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ANTIMICROBIAL POTENTIAL OF A NOVEL SIDDHA METALLO-MINERAL FORMULATION "*KAALAMEGA NARAYANA CHENDHOORAM*" AS MENTIONED IN "*ATHMARAKSHA MIRTHAM ENNUM VAITHIYA SAARA SANGERAHAM*" AGAINST A GRAM NEGATIVE ORGANISM *E.Coli* IN *IN-VITRO* STUDIES.

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ABSTRACT

AIM AND OBJECTIVE: The aim of this 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 organism *Escherichia coli* in in-vitro Studies. METHODS: Siddha system of medicine was a fruitful gift offered by the siddhars. It contains plants, metals, minerals and animal products in their medicinal preparations. It involved in treating various acute and chronic diseases. Siddha system of medicine had a history of antimicrobial medicine before the prehistoric era of antibiotics. Due to the lack of proper validations of medicine, it was not accepted by the scientific world. Thus an attempt was made in this paper to screen the efficacy of 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 organism Escherichia coli 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 organism showed the zone of inhibition in following concentration of Escherichia coli 250µg/ml, 500µg/ml, 1000 µg/ml as 15mm,16mm, 19mm .On increasing the concentration of the drug there is a gradual increase in the 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.

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INTRODUCTION:

Classification of *Escherichia coli:*

Scientific name	:	*Escherichia coli*
Class	:	Gammaproteobacteria
Order	:	Enterobacteriales
Phylum	:	Proteobacteria
Family	:	Enterobacteriaceae

It is a Gram-negative organism with a facultative anaerobic, rod-shaped, coliform bacterium in the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms)[1].

E. coli can live in a wide variety of substrates and then it uses the mixed-acid fermentation in anaerobic conditions, producing the lactate, succinate, ethanol, acetate, and carbon dioxide. Since they are many pathways in mixed-acid fermentation, which produce the hydrogen gas, these pathways require the levels of hydrogen to be low, as is the case when *E. coli* lives together with hydrogen-consuming organisms, such as methanogens or sulphate-reducing bacteria[2].

For the optimum growth of *E. coli* occurs at 37 °C (98.6 °F), but in certain laboratories the strains can multiply at the temperatures up to 49 °C (120 °F)[3]. *E. coli* grows in different types of defined laboratory media, includes lysogeny broth, or the medium which contains glucose, ammonium phosphate monobasic, sodium chloride, magnesium sulfate, potassium phosphate dibasic, and water. Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen, amino acids, and the reduction of substrates includes oxygen, nitrate, fumarate, dimethyl sulfoxide, and trimethylamine N-oxide[4].

Most of the *E. coli* strains do not cause disease, naturally they are living in the gut region[5].Some of the virulent strains of *E.coli* can cause the complications such as gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn's disease. The most common signs and symptoms includes severe abdominal cramps, diarrhea, hemorrhagic colitis, vomiting, and sometimes fever. Usually the symptoms will start appear in 3 to 4 days after being exposed to the bacteria. The symptoms abdominal pain, severe abdominal cramping, watery diarrhea, bright red bloody stools around a day later, nausea , vomiting, fever, fatigue, In some rarer cases, some types of virulent strains of *E.coli* are also responsible for bowel necrosis (tissue death) and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, sepsis, and Gram-negative pneumonia. Very young children are more susceptible to develop severe form of illness which includes hemolytic uremic syndrome. Thus the individuals of all ages are found to be very at risk to the severe consequences that may arise as a result of being infected with *E. coli*[6]. The mode of transmission of disease is due to contaminated water, contaminated food, Person to-person contact, Contact with animals. Certain risk factors includes People with a weakened immune system**,** Patients with decreased stomach acid, Young children and older people are more prone to these type of infections. The complications includes Mostly people will recover within a week, sometimes it may develop hemolytic uremic syndrome, anemia, kidney failure, central nervous system (CNS) problems, seizures, paralysis, brain swelling, and coma[7].

Siddha system medicine plays an important role in treating various acute and chronic ailments. The fruitfull effect of Siddha system of medicine palys an important role in treating drug resistance micro – oraganisms. Now a days these micro organisms are involved in producing resistance against the anti microbial drug. Thus there was a huge demand in the field of antimicrobials in the world of micro-organisms. The siddha system of medicine had a knowledge about the antimicrobial agent before the drug discovery of antimicrobials. But they cannot be used around all over due to the lack of screening of anti microbial effect of siddha medicines. Thus an attempt was made in this paper to validate the effect of traditional siddha medicine *KMNC* against the drug against microbials.

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 from 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[8]

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 profile:

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	: *Putru* [CANCER], *Elaippu* [Tuberculosis], *Kuttam 18* [Hansen's Disease]
Reference	: *"AthmaraksheMirutham Ennum Vaithiya Saara Sangeeraham"*[8].

Fig No: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:
1Muller 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%
1. *E.coli* (ATCC 25922)

PROCEDURE:

Petriplates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture of *E.coli* (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.Note: Concentration of stock 10mg/mL DMSO[9].

RESULTS AND DISCUSSIONS:

The antimicrobial effects of *KMNC* was carried out against the Gram negative micro organism *E.Coli* .The values were plotted in the table shown below.

GRAM NEGATIVE BACTERIA *E.Coli*:

FIG NO: 1.EFFEC OF *KMNC* ON *E.Coli*:

Sample	Concentration(µg/mL)	Zone of inhibition(mm)
KMNC	Streptomycin (100µg)	20
	250	15
	500	16
	1000	19

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

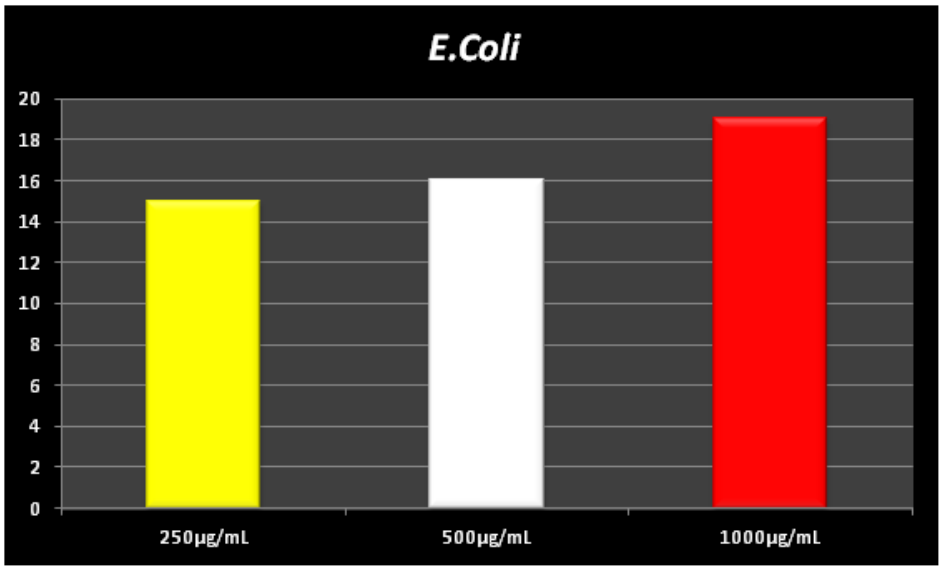

FIG NO: 2.ZONE OF INHIBITION WITH GRAPHICAL REPRESENTATION:

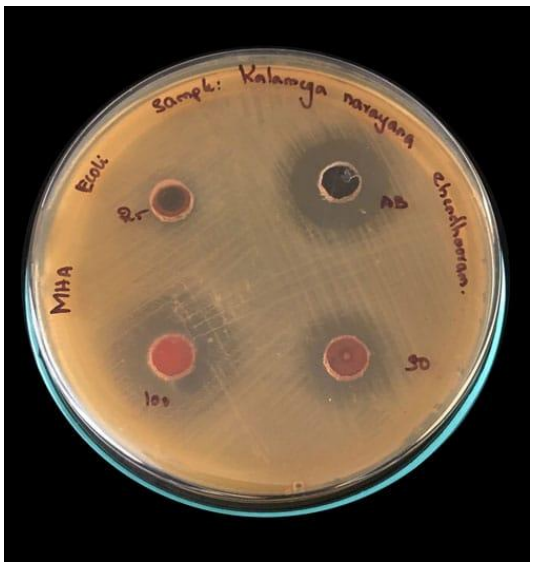

FIG NO: 3. GROWTH OF BACTERIA.

DISCUSSIONS

Due to the resistance created by the microbials, there was a huge demand in the field of antibacterials. The different concentrations of the *KMNC* were treated in the muller hinton Agar Medium along with the bacterial culture. The zone of inhibition was calculated in mm as streptomycin as a standard drug. The different concentration *KMNC* showed a better zone of inhibition in the culture media after the 24 hours of incubation at 37 degree Celsius. The results represents that, *KMNC* potencially inhibits the growth of above organism in the concentration of 250µl, 500µl and 1000µl / disc. Then the results are compared with the ranges of 14 mm – Low sensitive, 15 mm – Moderate, above 16 mm – Highly sensitive. The gram negative organism *E.coli* shows the zone of inhibition in following concentration *KMNC* in different concentration of 250µg/ml, 500µg/ml, 1000 µg/ml as the zone of inhibition of 15mm,16mm, 19mm. From the table, graph and growth medium of *E.coli* showed the zone of inhibiton of bacteria on increasing the concentration of the drug. 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. Thus the traditional medicine *KMNC* plays an important role in the field of drug resistance bacteria.

CONCLUSION

In the current modern World, they are large number micro organisms are made resistance against antimicrobials. The only treatment predicted for killing bacterias are antibiotics But the strains make resistance to them. So there is a huge demand in antibiotics in the modern world. Thus an attempt was made in this research to solve the problems created by the bacteria with less adverse effect and less cost effectiveness, the sample *KMNC* were prepared as a per the classical siddha literature and screened for antimicrobial activity against *E.coli* and streptomycin as a standard drug. The trail drug gave a better results in antimicrobial activity and also helpful in treating microbes which cause various infections.

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