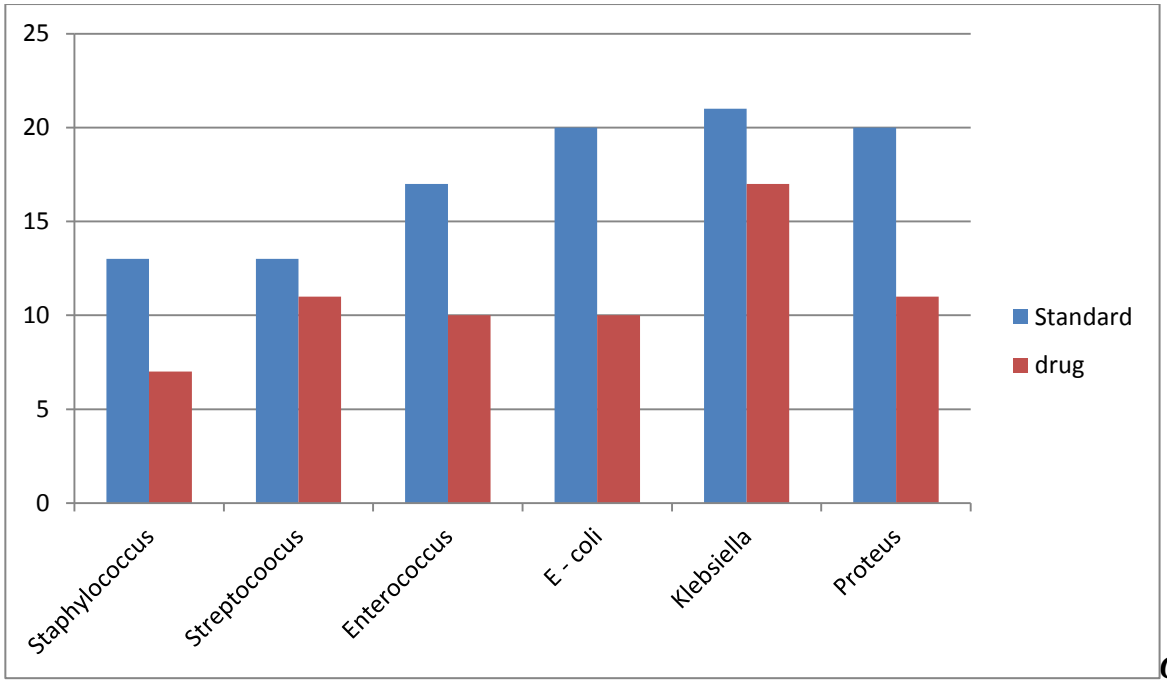
NC - ***Negative (plain disc)***

- - ***No Zone***

Mm - ***Millimetre***

G+ - ***Gram Positive organism***

G- - ***Gram Negative organism***



MIC Procedure

Two fold dilutions of the antibiotic solution in Mueller Hinton broth were prepared and describe below: Ten sterile tubes were placed in a rack and were labeled each 1 through 8 and last 9th labeled as antibiotic control and 10th was labeled as growth control. 10 ml of Mueller Hinton broth was added in each test tube(1to 8) 2mg of Sample was added to test tube no 1, 1mg of Sample was added to test tube no 2, 0.5 mg of Sample was added to test tube no 3, 0.25 mg of Sample was added to test tube no 4 , 0.12 mg of Sample was added to test tube no 5, 0.06mg of Sample was added to test tube no 6 , 0.03 mg of Sample was added to test tube no 7 and 0.01 mg of Sample was added to test tube no 8. The 9th received no antimicrobial agent and was served as a growth control. 10^{th} labeled test tube has only antimicrobial agent was served as a positive control. Each tube was inoculated (including the growth control except antibiotic control) with 0.1 ml of the culture of respective organism. The tubes were incubated at 37^0C for 24 hours. The tubes were examined for growth and were determined the MIC of tested antibiotics, which is bacteriostatic for the test organism. The tubes were examined for visible growth (cloudy) and was recorded growth as (+) and no growth as (-).

Minimum Inhibitory Concentration (MIC)

MIC (µg/ml) *Klebsiella pneumonia*	
Sample Concentration	
2.0	-
1.0	-
0.5	-
0.25	-
0.12	-
0.06	+
0.03	+
0.01	+
PC	+
NC	-

Result: Minimum Inhibitory Concentration is 0.12

Keys

\+ = Growth

\- = No Growth

PC = Bacteria only

NC = Sample only

DISCUSSION

Phytochemical Sceening revealed the presence of tannin and saponin. Several Studies Showed that antidysenteric and antidiarrhoeal properties of medicinal plants were due to tannin. Also the Antimicrobial Studies showed that there is a marked sensitivity against the bacteria Escherichia coli, Staphylococcus, Enterococcus, Klebsiella, Proteus vulgaris which are responsible for causing diarrhoea. Klebsiella pneumoniae is more sensitive to the trial drug. The Minimum inhibitory Concentration is 0.12 for Klebsiella pnemoniae. It can be recommended for dysentery and diarrhoeal diseases.

CONCLUSION

Thus we conclude that the *Seedhabethy Chooranam* is an effective medicine which can be used in the treatment of diarrhoea and dysentery and it has antimicrobial activity. This is only a

preliminary Study. Our work is done to explore the herbal, non invasive drug for dysentery and diarrhoea

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