ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

ANTIMICROBIAL ACTION AND PRELIMINARY ANALYSIS OF *NAAYURUVI KUZHI THYLAM*

S. Esaivani[1]*, T.Kavitha[1], R.Susila[1], A.Usha[1], R.Thilagavathi[2]

*[1]Research Officer, Siddha, Siddha Central Research Institute, Arumbakkam, Chennai, Tamil Nadu, India.

[2]Research Officer Siddha, Siddha Clinical Research Unit, Bengaluru, Karnataka, India.

ABSTRACT

Naayuruvi Kuzhi thylam (NKT) is a Siddha single herbal formulation indicated in literature for the management of Asthma (Suvasa irumal ICD-DBA1.3)). Since asthma can accompany with other comorbid bacterial infections, the present study was focused to scientifically evaluate the antimicrobial action of *Naayuruvi kuzhi thylam* (NKT) against selected pathogens and also its chemical analysis. The antimicrobial action of *Naayuruvi kuzhi thylam* (NKT) was performed against selected bacterial and fungal pathogens such as *Escherichia coli, Streptococcus aureus, Salmonella typhi* and *Candida albicans. Naayuruvi kuzhi thylam* (NKT) was prepared and a concentration of 1000µg, 500µg, 250µg, 100µg of NKT was inoculated in the Agar well medium. The antibacterial activity of the given sample was determined by disc diffusion method on Muller Hinton agar (MHA) medium and the zone of inhibition was determined using the standard antibiotic Streptomycin 10µg as control. All the tested pathogens showed maximum zone of inhibition (8mm) at a concentration of 1000µg of NKT. The chemical analysis revealed the presence of phosphate, sulphide, Calcium, potassium, ammonium, alkaloid, starch and aminoacids. The results conclude that the study drug NKT possess intermediate antimicrobial activity against the selected pathogens.

KEYWORDS: *Naayuruvi Kuzhi thylam* (NKT), Traditional medicine, Herbal medicine, Antimicrobial activity, Chemical analysis.

INTRODUCTION

Siddha is one among the well known global system of medicine which is constantly playing an important role in providing health care to large section of population, especially in developing countries. WHO estimates that nearly 4.3 billion people or 80% of the global population rely on traditional medicine for their primary health care needs.[1] The traditional *Siddha* system of medicine has several herbal formulations that are increasingly utilized to treat a wide variety of diseases. *Naayuruvi Kuzhi thylam* is one such formulation that has been indicated in Siddha literature *Gunapaadam Mooligai Vaguppu* for the treatment of cough, Bronchial Asthma and other respiratory ailments (*Kapha* diseases),[2] but the knowledge about their mode of action is relatively deficient. In recent years there is an emerging interest regarding the pharmacological evaluation of various drugs used in traditional system of medicine. In order to achieve this object an attempt was made to assess the antihistaminic and bronchodilator activity as on *Naayuruvi Kuzhi thylam* for its antihistaminic and antiasthmatic actions.

MATERIALS AND METHODS

Preparation of *Naayuruvi kuzhi thylam*

The whole plant of Achyranthes aspera (*Naayuruvi*) was dried and soaked in Cow's urine for one day and again dried for a day. (*Kuzhithylam* Apparatus). For Pit-oil apparatus a pot with a few holes in the bottom was taken and thin wires were passed through these and bended so that they converge at a point a few inches away from the base. The upper ends were suitably secured. Then the pot was filled with dried plant of *Achyranthes aspera* and the oil is prepared by destructive distillation by placing a collecting vessel in the centre of a pit and supporting the pot on it and cow dung cakes were arranged around and above the pot in a regular manner from the top to bottom of the apparatus. The cow dung cakes were ignited and *Kuzhi thylam* (Pit-oil) was collected.

Solvent extraction

Samples were directly used for counter current solvent extraction process (Soxhlet extraction) in which acetone and ethyl acetate were used for extraction. The extracts were evaporated at

50°C until solvent layer evaporated completely. The extracts were weighed and yield calculated. They were then diluted in DMSO to achieve a concentration of 100mg/ml.

Preparation of Inoculum

Stock cultures were maintained at 4°C on slant of nutrient agar. Active cultures for experiments were prepared by transferring a loopfull of cells from the stock cultures to test tubes of nutrient broth for bacteria that were incubated at 24hrs at 37°C. The assay was performed by agar disc diffusion method.

Agar Disc Diffusion Method

Antibacterial activity of the given sample was determined by disc diffusion method on Muller Hinton agar (MHA) medium. The Muller Hinton Agar medium is poured in to the petri plate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistured with the bacterial suspension. The disc were placed in MHA plate and different concentration [1000µg, 500µg, 250µg, 100µg] of each samples were placed in the disc. The plates were incubated for 24 hrs, at 37°C. Then the microbial growth was determined by measuring the diameter of zone of inhibition.

Chemical Analysis of *Naayuruvi kuzhi thylam*

50ml *Naayuruvi kuzhi thylam*, sample is mixed with 5gm of sodium carbonate and taken in a 100ml beaker and 100ml distilled water is added. The solution is boiled for 10 minutes, cooled and then filtered. The filtrate is called sodium carbonate extract. This preparation was used for the qualitative analysis of acidic/ basic radicals and biochemical constituents in it.[3]

Test for acid radicals

1. Test for sulphate

a. 2ml of the above prepared extract is taken in a test tube. To this added 2ml of 4% ammonium oxalate solution.

b. 2ml of sodium carbonate extract is added with 2ml of dilute hydrochloric acid is until the effervescence ceases off. Then 2ml of Barium Chloride solution is added.

2. Test for chloride

2ml of the sodium carbonate extract is added with dilute nitric acid till the effervescence ceases. Then 2ml of Silver Nitrate solution is added.

3. Test for Phosphate

2ml of the extract is treated with 2ml of ammonium molybdate solution and 2ml of concentrated nitric acid.

4. Test for carbonate

2ml of the extract is treated with 2 ml of Magnesium sulphate solution.

5. Test for sulphide

1gm of the substance is treated with 2ml of concentrated Hydrochloric acid.

6. Test for Nitrate

1gm of the substance is heated with the copper turnings and concentrated sulphuric acid and viewed the test tube vertically down.

7. Test for fluoride and oxalate

a. 2ml of the extract is added with 2ml of calcium chloride solution and heated.

b. 5 drops of clear solution is added with 2ml of dilute sulphuric acid and slightly warmed. To this, 1ml of dilute potassium permanganate solution is added

8. Test for Nitrate

3 drops of the extract is placed on a filter paper. On that, 2 drops of benzidine solution is placed

9. Test for borate

2 pinches of the substance is made into paste by using sulphuric acid and alchohol (95%) and introduced into the blue flame

Test for basic radicals

1. Test for lead

2ml of the extract is added with 2ml of potassium Iodide solution.

2. Test for Copper

a. One pinch of substance is made into paste with concentrated hydrochloric acid in a watch glass and introduced into the non-luminous part of the flame.

b. 2ml of the extract is added with excess of ammonia solution.

3. Test for Aluminum

To the 2ml of the extract sodium hydroxide solution is added in drops to excess.

4. Test for Iron (Ferric)

a. To the 2ml of the ammonium thiocyanate solution is added.

b. (ferrous): To the 2ml of extract, 2ml of Ammonium thiocyanate is added.

5. Test for Zinc

To the 2ml of the extract sodium hydroxide solution is added in drops to excess.

6. Test for Calcium

2ml of the extract is added with 2ml of 4% Ammonium oxalate solution

7. Test for Magnesium

To 2ml of extract, sodium hydroxide solution is added in drops to excess.

8. Test for Ammonium

To 2ml of extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added.

9. Test for potassium

A pinch of substance is treated with 2ml sodium nitrate solution and then treated with 2ml of cobalnitrate in 30% glacial acetic acid.

10. Test for sodium

2 pinches of the substance is made into paste by using hydrochloric acid and introduced into the blue flame.

11. Test for Mercury

2ml of the extract is treated with 2ml of sodium hydroxide

12. Test for Arsenic

2ml of extract is treated with 2ml of solution nitrate solution.

4. Phytochemical analysis

The Phytochemical screening of the extract gives general idea regarding the nature of chemical constituents present in the crude drug. The phytochemical tests were done as per standard methods as follows.[4]

Table 1: Preliminary phytochemical analysis of *Naayuruvi kuzhi thylam*

S.No	Phytochemical screening test	Indication
1.	**Test for reducing sugar**: 5ml of benedict solution is taken in a test tube & allowed to boil for 2 mins and added 8-10 drops, again boiled for 2 mins. The color changes are noted.	Appearance of green or brick red color
2.	**Test for alkaloides:** a) 2ml of extract is heated with 2ml of potassium iodide solution.	Appearance of Red color
	b) 2ml of extract is heated with 2ml of picric acid.	Appearance of Yellow color
	c) 2ml of extract is heated with 2ml of phospho tungstic acid.	Appearance of White precipitate
3.	**Test for Tannin:** 2ml of extract is treated with 2ml of ferric chloride solution.	Appearance of Blue precipitate
4.	**Test for unsaturated compound:** 2ml of extract is treated with 2ml of potassium permanganate solution.	Potassium permanganate solution is decolorized.
5.	**Test for Amino acid:** Placed 2 drops of extract on a filter paper and dry it well. After drying 1% ninhydrine is sprayed over the same and dried well.	Appearance of Violet color
6.	**Test for Albumin:** 2ml of extract is added with 2ml esbatch's agent.	Appearance of Yellow color precipitate.
7.	**Test for Starch:** 2ml of extract is heated with iodine solution.	Appearance of Blue color
8.	**Test for type of compound :** 2ml of extract is treated with 2ml of ferric chloride solution.	Appearance of Green/Red/Violet /blue

RESULTS

Table 2: Determination of zone of inhibition of ethyl acetate extract of *Naayuruvi kuzhi thylam*

S.no	Microorganisms	Zone of inhibition (mm)					
		Different concentrations of Ethyl acetate extract of *Naayuruvi kuzhi thylam*				Negative Control DSMO	Positive control Streptomycin
		1000µg	500µg	250µg	100µg		10µg
1.	*Escherichia coli*	8	8	6	5	-	25
2.	*Streptococcus aureus*	8	7	6	-	-	20
3.	*Salmonella typhi*	8	7	7	6	-	15
4.	*Candida albicans*	8	8	7	6		22

Table 3: Chemical analysis of *Naayuruvi kuzhi thylam*

Test for acid radicals	Inference
Sulphate	+
Chloride	-
Phosphate	+
Carbonate	-
Nitrate	-
Fluoride and oxalate	-
Nitrate	-
Borate	-
Test for basic radicals	
Lead	-
Copper	-
Aluminum	-
Iron	-
Zinc	-
Calcium	+
Magnesium	+
Ammonium	+
Potassium	-
Sodium	-
Mercury	-
Arsenic	-

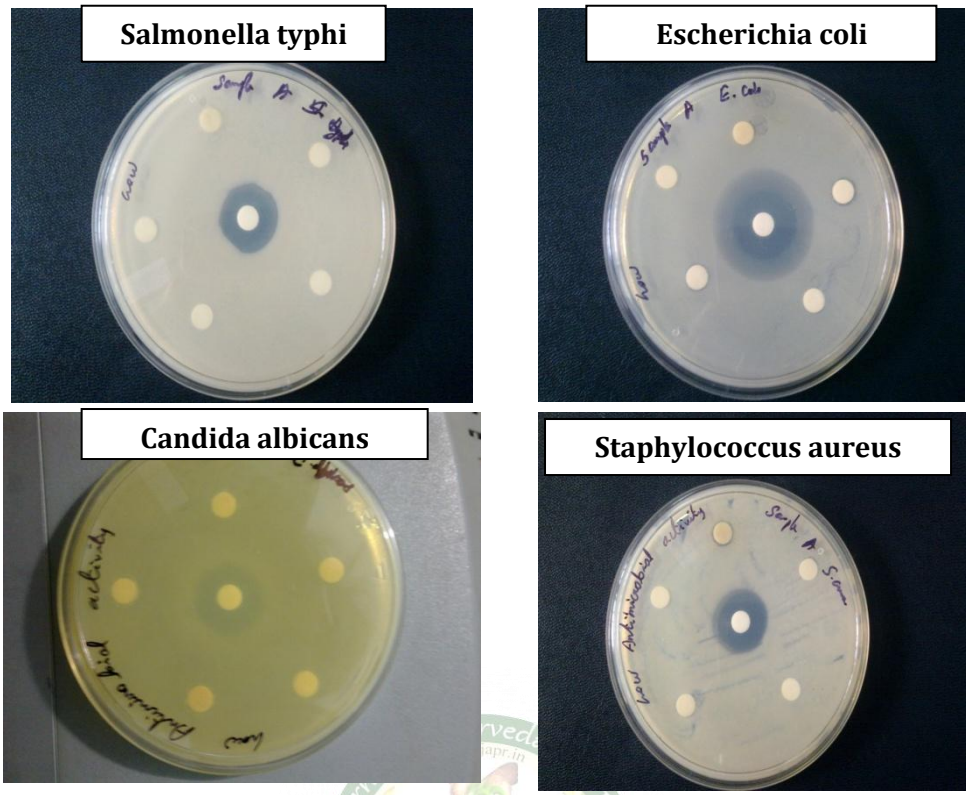
The chemical analysis reveals the presence of acid radicals phosphate and sulphide, basic radicals such as Calcium, potassium and ammonium (Table-3).

Table 4: Preliminary Phytochemical screening

S.No	Phytochemical screening test	Inference
1.	Reducing sugar	-
2.	Alkaloides	+
3.	Tannin	-
4.	Unsaturated compound	-
5.	Amino acid	+
6.	Albumin	-
7.	Starch	+

Preliminary Phytochemical screening revealed the presence of alkaloid, starch and aminoacids (Table-4).

Antimicrobial activity of *Naayuruvi kuzhi thylam* against selected pathogens 1000µg of NKT with Positive control Streptomycin



DISCUSSION

Herbs have been utilized for a very long while as rich wellsprings of horticulturally, biotechnology and therapeutically significant secondary metabolites. These are subsequently potential sources of medicinally useful products.[5-6] *Naayuruvi Kuzhi thylam* consist of a single herb *Achyranthes aspera* (Amaranthaceae) a significant plant found throughout in India. The parts which are utilized restoratively are seeds, roots and shoots. For the most recent couple of decades, broad research work has been completed to demonstrate the pharmacological properties of various medicinal formulations of *Achyranthes aspera*. Various phytoconstituents such as oleonolic corrosive, saponins, alkaloids, dihydroxy ketones, long chain compounds and numerous compounds have been isolated. These concentrates have more prominent insignificant antibacterial action.[7] Antimicrobial agents are essentially the disinfectants that are utilized to kill wide varieties of microorganisms inside the body. Antibacterial agents can likewise be further sub-isolated as bactericidal that are used for kill bacteria, and the bacteriostatic agent that slows down bacteria growth.[8] Since the use of synthetic chemicals for the control of pathogenic microorganisms is limited because of their carcinogenic effects, acute toxicity, and environmental hazard potential. In this regard, the utilization of herbal oils to control epidemic multidrug-resistant pathogenic microorganisms can be useful to combat various infectious diseases.[9]

The organisms that were included in the study consisted of both Gram-positive (S. aureus) and Gram-negative (*Escherichia coli, Salmonella typhi*) and a fungal pathogen *Candida albicans*. Streptococcus aureus sets the stage for lower respiratory tract infections which include bronchitis, bronchiolitis, pneumonia with symptoms of cough, fever, chest pain and wheezing.[10-11] *Salmonella* represents a large genus of global public health significance and is the leading cause of food borne illnesses responsible for thousands of deaths worldwide.[12] Although most *E. coli* strains are harmless, certain strains are pathogenic and cause diseases such as watery diarrhea, bloody diarrhea, urinary tract infection, meningitis, and sepsis, which can lead to death.[13,14]

All the tested pathogens showed maximum zone of inhibition (8mm) at a concentration of 1000µg of NKT and the positive control drug Streptomycin showed a greater zone of inhibition ranging from 15mm to 25mm at a concentration of 10µg. Hence NKT has a moderate antimicrobial action against these selected pathogens (Table-2). It may be worth to test against other microbes responsible for upper and lower respiratory illnesses.

The chemical analysis reveals the presence of acid radicals, phosphate and sulphide, basic radicals such as calcium, potassium and ammonium. Preliminary Phytochemical screening revealed the presence of alkaloid, starch and aminoacids. These minerals and phytochemicals may be responsible for the pharmacological property of NKT as indicated in the literature.

CONCLUSION

This preliminary work on the screening of *Naayuruvi Kuzhi thylam* a Siddha single herbal preparation for phytochemical, chemical analysis and antimicrobiological activity has demonstrated the presence of various constituents that responsible for its antimicrobial action against the selected pathogens. Since the present study has demonstrated moderate antibacterial activity against *S. aureus*, *Escherichia coli, Salmonella typhi* and *Candida albicans* when compared with the control drug streptomycin, the study needs to be progressed in future with other respiratory, gastro intestinal and skin pathogens to explore the pharmacological action of *Naayuruvi Kuzhi thylam*. Further, as herbal drugs are major drugs for future perspective because of its safety against synthetic drugs more such studies should be conducted to know spectrum of activity, efficacy and safety.

ACKNOWLEDGEMENT

Dr.K.Kanakavalli, Director General, Central Council for Research in Siddha, Arumbakkam, Chennai-106, Dr. P.Parthiban, Joint Director, Director of Indian Medicine and Homoeopathy, Arumbakkam, Chennai and Dr.P.Sathiyarajeswaran, Director i/c, Siddha, Central Council for Research in Siddha, Arumbakkam, Chennai for their continuous support and guidance throughout the study

REFERENCES

1. WHO global report on traditional and complementary medicine. Geneva: World Health Organization; 2019. Licence: CC BY-NC-SA 3.0 IGO
2. Murugesa Mudaliar KS. Text book of Materia Medica (Gunapadam). 5th ed. Mooligai, Department of Indian Medicine and Homeopathy; 2008.
3. Anonymous. Bio chemical Standards of Unani formulations. Part 3. Ministry of Ayush; 1998.
4. Journal article on the Internet Regupathi T, Chitra K. Physicochemical Analysis of Medicinal Herbs. 2014, http://shodhganga.inflibnet.ac.in/itstream/1060 3/7 7695/14/2%20annexure.pdf.
5. Dung NX, Loi DT. Selection of traditional medicines for study. J Ethnopharmacol 1991; 32: 57-70.
6. Deans SG and Svoboda, KP. Antibacterial activity of summer savory essential oil and its constituents. J. Hort. Sct *1989;* 64: 205-21.
7. Singh P, Shrivastava S, RL Khosa. Achyranthes aspera-important medicinal plant. J Naat Prod Resour 2011; 2-10.
8. Shahazadi Parveen. Phytochemical Screening and Antimicrobial Activity of Herbal Plant Extracts-Achyranthus aspera. Int J Pharm Clin Res 2018; 10(7): 201-209.
9. Mulyaningsih S, Sporer F, Zimmermann S, Reichling J, Wink M. Synergistic properties of the terpenoids aromadendrene and 1,8-cineole from the essential oil of Eucalyptus globulus against antibiotic-susceptible and antibiotic-resistant pathogens. Phytomed 2010; 17(13)1061–1066.
10. Carolyn M. Slupsky. Streptococcus pneumoniae and Staphylococcus aureus pneumonia induce distinct metabolic responses. J Proteome Res 2009; 8(6):3029-36.
11. Scott JA, Wonodi C, Moïsi JC, Deloria-Knoll M, DeLuca AN, Karron RA. The definition of pneumonia, the assessment of severity, and clinical standardization in the pneumonia etiology research for child health study. *Clin Infect Dis* 2012;54 Suppl 2:S109-16.
12. Jajere SM. A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. Vet World. 2019;12(4):504-521.
13. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. Clin Microbiol Rev. 1998; 11(1): 142–201. Erratum in: Clin Microbiol Rev. 1998; 11(2): 403.
14. Gyles CL. Shiga toxin-producing *Escherichia coli*: an overview. J Anim Sci. 2007; 85 (13 Suppl): E45–E62.

Cite this article as:
S. Esaivani, T.Kavitha, R.Susila, A.Usha, R.Thilagavathi. Antimicrobial Action and Preliminary Analysis of Naayuruvi Kuzhi Thylam. International Journal of Ayurveda and Pharma Research. 2020;8(4):1-6.
Source of support: Nil, Conflict of interest: None Declared

***Address for correspondence**
Dr S. Esaivani
Research Officer (Siddha)
Siddha Central Research Institute,
Arumbakkam, Chennai.
Email: esaivani08@gmail.com
Pn.no: 9597322218