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PRELIMINARY PHYTOCHEMICL AND ANTI MICROBIAL STUDIES ON *LASUNA THAABITHA CHOORANAM*- A SIDDHA DRUG.

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ABSTRACT

Lasuna Thaabitham in siddha (Tonsillitis) is an inflammation of the two oval shaped pads of tissue at the back of the throat. It is mostly caused by bacteria or viral infection. This condition is more prevalent in children. Although self limiting in nature, it is treated by antibiotics and anti inflammatory drugs to avoid clinical manifestations and complications. Chronic condition can be treated by tonsillectomy (surgical removal of the tonsils). In siddha system of medicine, there are many single herbs as well as polyherbal formulations that are effective in the management of *lasuna thaabitham*. The role of aromatic herbs such as *Val milagu* (*Piper cubeba*), Sittrarathai (*Alpinia officinarum*), *Jathikaai* (*Myristica fragrans*),etc. Are note worthy are effective in the treatment of *lasuna thaabitham*. This paper is intended to study the preparation of *Lasuna thaabitha chooranam* and also to scientifically validate its efficacy through preliminary anti-microbial and phytochemical analysis of the aforesaid preparation.

KEYWORDS

Lasuna thaabitha chooranam, Tonsillitis, Siddha system, Anti-microbial Study.

INTRODUCTION

Siddha system is the oldest system of medicine being practiced in South India for centuries. A great attention towards our system is being shown nowadays due to the potency of curing diseases with herbs, metals, minerals and animal sources. It is the concept of developing a healthy soul in a healthy body. This paper aims at the cure of *lasuna thaabitham* (Tonsillitis). Tonsillitis is most commonly caused by viral or bacterial infection. Symptoms may include sore throat, and fever. The overwhelming majority of people recover completely with or without medication. In 40%, symptoms will resolve in three days, and within one week in 85% of people, regardless of whether streptococcal infection is present or not.

LASUNA THAABITHAM IN *SIDDHA*

Lasunam+Thaabitham, where Lasunam means Tonsils and *Thaabitham* means inflammation. It occurs due to the elevation in *KABHAM*. The synonyms are *Annakku Thooru, Unnakku Alarchi, etc.*

TONSILLITIS

Tonsil + itis = inflammation of the tonsil caused by bacterial and viral infection particularly, streptococcus which includes the following signs and symptoms:

- Difficulty in breathing
- Disrupted breathing during sleep (obstructive sleep apnoea).
- Infection that spreads deep into surrounding tissue (tonsillar cellulitis).
- An Infection that results in a collection of pus behind a tonsil.

If tonsillitis caused by group A streptococcus or another strain of streptococcal bacteria and isn't treated or if antibiotic treatment is incomplete, then the child is at the risk of developing complications such as

- Rheumatic fever
- Post streptococcal glomerulonephritis (Acute)

LASUNA THAABITHA CHOORANAM

Lasuna Thaabitha chooranam has its reference in the siddha text (*Sarabendhirar Vaithiya Muraigal*). The main aromatic ingredients are:

BOTANICAL NAME	ALKALOIDS	USES IN SIDDHA
Piper cubeba (*Vaal milagu*)	Piperine, pipernonaline, piperidine, piperettine.	Cold, cough, Fever.
Alpinia officinarum (*Sittrarathai*)	Galagin, galangal, essential oil	Cough, fever, Asthma, expectorant action.
Myristica fragrans (*Jaathikaai*)	Saponin, Tannin, anthraquinone, cardiac glycoside	Respiratory disorders, asthma, cough.

The drug was subjected to both qualitative and quantitative phytochemical analysis.

QUALITATIVE ANALYSIS

PROCEDURE

Test for Carbohydrates - Benedict's test (Brain & Turner, 1975)

To 0.5 ml of test drug about 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic c colored precipitate indicates the presence of sugar.

Glycosides (Ansari, 2006)

Keller-Killiani Test: 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.

Steroids (IP, 1996)

Salkowski Test: 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.

Alkaloids (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.

Flavanoids (Kokate, 1994)

Shinoda Test:

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.

Tannins (Mukherjee, 2002)

Lead Acetate Test: On addition of lead acetate solution to the extract white precipitate appeared.

Saponin (Ansari, 2006)

Foam Test: Drug extract was shaken vigorously with water. No persistent foam was formed.

Protein (Ansari, 2006)

Biuret test With 3 ml of test solution, few drops of 4% NaOH and 1% $CuSO_4$ solution were added. The tubes were observed for violet or pink colour formation.

Phenol (Mukherjee, 2002)

Ferric chloride test

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 color indicates the presences of phenolic compounds.

Test for Glycosides (Horbone, 1984)

0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

Test for Triterepnoids (Horbone, 1984)

To the 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.

RESULT OF QUALITATIVE ANALYSIS:

TEST NAME	RESULT
Carbohydrate	Present
Protein	Absent
Alkaloid	Absent
Flavanoid	Present
Glycoside	Absent
Steroid	Absent
Saponin	Present
Phenol	Absent
Tannin	Present
Terpenoid	Present

QUANTITATIVE ANALYSIS:

PROCEDURE

Quantitative Estimation of carbohydrate

The total sugar content was estimated by Anthrone method (Roe, 1955). A known amount of the sample was taken, ground well with 80% ethanol and was centrifuged at 4000 rpm. From the supernatant, 0.5 ml was taken and 5 ml of anthrone reagent was added. The tubes were kept in a boiling water bath for 15 min. After that, they were kept in a dark room for another 15 minutes. The colour intensity developed was read in a spectrophotometer at 650 nm.

Quantitative Estimation of flavanoids: (Evans, 1996)

Total flavonoid content was determined by Aluminium chloride method using catechin as a standard. 1ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). After 5 min 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as catechin equivalents (mg catechin/g dried extract).

Quantitative Estimation of Saponins: (Evans, 1996)

Methanolic and water 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 600c for 10min, absorbance was measured at 544nm against reagent blank. Diosgeninis used as a standard material and compared the assay with Diosgenin equivalents.

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).

Total terpenoid determination

Total terpenoid content was determined by the method of Ghorai et al (2012)17. To 1 mL of the plant extract, 3 mL of chloroform was added. The sample mixture was thoroughly vortexed and left for 3 min and then 200 µl of concentrated sulfuric acid (H2SO4) was added. Then it was incubated at room temperature for 1.5h-2h in dark condition and during incubation a reddish brown precipitate was formed. Then carefully and gently, all supernatant of reaction mixture was decanted without disturbing the precipitation. 3 mL of 95% (v/v) methanol was added vortexed thoroughly until all the precipitation dissolve in methanol completely. The absorbance was read at 538 nm using UV/visible spectrophotometer. The total terpenoid content was calculated by calibration curve of Linalool and the results were expressed as Linalool equivalent (mg/g).

RESULT OF QUANTITATIVE ANALYSIS

TEST NAME	RESULT	BIOLOGICAL SIGNIFICANCE
Carbohydrate (mg/ g)	39	Effective in treating cancer and coeliac disease
Flavanoid (mg/ g)	50	Has wound healing and anti inflammatory property.It also treats cardiovascular diseases, cancer.
Saponin (mg/ g)	2	Anti cancer, anti viral property and treats polycystic kidney
Tannin (mg/ g)	35	Oesophageal cancer, diseases involving gastro intestinal tract
Terpenoid (mg/ g)	130	Anti allergic, anti viral, bactericidal and fungicidal property.

ANTIMICROBIAL ACTIVITY

PROCEDURE

Antibacterial Activity Procedure:

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) and fungi on Sabouraud Dextrose Agar (SDA).

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 33.9 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^{0}C for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured onto 100 mm 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.

ANTIMICROBIAL RESULTS

Sample Code	Bacteria Strains (mm)				
	Staphylococcus aureus (G+)	*Bacillus subtilis (G+)*	*Klebsiella pneumonia (G-)*	*E.coli (G-)*	Proteus vulgaris (G-)
LTC5	-	-	-	-	-
LTC10	-	-	-	8	7
LTC15	-	-	-	8	8
LTC20	7	9	9	9	10
LTC25	8	11	11	10	11
+ve	22	23	23	21	20
-ve	-	-	-	-	-

Keys

+ve - ***Positive (standard- streptomycin)***

-ve - ***No Zone (water)***

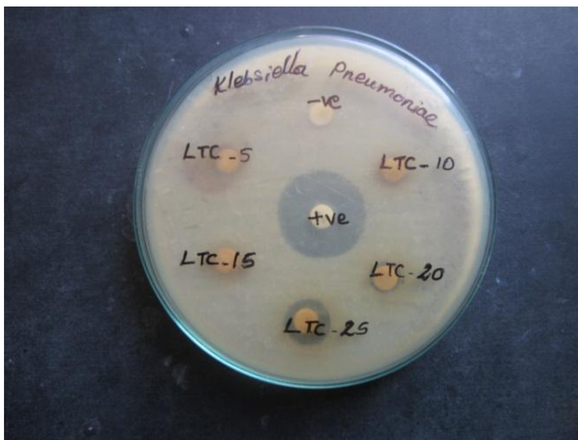
Mm - ***Millimetre***

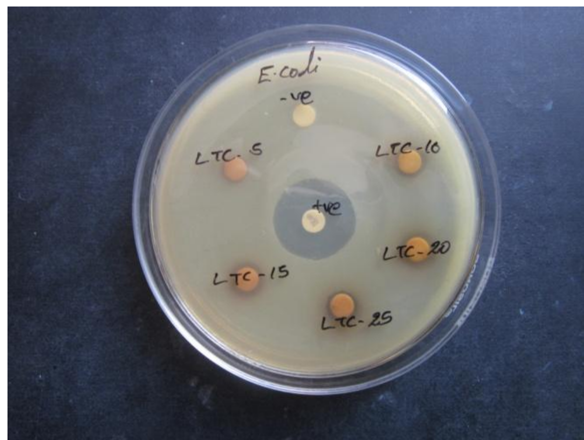
G+ - ***Gram Positive organism***

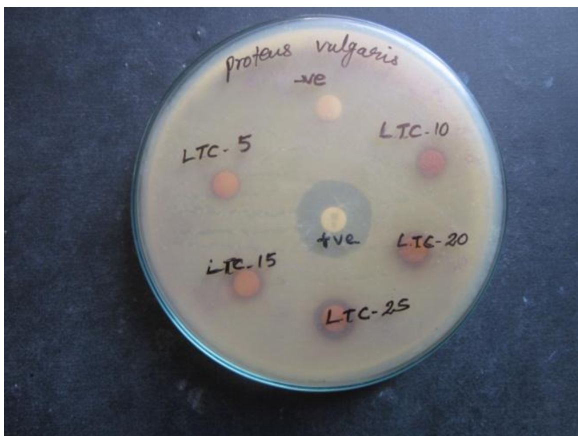
G- - ***Gram Negative organism***

Staphylococcus aureus
-ve
LTC-5
LTC-10
+ve
LTC-15
LTC-20
LTC-25

Bacillus subtilis
-ve
LTC-5
LTC-10
+ve
LTC-15
LTC-20
LTC-25


Klebsiella Pneumoniae
-ve
LTC-5
LTC-10
+ve
LTC-15
LTC-20
LTC-25


E.coli
-ve
LTC-5
LTC-10
+ve
LTC-15
LTC-20
LTC-25


proteus vulgaris
-ve
LTC-5
LTC-10
+ve
LTC-15
LTC-20
LTC-25

DISCUSSION AND CONCLUSION

Evaluation of traditional medicine can be done by the proper documentation of the literary evidences and scientific validations through universally accepted parameters. In that way the authors have done the preliminary phytochemical and anti microbial screening of the trial drug, *Lasuna Thaabitha Chooranam*. From these results, it is clear that the preparation mentioned here proves to be an effective medicine which can be used in the management of *Lasuna Thaabitham*. This is only preliminary study with available infrastructure. More work is to be done to explore the efficacy of this safe, economical, herbal, non invasive drug for tonsillitis.

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