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ANTIMICROBIAL AND PHYTOCHEMICAL ANALYSIS OF *KADHAGAADHI CHOORANAM*

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ABSTRACT

Purpose

Siddha medicine is one of the most ancient medicine systems of India. The word Siddha means established truth. Fundamental principles of Siddha include theories of five elements *(Aimbootham),* three humors *(Mukkuttram).* Eight fold diagnostic method *(Envagai thervukal)* is used to identify the etiology, diagnosis and prognosis and also to choose the treatment modality. This study intends to suggest the Siddha formulation of *kadhagaadhi chooranam* used for the treatment of Urinary Tract Infection, Respiratory disease and *Bramiyam (Gonorrhoea).*

Objective

This work is done to find out the efficacy of the test drug against *Bramiyam* by determining the antimicrobial sensitivity, thin layer chromatography (TLC), Minimum Inhibitory Concentration (MIC) and Qualitative and quantitative Phytochemical estimation.

Methodology

The *Kadhagaadhi chooranam* referenced from *"Agasthiyar Attavanai vaagadam",* is primarily indicated for the management of *Bramiyam* (Gonorrhorea). The test drug was prepared againt UTI. The test drug was subjected to antimicrobial assay, phytochemical analysis, Thin Layer Chromatography (TLC) and Minimum Inhibitory Concentration(MIC).

Result

The *Kadhagaadhi chooranam* is sensitive to *E-coli, Streptococcus mutans, Bascillus subtilis, Klebsiella pneumonia, Proteus vulagaris.* The preliminary phytochemical shows the presence of phenol. MIC shows the 0.5 The concentration of the bacterial growth.

Key words

Kadhagaadhi chooranam, antimicrobial analysis, phytochemicals, TLC, MIC, Siddha medicine.

INTRODUCTION

Siddha is the oldest healing system of medicine and it has fundamental aspect of drug formulation

A urinary tract infection (UTI) is an infection in any part of urinary system. Most infection involve the lower part of urinary tract. Women are greater risk of developing a UTI than are men. However, serious consequences canoccure if a UTI spreads to your kidneys. UTI symptoms are urgency to urination burning sensation when urinating, cloudy urine, bloody urine.

Gonorrhoea is the sexual transmitted disease sometimes referred to as "the clap". It is caused by the bacteria "*Neisseria gonorrhea"*. It is more complicated in women than male. It spreads by having unprotected vaginal, anal or oral sex with someone who has gonorrhoea or from mother to child during child birth. The symptoms usually occur within two to fourteen days after the exposure.

Microorganisms are the causative agents of almost all kind of acute and chronic disease. Plant based antibacterial have enormous therapeutics potential. In this work, we have selected the *Kadhagaadhi chooranam* referenced from Agasthiyar Attavanai vaagadam which is mentioned for Gonorrhoea. The main ingredient of the formulation is the seeds of *Strychnos potatorum* which consists of the chemical constituents such as strychnine, β-sitosterol, oleanolic acid, and brucine which are well know to have antibacterial activities. Based on this, phytochemical estimation was done to the test drug. *Kadhagaadhi chooranam* is tested against gram positive *Staphylococcus aureus, Bacillus subtilis, Streptococcus mutans* and gram negative bacteria *E-coli, Klebsiella pneumonia, Proteus vulgaris.*

MATERIALS AND METHODS

Collection

The Strychnos potatorum seeds (*Thetran vidhai),* Asparagus racemosus root *(Thannervittan kizhangu ver),* Cuminum cyminum fruits *(seeragam vidhai) and* sugar *(sarkarai)* were collected from the traditional raw drug store, Tirunelveli, Tamilnadu.

Authentication

The raw drugs were authenticated by faculties of Department of Botany and Department of Gunapadam, GSMC, Palayamkottai, Tirunelveli. The polyherb preparation *Kadhagaadhi chooranam* was referenced from *"Agasthiyar Attavanai Vaagadam"* .

Purification

- The *Strychnos potatorum* seeds purified by soaked in the milk for 24 hour. After 24 hour, the seed had been washed with pure water and dried in shade.
- The *Asparagus racemosus* root was collected and dried in shade.
- The *Cuminum cyminum* fruits were to be roasted.

These are the purification process to be carried out.

Preparation

Table no:1 List of ingredients of *Kadhagaadhi chooranam*.

Herbs	**Part used**	**Quantity**	**Suvai**	**Thanmai**	**Pirivu**
Strychnos potatorum	Root	2 gram	Kaippu(Bitter)	Veppam	Karppu(Pungent)
Asparagus racemosus	Seed	17.5gram(*Arai palam)*	Inippu(Sweet)	Thatpam	Inippu(Sweet)
Cuminum cyminum	Fruit	17.5gram(*Arai palam)*	Karppu,Inippu (Pungent, sweet)	Thatpam	Inippu(Sweet)
Sacchasum officinarum(Sugar)	Sugar	17.5gram(*Arai palam)*	Inippu(Sweet)	Seetham	Inippu(Sweet)

All the raw drugs were powdered separately, mixed and subject to *vasthirakaayam* Process (Powder is sieved by using cloth) where the powdered chooranam becomes more finer in this process.

Then the fine powder was tested for antimicrobial analysis, phytochemical analysis, TLC(Thin Layer Chromatography) and MIC(Minimum Inhibitory Concentration).

MICROBIAL ANALYSIS

Dilution : 0.1g in 1ml

Test Organism:

The test microorganisms used for antimicrobial analysis *Microorganism Name* 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. Microorganism name 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). The zone of inhibition for each microorganism in the following table no 3. And following figures 1,2. **Table 2. Zone of inhibition produced in the test Bacterial Strain Name**

Samples	Strains					
	Staphylococcus aureus (G+) MTCC 916	**Bacillus subtilis**G+)	**E- coli** (G-) MTCC 1671	**Klebsiella pneumonia** (G-) MTCC 503	**Proteus vulgaris**(G-)	**Streptococcus mutans (G+)** MTCC 497
A.Aq 25 µl	10	10	12	11	10	9
A.Aq 50 µl	11	11	16	13	11	11
A.Aq 75 µl	12	17	18	14	13	12
A.Aq 100 µl	14	20	19	16	17	14
PC	20	24	20	20	23	15
NC	-	-	-	-	-	-

Keys

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

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

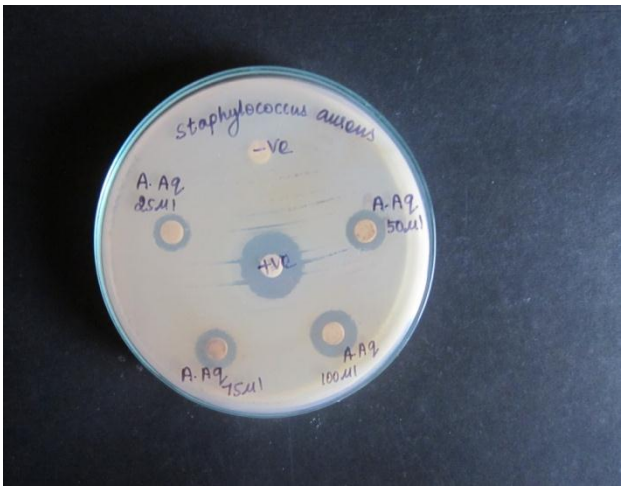
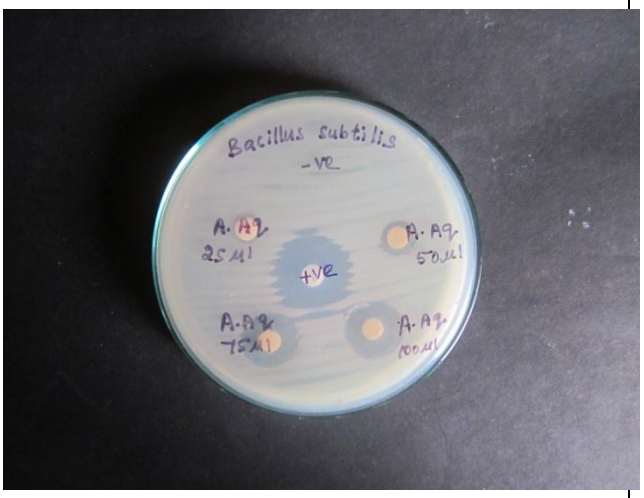
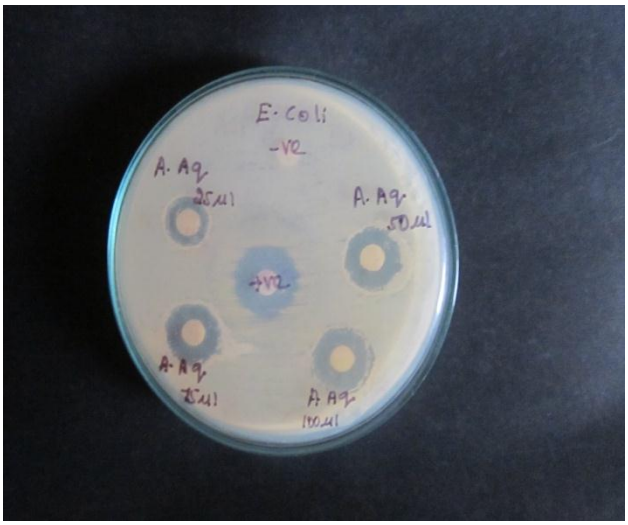
- **-** ***No Zone***

Mm **-** ***Millimetre***

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

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

Figure no.2-Zone of inhibition of test drug against tested organisms

Zone of inhibition for Staphylococcus aureus(G+)	**Zone of inhibition for Bacillus subtilis(G+)**
	
Zone of inhibition for streptococcus mutans(G+)	**Zone of inhibition for E-coli(G-)**
	

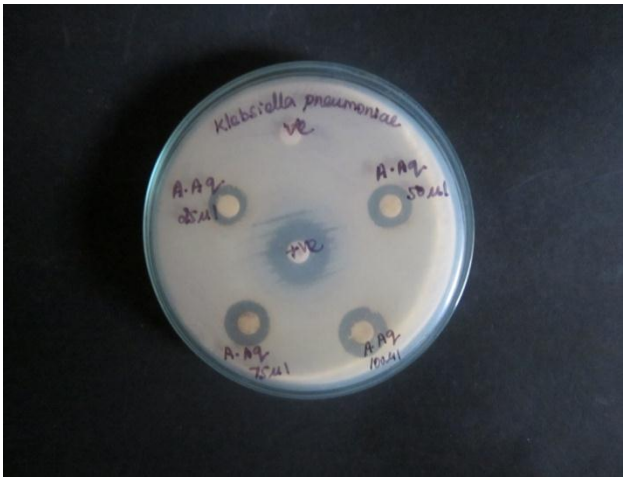
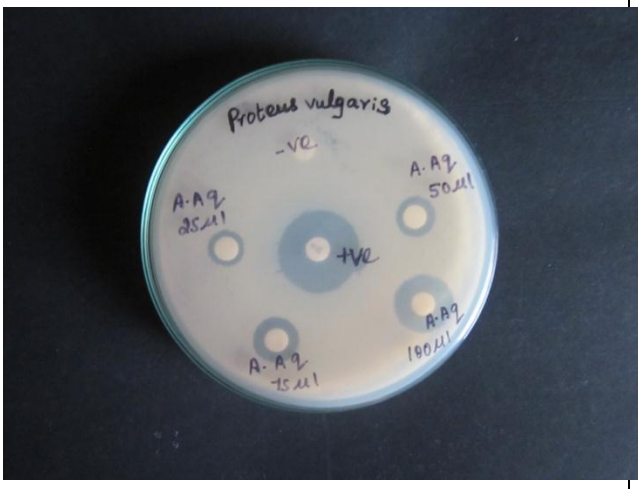
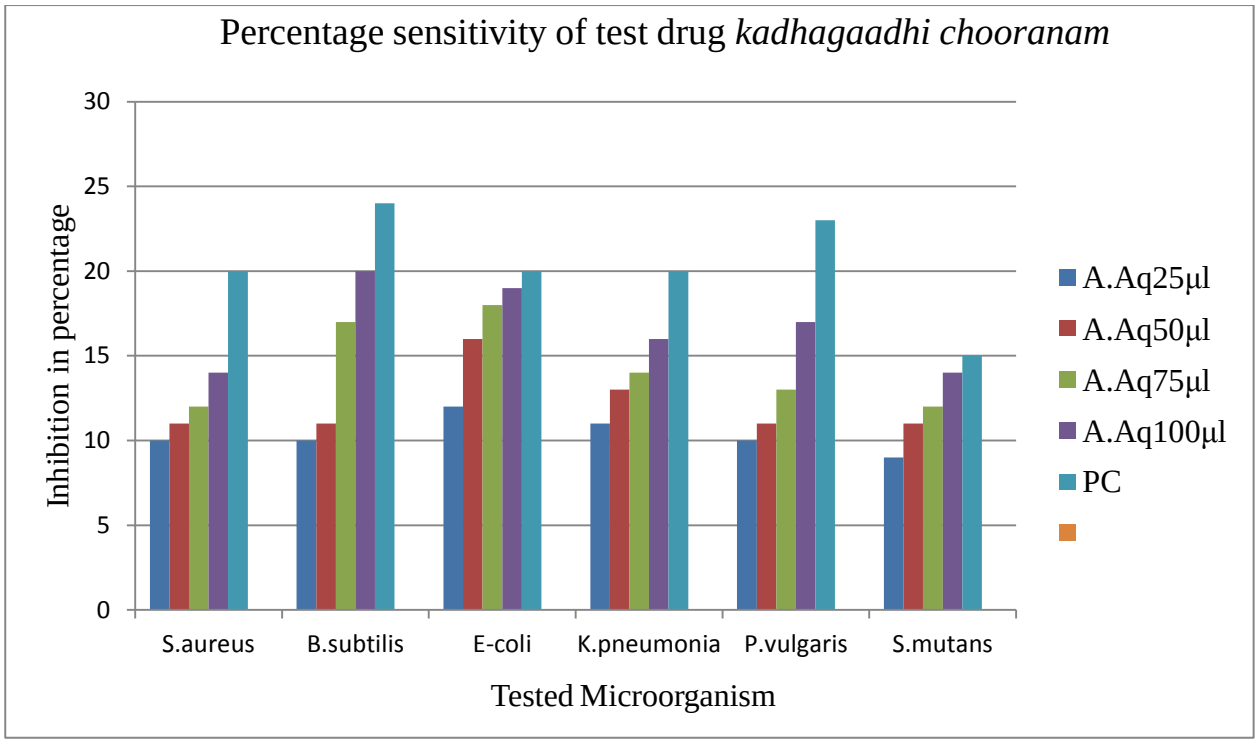
Zone of inhibition for Klebsiella pneumonia(G-)	Zone of inhibition for Proteus vulgaris(G-)
	

Figure 3- Percentage representation of Microbial sensitivity of drug



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.

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FLAVONOIDS

Shinoda Test: (Kokate, 1994)

To the extract, 5 ml of 95% ethanol and few drops of concentrated hydrochloric acid was added. To this solution 0.5 gm of magnesium turnings were added. Pink colouration indicated the presence of flavanoids.

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.

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PHENOL

Ferric chloride test: (Mukherjee, 2002)

The extract was diluted to 5 ml with distilled water. To that a few drop of neutral 5% ferric chloride solution was added. A dark green colour indicates the presences of phenolic compounds.

TANNINS

Lead Acetate Test: (Mukherjee, 2002)

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

TERPENOIDS (Horbone, 1984)

To the 5ml test solution 2ml chloroform was added with few drops of conc. Sulphuric acid (3ml) at the side of the test tube. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

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.

SAPONINS

Foam Test: (Ansari, 2006)

Sample extract was added with distilled water and shaken vigorously. Observe the stable foam formation.

QUINONES

1ml sample was mixed with 1ml of concentrated sulfuric acid and stand for few minutes. Observe the formation red color indicates the presence of quinines in the sample.

.**ANTHOCYANIN**

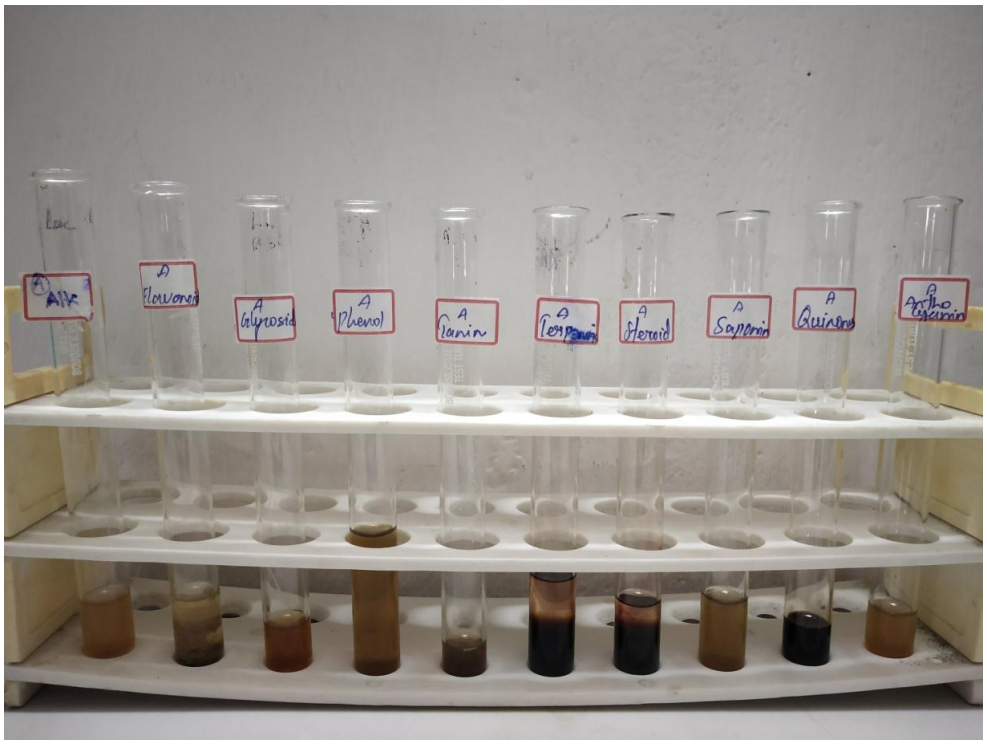
2ml of 2N Hcl was mixed with 2ml of test sample. Observe the pink red color turns into blue violet color indicates the presence of anthocyanin in sample.

PHYTOCHEMICAL RESULTS

Table 3: Qualitative Phytochemical analysis of plant samples

TEST NAME	Aqueous A
Alkaloids	Absent
Flavonoids	Absent
Glycosides	Absent
Phenol	Present
Tannin	Absent
Terpenoids	Absent
Steroids	Absent
Saponin	Absent
Quinones	Absent
Anthocynains	Absent

Figures 4: Qualitative Phytochemical analysis of plant samples



PHYTOCHEMICAL ANALYSIS - Quantitative

Quantitative Estimation of Phenolic Compounds: (Evans, 1996)

- The total phenolics content in different solvent extracts was determined with the Folin- Ciocalteu's reagent (FCR)**.** In the procedure, different concentrations of the extracts were mixed with 0.4 ml FCR (diluted 1:10 v/v).
- After 5 min 4 ml of sodium carbonate solution was added. The final volume of the tubes were made upto 10 ml with distilled water and allowed to stand for 90 min at room temperature.
- Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using Gallic acid solutions as standard (0 to 250µg/µl)

QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS

Table 4: Qualitative estimation of Phytochemical constituents

SAMPLE : DYS	
Test	**Aqueous**
Phenol µg / ml	**25.62 ± 0.3**

MINIMUM INHIBITORY CONCENTRATION

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 (2µg/ml) *E.coli*	
Sample Concentration	MIC
2.0	-
1.0	-
0.5	-
0.25	+
0.12	+
0.06	+
0.03	+
0.01	+
PC	+
NC	-

Result

0.5 Concentration is Minimum inhibitory of the bacterial growth of E-coli.

Keys

\+ = Growth

\- = No Growth

PC = Bacteria only

NC = Sample only

THIN LAYER CHROMATOGRAPHY ANALYSIS

Mobile phase was prepared by dissolving the Methanol and water at 6:3 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,

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

RESULT

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

$Rf = \frac{3.7}{3.4}$ =1.1

Figure 5: Thin layer Chromatography

CONCLUSION

This study shows that the trial drug is more sensitive to E-coli, Bacillus subtilis, proteus vulgaris. It is sensitive to Klebsiella pneumonia. In phytochemical studies, phenol is present. In MIC studies 0.5 concentration is the Minimum inhibitory the bacterial growth. In TLC studies, based on the Rf value of 1.1, test sample shows the presence of phenolic components. The test drug *Kadhagaadhi chooranam* is efficiently acts against E-coli. As mentioned in our Siddha literature, indicated for gonorrhoea. Further, studies will be conducted against gonorrhoea, as a support to prove the efficacy of the test drug *Kadhagaadhi chooranam*.

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