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AN EFFECT OF ANTIMICROBIAL POTENTIALS OF A NOVEL SIDDHA METALLO-MINERAL FORMULATION "*KAALAMEGA NARAYANA CHENDHOORAM*" AS MENTIONED IN "*ATHMARAKSHA MIRTHAM ENNUM VAITHIYA SAARA SANGERAHAM*" AGAINST A GRAM NEGATIVE ORGANISMS *KLEBSIELLA PNEUMONIA*, *PSEUDOMONAS AEROGINOSA* IN *IN-VITRO* STUDIES.

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ABSTRACT

Aim And Objective: The aim of the present study is to validate the Anti microbial potentials of a novel siddha metallo-mineral formulation *Kaalamega Narayana Chendhooram* as mentioned in *Athmaraksha Mirtham Ennum Vaithiya Saara Sangeraham* against a Gram negative organisms such as *Klebsiella Pneumonia*, *Pseudomonas Aeroginosa* in *in-vitro* Studies. **Methods:** Siddha system of medicine is one of the traditional system of medicine plays an important role in treating various acute and chronic ailments without any adverse effect. The super natural scientists gave a boon of siddha system of medicine which have the solutions for various diseases including microbial infections. There is a huge need for antimicrobials due to the resistance of micro-oraganism. Thus an attempt was made to screen the anti microbial potentials of *Kaalamega Narayana Chendhooram* as mentioned in *Athmaraksha Mirtham Ennum Vaithiya Saara Sangeraham*, a novel siddha metallo-mineral formulation against a Gram negative organisms *Klebsiella Pneumonia*, *Pseudomonas Aeroginosa* in *In-vitro* Studies of Muller Hinton Agar Medium with streptomycin as a standard drug and test drug with the different concentration of the drug as 250µg/ml, 500µg/ml, 1000 µg/ml were added and zone of inhibitions were measured in mm. **Results:** At the end of this research study, that the trail drug of a novel siddha metallo-mineral formulation *Kaalamega Narayana Chendhooram* as mentioned in *Athmaraksha Mirtham Ennum Vaithiya Saara Sangeraham* through Muller Hinton Agar Medium with streptomycin as standard drug. The gram negative organisms shows the zone of inhibition in following concentration *Klebsiella Pneumonia* 250µg/ml, 500µg/ml, 1000 µg/ml as 17mm,19mm, 23mm , *Pseudomonas Aeroginosa* 250µg/ml, 500µg/ml, 1000 µg/ml as 19mm, 20mm, 23mm. On increasing the concentration of the drug there is a gradual increase zone of inhibition of micro organisms. **Conclusion:** The present in-vitro study of a potent siddha metallo-mineral formulation *Kaalamega Narayana Chendhooram* as mentioned in *Athmaraksha Mirtham Ennum Vaithiya Saara Sangeraham* through a Muller Hinton Agar Medium with streptomycin as a standard drug was found to be a potent anti-microbial medicine.

KEYWORDS: *Kaalamega Narayana Chendhooram, KMNC, Chendhooram, Siddha,* metallo-mineral formulation, Gram negative organisms, Muller Hinton Agar Medium, Anti microbial activity, *Klebsiella Pneumonia*, *Pseudomonas Aeroginosa.*

INTRODUCTION

Bacteria are ubiquitous pathogen which involving in causing different types of infections in human beings. Anti- biotics are generally used to treat microbial infections in World wide. In due course the organisms made resistance with that antimicrobials.[1]

From the beginning of earliest civilization of human beings in this planet, plants and natural products are playing a marvellous role in maintaining, improving human health. In addition to that, antibiotics have many adverse effects such as hypersensitivity, depletion of beneficial gut flora, immune suppression and allergic reaction. Therefore there is a urgent need to develop an

alternative antimicrobial drug for treating various microbial infections.[2]

Siddha system of medicine is an unique system among the Indian system of medicine. It have been developed by the Siddhar's the ancient supernatural spiritual saints of India. It constitutes the drug sources which are obtained from plant, mineral, metal and animals.[3]

The Siddha system owes its origin to the pre-antibiotic era with enormous collection of classical literature, has in store of enormous number of herbal, metallo-mineral, aquatic and animal products which are used for the spectacular in the prevention and also in the treatment of various infections. The medicines are selected on the basis of ability to pacify the derangements of humors like vatham, pitham, and kabam pertaining to its signs and symptoms of disease, and it also used to mold our immune system and provide an unfavorable environment for the pathogens growth. The medicines obtained from the natural source cannot be used as antibiotics because they aren't scientifically validated. The presence of various biological active compounds in traditional system of medicine have various actions including anti-allergic, anti-inflammatory, expectorant and immuno-modulatory properties. Thus an attempt was made in this research to screen the antimicrobial activity against the gram negative organisms. The gram negative organisms are *Klebsiella Pneumonia*, *Pseudomonas Aeroginosa.*

1. *Klebsiella Pneumonia*
Classification of *Klebsiella Pneumonia*
Domain: Bacteria;
Phylum: Proteobacteria;
Class: Gammaproteobacteria;
Order: Enterobacteriales;
Family: Enterobacteriaceae;
Genus: *Klebsiella*;
Species: *K. pneumonia*

Klebsiella pneumonia is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod-shaped bacterium. It appears like a mucoid lactose fermenter on MacConkey agar.It is present in the flora of the mouth, skin, and intestine.[4]

It may cause illness and affects the middle-aged people and older men with debilitating diseases. It vigorously acts on the persons with diabetes, alcoholism, malignancy, liver disease, chronic obstructive pulmonary diseases, glucocorticoidtherapy, renal failure, and certain occupational exposures (such as papermill workers). Many of these infections are obtained when a person is in the hospital for some other reason (a nosocomial infection).

In addition to that of pneumonia, Klebsiella sp can also cause infections in the urinary tract, lower biliary tract, and surgical wound sites. The range of clinical diseases includes pneumonia, thrombophlebitis, urinarytractinfection, cholecystitis, diarrhea,upper respiratory tract infection, wound infection, osteomyelitis, meningitis, and bacteremia and sepsis.[5]

2. *Pseudomonas aeruginosa*
Classification of *Pseudomonas aeruginosa*
Domain-Bacteria
Phylum-Proteobacteria
Class-Gamma proteobacteria
Order-Pseudomonadales
Family-Pseudomonadaceae
Genus-Pseudomonas
Species Group-Pseudomonas aeruginosa

Pseudomonas aeruginosa is a common encapsulated, Gram-negative, rod-shaped bacterium which can cause the diseases in plants and animals, including humans. The medical importance of P. aeruginosa is a multidrug resistant pathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and in association with the serious illnessessuch as hospital-acquired infections, ventilator-associated pneumonia and various sepsissyndromes. The symptoms are mostly generalized in the form of inflammation, Pneumoniaand sepsis. If such colonizations occur in critical body organs, such as the lungs, the urinary tract, Gastrointestinal infection, Skin and soft tissue infections, and kidneys, the results can be fatal.[6]

MATERIALS AND METHODS

The different types of *kmnc* preparations were available in differen classical siddha literatures. they are listed as below

- Vaiththiya Viththuvan Mani S.Kannuchamipillai, Chikichcha Raththina Theepamennum Vaithya Nool, Page No: 247, B.Rathna Nayaagar & Sons, ThirumakalVilasa Achchakam, Chennai 79.
- Kandhasamy Mudhaliyaar, Athmaraksha Mirtham Ennum Vaithiya Saara Sangeraham, First edition 1931, Page No: 496, B.Rathna Nayaagar & Sons,Thirumakal Vilasa Achchakam, Chennai 79.
- Vaiththiya Viththuvan Mani S.Kannuchamipillai, Kannusamy Paramparai Vaithiyam, Page No : 327, B.Rathna Nayaagar & Sons, Thirumakal Vilasa Achchakam, Chennai 79.
- Vaiththiya Viththuvan Mani S.Kannuchamipillai, Kannusamiyam, Page No : 120, B.Rathna Nayaagar & Sons, Thirumakal Vilasa Achchakam, Chennai 79.
- Vaiththiya Viththuvan Mani S.Kannuchamipillai, Kannusamy Paramparai Vaithiyam, Page No : 327, B.Rathna Nayaagar & Sons, Thirumakal Vilasa Achchakam, Chennai 79.

All the above mentioned the classical siddha text books shows the same ingredients and the same indications of *KMNC* but all the above preparations follows different medicinal preparation methods.

The current research derived the medicinal preparation the siddha text, Kandhasamy Mudhaliyaar, Athmaraksha Mirtham Ennum Vaithiya Saara Sangeraham, First edition 1931, Page No: 496, B.Rathna Nayaagar & Sons,Thirumakal Vilasa Achchakam, Chennai 79.

Selection of the Drug

For this present study, the metello-mineral formulation **"*Kaalamega Narayana Chendhooram*"** was taken as the compound drug preparation for oral cancer mentioned in the classical Siddha literature "*Athmarakshamirtham Ennum Vaithiya Saara Sangeraham"* written by *Kandhasamy Mudhaliyaar*, pg no:493,First Edition 1931.

Ingredients of the drug

1. Purified *Vediuppu [Potassium nitrate]* – 840 gm
2. Purified *Thurusu [Cupric sulphate]* – 210 gm
3. Purified *Padikaaram [Aluminium potassium sulphate (Alum)]* – 840 gm
4. Purified *Vengaram [Sodium bicarbonate* (Borax)*]* – 210 gm
5. Purified *Navacharam [Ammonium Chloride]*-210gm
6. Purified *Pooneeru [Impure Sodium Carbonate (Fullers Earth)]* – 105 gm
7. Purified *Jaathilingam* [Red sulphate of mercury]-525gm
8. Purified *Gandhagam [Sulphur]* – 420 gm
9. Purified *Kalluppu [Sodium chloride]*- 210 gm
10. Purified *Rasam [Hydragyrum]* – 1050 gm
11. Purified *Aritharam [Tri sulphate of Arsenic* (Yellow Orpiment)*]*- 350 gm
12. Purified *Manosilai [Di suphate of Mercury* (Red Orpiment)*]*- 140gm.[7]

Collection of the raw materials

All the raw materials were purchased from R.N. Rajan country drug store, Parrys corner, Chennai.

Identification and Authentication of the drug

The raw materials were identified and authenticated by the experts of *Gunapadam*, Government Siddha Medical College, Arumbakkam, Chennai- 106.

The specimen sample of each raw material has been kept in the PG *Gunapadam* department individually for future reference.

Procedure

- 840 gm of 8th solution of *Vediuppu [Potassium nitrate]* and *Padigaram* [Aluminium potassium sulphate *(Alum)]* were taken.
- Along with that, 210 gm of *Thurusu [Cupric sulphate], Vengaram [Sodium bicarbonate* (Borax)*], Navacharam [Ammonium Chloride], Kalluppu [Sodium chloride Impura]* were taken and then mixed with 105 gm of *Pooneeru [Impure Sodium Carbonate (Fullers Earth)]*.
- Above ingredients were ground into fine powder and divided into 3 parts.
- First part of the powder was underwent distillation process, the end product was mixed with 2nd part of powder and dried.
- Second part of the powder was underwent distillation process, the end product was mixed with 3rd part of powder and dried.
- Third part of the powder was undergoes distillation process, the final end product was taken and kept in a sealed bottle.
- The *Jaathilingam* [Red sulphate of mercury]-525 gm, *Aritharam* [Tri sulphate of Arsenic (Yellow orpiment)]-350 gm, *Gandhagam [Sulphur]* 420 gm, *Rasam* (Mercury)- 1050 gm, *Manosilai [*Di sulphate of mercury (Red Orpiment)*]* 140 gm wereground ,along with the end product of distillation for 12 hours (4 *saamam*) and made into fine powder and dried.
- Dried powder was kept in a mud pot which was sealed with 7 mud pasted plaster.
- Another mud pot with small quantity of sand was taken and above preparation was kept into it and sealed the lid with mud pasted plaster.
- The mud pot was ignited by using *Aavarai* stick for 30 hours (10 *saamam*), after 30 hours "*Chendhooram*" was obtained

Drug name	*Kaalamega Narayana Chendhooram*
Dosage	244 mg of *Chendhooram* [1/2 *Panavedai*]
Route	Enteral (oral)
Adjuvant	*Thipili chooranam* with honey (bd for 48 days – 1 *mandalam*)
Indications	*Kannaputru* [ORAL CANCER], *Elaippu* [Tuberculosis], *Kuttam 18* [Hansen's Disease]
Reference	*"AthmarakshaMirutham Ennum Vaithiya Saara Sangeeraham*".[13]

Fig. 1: Final Product Of Kmnc Chendhooram.

Antimicrobial activity

Agar- well diffusion method

The study was conducted in Biogenix Research Centre, Trivandrum, Kerala, India.

Principle

The antimicrobials present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Materials Required

1. Muller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.8 g of the commercially available Muller Hinton Agar Medium (MHI Agar Media) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Nutrient broth (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HI Media) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

3. Streptomycin (standard antibacterial agent, concentration: 10mg / ml)

4. Culture of test organisms; growth of culture adjusted according to McFards Standard, 0.5%.

Gram negative organisms:

1. *Pseudomonas aeroginosa* (ATCC 27853)
2. *Klebsiella pneumonia* (ATCC 13883)

Procedure

Petriplates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture of, *Pseudomans aeroginosa, Klebsiella pneumoniae* (growth of culture adjusted according to McFards Standard, 0.5%). Wells of approximately 10mm was bored using a well cutter and different concentrations of sample such as 250µg/mL, 500µg/mL, 1000µg/mL were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Streptomycin was used as a positive control.[8]

RESULTS AND DISCUSSIONS

Sample	Concentration(µg/mL)	Zone of inhibition(mm)
KMNC	Streptomycin (100µg)	24
	250	19
	500	20
	1000	23

Fig. 2: Gram Negative Bacteria *Pseudomonas aeroginosa*.

14 mm – Low sensitive, 15 mm – Moderate, above 16 mm – Highly sensitive

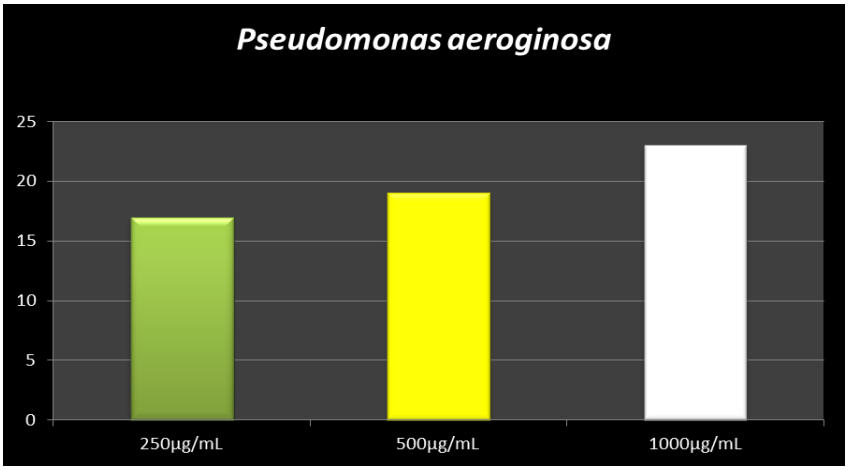

Fig 3: Zone of inhibition.

Sample	Concentration(µg/mL)	Zone of inhibition(mm)
KMNC	Streptomycin (100µg)	22
	250	17
	500	19
	1000	23

Fig 4. Gram Negative Bacteria *Klebsiella pneumoniae*.

14 mm – Low sensitive, 15 mm – Moderate, above 16 mm – Highly sensitive

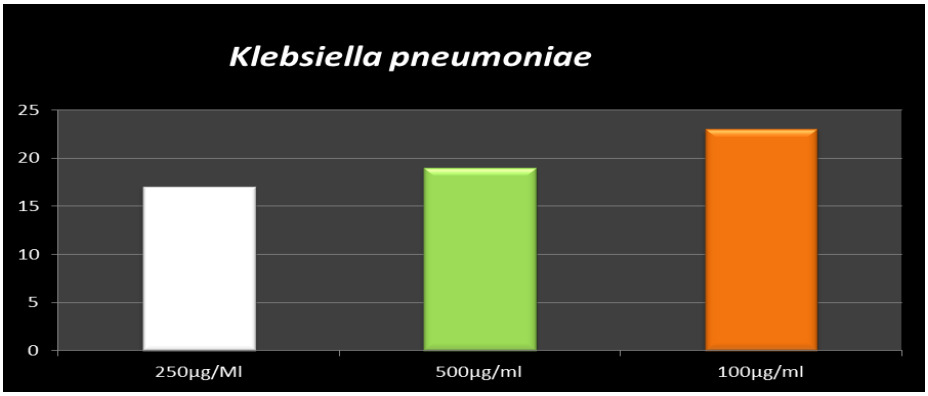

Fig. 5: Zone of inhibition.

Fig. 6: Disc shows the zone of inhibitions of micro-organisms.

DISCUSSIONS

The development of resistance against the presently available antibiotics arises the necessity of rediscovery of new anti-bacterial agents in traditional systems of medicine. Different dosages of test drug against the microbes in antimicrobial activity of *KMNC* was compared with Standard drug Streptomycin (100µg)/ml disc for the following pathogens, they are *Pseudomonas aeruginosa, Klebsiella pneumoniae,* The results represents *KMNC* potencially inhibit the growth of all above organism in 250µl, 500µl and 1000µl / disc. 14 mm – Low sensitive, 15 mm – Moderate, above 16 mm – Highly sensitive. The gram negative organisms shows the zone of inhibition in following concentration *Klebsiella Pneumonia* 250µg/ml, 500µg/ml, 1000 µg/ml as 17mm,19mm, 23mm, *Pseudomonas Aeroginosa* 250µg/ml, 500µg/ml, 1000 µg/ml as 19mm, 20mm, 23mm. On increasing the concentration of the drug there is a gradual increase zone of inhibition of micro organisms. The findings reveal that the Siddha drug *KMNC* have anti microbial potency against bacterial pathogens which is used in the treatment of diseases.

CONCLUSION

Antibiotics are used for various microbial infections all around the World wide. Micro organisms made resistance against the antibiotics. Thus an urgent need for screening antimicrobial activity was made in this research. The current study showed that the *KMNC* is more effect against the gram negative organisms such as *Pseudomonas Aeroginosa and Klebsiella Pneumonia.* This study will help for treating various infections caused by micro-organisms.

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