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A PRELIMINARY STUDY ON *DHUVATHI CHOORANAM* FOR THE TREATMENT OF *RATHA PREMEGAM*

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ABSTRACT

Siddha system, being largely therapeutic in nature, is one of the ancient system of Indian medicine. The term siddha means perfection or achievement. In our system, many diseases have been explained along with treatment by herbs, metals and minerals that are available in the nature. They can be cured by internal and external medicines. In this paper, we studied about *Ratha premegam* (infectious venereal diseases associated with hematuria). In siddha system of medicine, there are many formulations for the management of this condition. Here, we aim at proving the efficacy of *Dhuvadhi chooranam* by scientifically evaluating its phytochemicals, Thin layer chromatography, and Antibacterial activity. The results of phytochemical analysis shown the presence of tannins in it. When it was subjected to antimicrobial testing it is found to be effective against Escherichia coli, Staphylococcus aureus and Streptococcus mutans which are effective in hematuria associated with infectious venereal diseases. Hence Dhuvadhi chooranam could be better medicine for this condition.

KEY WORDS

Dhuvadhi chooranam, R*atha pramegam*, hematuria, venereal diseases.

INTRODUCTION

Ratha pramegam in siddha is the condition in which blood is seen in urine due to infectious venereal diseases. Blood or red blood cells can enter and mix with urine through urinary system, reproductive system and integumentary system. Urinary causes are generally divided into glomerular and non glomerular causes. And here we speak about presence of blood in urine due to sexually transmitted diseases (*megam)* and its treatment by Dhuvadhi chooranam.

As the prevalence of this condition is known to increase, the management becomes the need of the hour. Nowadays people are turning towards siddha system of medicine due to its efficacy.

RATHA PREMEGAM

In Siddha, it is the urinary disease marked by the discharge of blood or bloody urine due to rupture of small blood vessels. It can be compared with *muyar kuruthi neer (aarga megam),* which is defined as reddish colour in urine similar to that of rabbit's blood.

DHUVATHI CHOORANAM

Dhuvathi chooranam has its reference from the Siddha literature- *Kannusamiyam vaidhiya sekaram.* The ingredients are:

INGREDIENTS	ACTION	SUVAI
Padikaram (Potash Alum)	Astringent, styptic, antiseptic, caustic, anti spasmodic.	Sour, sweet, astringent
Val milagu (Piper cubeba)	Stimulant, carminative, diuretic, expectorant.	Pungent

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.

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.

Kokate, C. K. 1994. Practical Pharmacognosy, 4th edition, Vallabh Prakashan, New Delhi. 4 - 29.

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.

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.

Horbone, J.B., In: Phytochemical methods, 2nd edition. Chapman and Hall, New York,1984.

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.

Figures: Qualitative Phytochemical analysis of plant samples.

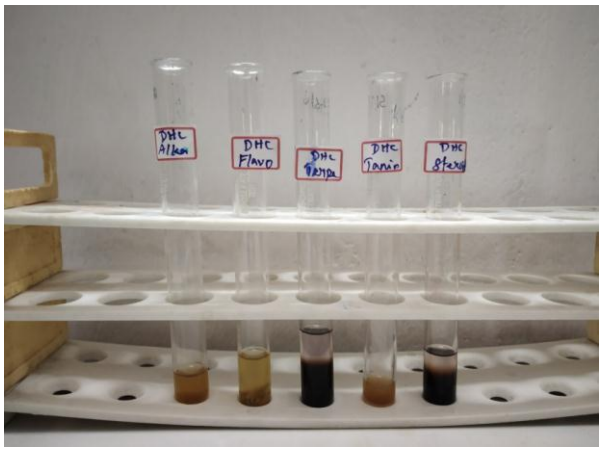


Table: Qualitative Phytochemical analysis of plant samples

TEST NAME	Aqueous DHC
Alkaloid	Absent
Flavonoids	Absent
Tannin	Present
Terpenoiods	Absent
Steroids	Absent

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 mixture 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.

Table: Qualitative estimation of Phytochemical constituents

SAMPLE : NCC	
Test	**Aqueous**
Tanin 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.**

$Rf = \frac{2}{1.5}$ =1.8

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 toyield 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)

ANTIMICROBIAL RESULTS

Samples	Strains		
	Staphylococcus aureus (G+) MTCC 916	**Streptococcus mutans** (G+) MTCC 497	**E- coli** (G-) MTCC 1671
SP.Aq 25 µl	8	10	9
SP . Aq 50 µl	11	13	11
SP .Aq 75 µl	15	15	16
SP .Aq 100 µl	18	17	19
PC	17	18	**19**
NC	-	-	-

Maximum Zone (19) observed in E.coli

Keys

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

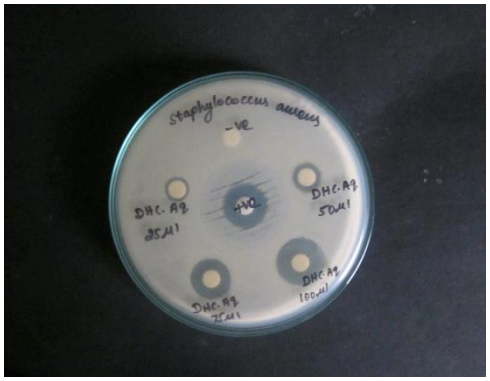
NC - Negative (plain disc)

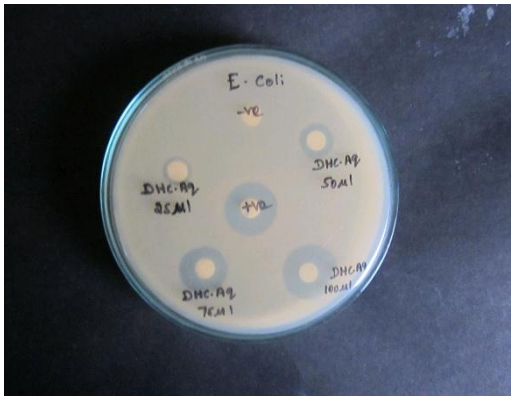
- - No Zone

Mm - ***Millimetre***

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

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





RESULTS AND DISCUSSION:

The above phytochemical study shows the presence of tannin in Dhuvathi chooranam. Many researches have been done stating that tannins are very good in the management of venereal diseases.

Further anti microbial assay has been best in positive control of the samples for Streptococcus , Staphylococcus and E.coli having zones around 18 , 17 and 19 respectively. Therefore this Dhuvadhi chooranam will be effective in the management of *ratha premegam* and helps to reduce the prevalence level of blood in the urine.

CONCLUSION:

The presence of tannins through phytochemical study and antibacterial test against oragnism Streptococcus , Staphylococcus and E.coli with best zone result in our medicine helps in treating

venereal diseases through haematuria which in turn reduces the prevalence of the *ratha premegam* . This is our preliminary research for this medicine further research will be done in our upcoming days.

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