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Antimicrobial and antioxidant evaluation of a retrospective siddha formulation *Dhasalavana dhravagam* used for the treatment of infectious disease

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ABSTRACT

The main objective of the present study is to evaluate the antimicrobial and antioxidant potential of Dhasalavana dhravagam (DLD) a well-known classical drug selected from the Siddha classical literature, "Kannusamiyam Ennum Vaidhya Segaram. The antimicrobial property of the DLD was studied against Gram-negative and Gram-positive microorganisms using the agar well diffusion method. The result of antimicrobial screening study projects that the drug DLD exhibited bigger zone of inhibition of about 08- 26 mm against Staphylococcus aureus followed by this 09- 25 mm of zone against Klebsiella pneumonia, 09- 24 mm of zone against Bacillus Subtilis and 07- 22 mm zone against Salmonella typhimurium at the concentration of 250mg/ml. Antioxidant potential of DLD was evaluated by using DPPH radical scavenging assay. The result obtained from the study shows that DLD possess potential antioxidant activity (91.3%) relatively near to the standard drug. From the study it was concluded that the drug DLD has promising antimicrobial, antioxidant activity and hence this siddha formulation may serve as a prospective drug for treating several infectious diseases in near future.

Keywords: Dhasalavana dhravagam, Antimicrobial activity, Gram-negative, Gram-positive Antioxidant activity, DPPH.

INTRODUCTION

In twenty-first century prediction of relationship between the host –pathogen interaction and its underlying mechanism continues to be a greater challenge for researchers and health care professionals. The infectious disease paradigm is shifting as our understanding of the relationship between host, infection-causing pathogens, and chronic diseases becomes more nuanced and complex [1].

Infectious diseases remain key agents of the debilitating poverty afflicting so much of the world today. Each year these diseases kill almost nine million people, many of them children under five, and they also cause enormous burdens through life-long disability. Stepping up research into their causes and how to effectively treat them and prevent them from spreading could have an enormous impact on efforts to lift people out of poverty and to build a better world for future generations [2].

Since years together herbal preparation being an integral part of health and wellbeing. An herb rich in active phytoconstituents becomes a valuable lead in the field of infectious disease research. Focus towards infectious

disease grabs higher attention especially in the developing countries is majorly because of its prevalence in the marginalized communities. According to the recent survey health care expenditure on infectious disease has been increased several folds in developing countries. Siddha system of traditional medicine was originated from Tamil nadu which has greater ailment for the treatment of several dreadful infectious diseases as per the vedic literatures.
Dhasalavana Dhravagam has been selected from the Siddha classical literature, "*Kannusamiyam Ennum Vaidhya Segaram*". Ingredients of the test drug are *Vediuppu* (Salt petre), *Padikaram* (Alum) *Kalluppu* (Rock salt), *Indhuppu* (Halite), *Navacharam* (Sal ammoniac) and *Kariuppu (*Common salt), *Vengaram* (Borax), *Annabedhi* (Green vitriol), *Pooneeru* (Fullers earth), *Thurusu* (Blue vitriol).

MATERIALS AND METHODS

2.1.Collection of the drugs

All the raw materials were obtained from Country drug shop, Ramaswamy chetti, Parrys, Chennai. In this preparation fullers earth was collected from Maamandore.

2.2. Identification and Authentication

All the raw drugs were identified and authenticated by the experts of Gunapadam (Pharmacology) at Government Siddha Medical College, Arumbakkam, Chennai. The specimen samples of the identified raw drugs were preserved in the laboratory of P.G Gunapadam for future references.

2.3. Purification of Raw drugs

All the ingredients of the trial drug were purified according to the Siddha classical texts. Salt petre: The drug was soaked in lemon juice, dried in sunlight until the moisture content was lost. .Alum: The drug was dissolved in pure water and filtered. Then it was allowed to boil in a pan until its moisture content was lost. When the mixture attained a thick molten consistency, it is allowed to cool and stored. Borax: The drug was powdered well and fried in a pan to complete dehydration. Sal ammoniac: It was ground with cow's urine for three hours and allowed to dry. Common salt: Common salt is dissolved in sea water and filtered. The filtrate is boiled till it reaches semi consistency state. It is dried in sunlight and it attains the solid state as purified salt. Blue vitriol: It is fried, till it turns to whitish.Fuller's earth : According to Bogar fuller's earth is dissolved in lemon juice and fitered. The filterate is boiled till the water completely evaporates to get purified form. Halite: It is kept soaked in vinegar for three days and insolated to get purified and detoxified form. Rock salt: It is dissolved in vinegar and clean with a cloth, dried in sunshade. Green vitriol: It is dissolved in water. A small quantity of sulphuric acid is added to it filtered and heated till it attains the consistency of dry salt [3].

2.4. Preparation of *Dhasalavana Dhravagam*

The following ingredients were mixed and grinded together 1.purified salt petre *(Suththitha Vediyuppu)*- 120gm,2.Purified Alum *(Suththitha Padigaram)*- 120gm 3.Purified Rock salt *(Suththitha Kalluppu)*- 40gm ,4.Purified Halite *(Suththitha Indhuppu)*- 40gm ,5.Purified Sal ammoniac *(Suththitha Navacharam)*-20gm ,6.Purified Common salt *(Suththitha Kariyuppu)*-20gm ,7.Purified Borax *(Suththitha Vengaram)*- 15gm ,8. Purified Green vitriol *(Suththitha Annabedi)* -50gm, 9.Purified Fullers earth *(Suththitha Pooneeru)* -5gm and 10.Purified Blue vitriol *(Suththitha Thurusu)*-5gm.

2.5. Processing of *Dhasalavana Dhravagam*

The final mixture is transferred to the *Valaiyanthiram* (Distillation apparatus) made of earthern distillation set up and intensely heated. During the process of heating, the salts were completely decomposed and expel the acidic fumes. The fumes were condensed at the condenser submerged in cold water and collected in the vessel.

2.6. Preparation of extract from *Dhasalavana Dhravagam*

Required volume of *Dhasalavana Dhravagam* was taken in a 250 ml clean beaker and 50 ml of distilled water was added, boil the solution for 20 minutes and allowed to cool and filter. The final extract was used for the antimicrobial and antioxidant evaluation.

2.7.Collection of Microorganism

To evaluate the microbial studies, the cultures were procured from various hospitals and laboratories in and around Chennai. The organisms used were *proteus vulgaris, Enterobacter aerogens, Escherichia coli, Pseudomonas*

aeruginosa, *Klebsiella pneumonia*, *Salmonella typhimurium*, *Staphylococcus aure*us and *Bacillus subtilis*. All the organisms were confirmed using specific biochemical tests.

2.8. Antimicrobial activity

The cup plate antibacterial susceptibility test method was followed. Muller Hinton agar plates were prepared and swabbed with different isolates of log phase cultures of above organisms. The plates were allowed to stand for few minutes. Wells were made over the plates at an equidistant position. Wells were loaded with 50 µl of the drug at the concentration of 250mg/ml Dimethylsulphoxide (DMSO) was used as the solvent. Control well was also included using the solvent. The ampicillin 10 mcg disc used as standard for comparison. All the plates were kept at 37ºC for 18-24 hrs. The zone of inhibition was measured using the vernier caliper [4,5,6].

2.9. DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay

The antioxidant activity of DLD was determined using the 1, 1-diphenyl-2 picrylhydrazyl (DPPH) free radical scavenging assay [7]. 100µl of Dhasalavana dhravagam extract was mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control subsequently, at every 5 min interval, the absorption maximum of the solutions were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicates [8,9].

Free radical scavenging activity was calculated by the following formula:

% Inhibition = (*Abs* of Control – *Abs* of Test) /*Abs* of Control × 100

RESULTS

3.1. Effect on DLD on Antimicrobial activity

The test drug DLD exhibited significant zone of inhibition in four organisms out of eight selected for microbial screening in which the maximum inhibition zone diameter (IZD) of about 26 mm against *Staphylococcus aureus* at the volume of 60 µl/well and the minimum IZD of about 09mm at the volume of 10 µl/well against the same. Similarly the second best IZD of about 09- 25mm exhibited against *Klebsiella pneumonia* at the volumes ranges from 15-60 µl/well. The third best IZD of about 09- 24mm exhibited against *Bacillus Subtilis* at the volume ranges from 10-60 µl/well. The fourth best IZD of about 07-22mm exhibited against *Salmonella typhimurium* at the volume ranges from 10-60 µl/well. The ampicillin 10 mcg disc used as standard for comparison shown 22mm of IZD against all the organisms selected for the study. All IZD corresponding to test organisms are tabulated in Table 1 and represented in figure 1-4. No significant IZD was observed in the screening against the following organisms namely *Proteus vulgaris*, *Enterobacter aerogens* ,*Escherichia coli* and *Pseudomonas aeruginosa*.

Table 1: Antimicrobial Activity of *Dhasalavana dhravagam*

S.No	Organisms	Inhibition Zone Diameter (IZD) in mm					
		60 µl/well	50 µl/well	25 µl/well	15 µl/well	10 µl/well	Standard Ampicillin 10µg/disc
1.	*Proteus Vulgaris*	-	-	-	-	-	22
2.	*Enterobacter aerogens*	-	-	-	-	-	22
3.	*Escherichia coli*	-	-	-	-	-	22
4.	*Pseudomonas aeruginosa*	-	-	-	-	-	22
5.	*Klebsiella pneumonia*	25	20	14	09	-	22
6.	*Salmonella typhimurium*	22	19	15	12	07	22
7.	*Staphylococcus aureus*	26	24	14	13	08	22
8.	*Bacillus Subtilis*	24	20	18	13	09	22

Figure 1: Antimicrobial activity of DLD against *Klebsiella pneumonia*

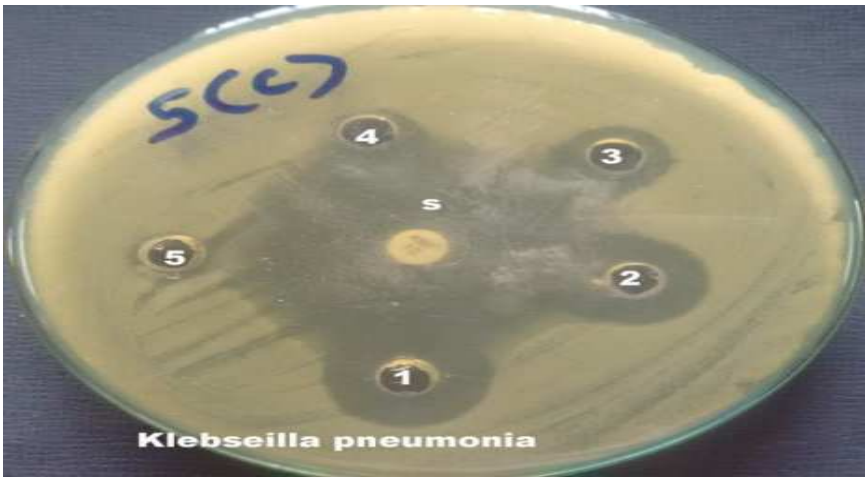
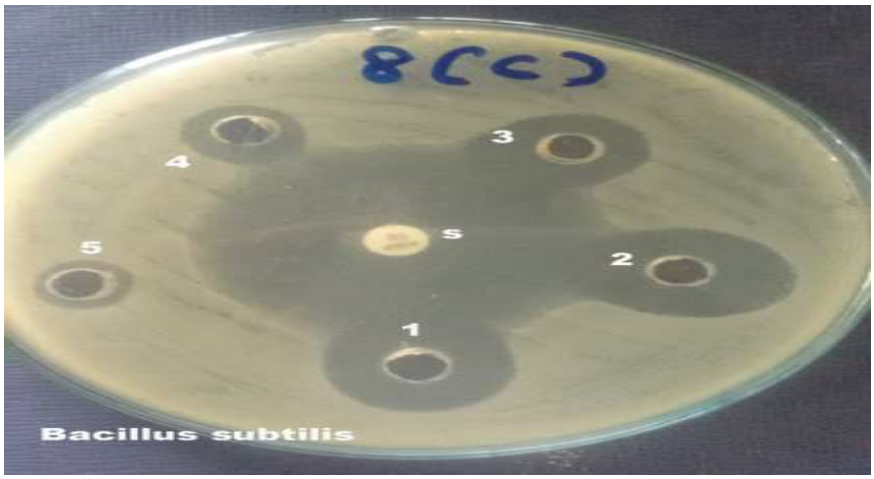


Figure 2: Antimicrobial activity of DLD against *Salmonella typhimurium*

Figure 3: Antimicrobial activity of DLD against *Staphylococcus aureus*

Figure 4: Antimicrobial activity of DLD against *Bacillus subtilis*



3.2. Effect on DLD on DPPH radical scavenging assay

The results of DPPH radical scavenging activity shows that the percentage inhibition of DLD ranges from 68.75 to 91.3 % in which the highest activity was detected at the time of 30th minute. Similarly the percentage inhibition of standard BHT ranges from 87.5 to 98.2 %. The results were tabulated in Table 2.

Table 2: Percentage inhibition of *Dhasalavana dhravagam* on DPPH radical scavenging assay

Time in Minutes	Absorbance of DLD	Absorbance of Standard BHT	Percentage Inhibition of DLD	Percentage Inhibition of Standard BHT
0	0.35	0.14	68.75	87.5
5	0.27	0.11	78.8	90.1
10	0.23	0.09	79.4	91.6
15	0.19	0.07	83.0	93.75
20	0.13	0.06	88.39	94.6
25	0.11	0.04	90.1	96.4
30	0.10	0.02	91.3	98.2

DISCUSSION

A bacterium which belongs to the genus *Klebsiella* may frequently cause human nosocomial infections. In particular, the medically most important *Klebsiella* species, *Klebsiella pneumoniae*, accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicemias, and soft tissue infections. The principal pathogenic reservoirs for transmission of *Klebsiella* are the gastrointestinal tract and the hands of hospital personnel. Because of their ability to spread rapidly in the hospital environment, these bacteria tend to cause nosocomial outbreaks [10].

According to WHO fact sheet 2013, salmonellosis, is one of the most common and widely distributed foodborne diseases, with tens of millions of human cases occurring worldwide every year. Most cases of salmonellosis are mild, however, sometimes people die from salmonellosis. The severity of the disease may depend on host factors and the strain of salmonella.

The results obtained from the antimicrobial screening shows that the drug DLD is highly sensitive at the concentration of 50µl, 60µl / well and low sensitive in 25µl/well against *Klebsiella pneumonia* similarly DLD is highly sensitive at the concentration of 50µl, 60µl/ well and moderately sensitive in 25µl/well against *Salmonella typhimurium*. Screening results against *Staphylococcus aureus* shows that DLD is highly sensitive at the concentration of 50µl, 60 µl / well and low sensitive in 25µl/well whereas with respect to *Bacillus subtilis* DLD is highly sensitive at the concentration of 25µl, 50µl, 60 µl / well. No significant sensitivity was observed in in the screening against *Proteus vulgaris*, *Enterobacter aerogens*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Oxygen-free radicals, more generally known as reactive oxygen species (ROS) along with reactive nitrogen species (RNS) are well recognized for playing a dual role as both deleterious and beneficial species. The production of ROS/RNS through either endogenous or exogenous insults is commonly known as oxidative stress. Oxidative stress in turn damages the cell membrane and leads to degenerative changes in tissues and organs [11].

In fast moving life style increased consumption of fast foods, lack of stress management ultimately contributes to increased production of reactive oxygen species in the body. This oxidative stress can lead to chronic diseases including cancer, diabetes, cardiovascular, neurological and pulmonary diseases [12]. The free radicals are scavenged from the body by various mechanisms to enable good functioning of the organs. Glutathione and vitamin E plays an important role in antioxidant defence against various free radicals.

The DPPH radical scavenging activity revealed that the presence of functional groups in *Dhasalavana Dhravagam.* May be functional group like phenolic group of DLD exerts antioxidant activity, they are responsible for properties like anti carcinogenic, anti-mutagenic, anti-inflammatory activity, apoptosis inducing and regulates carcinogen metabolism [13].The antioxidant potential of phenols, especially polyphenols scavenge the free radicals produced by oxidative stress, lipid peroxidation may damage the cells leading to cancer and neurodegenerative diseases like Parkinson's [14].

CONCLUSION

From the antimicrobial study it was concluded that DLD is highly sensitive against *Klebsiella pneumonia*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus subtilis* indicating its promising antimicrobial potency against selective gram-positive and gram-negative organism .Further the test drug fails to prove its antimicrobial activity against *Proteus vulgaris*, *Enterobacter aerogens*, *Escherichia coli* and *Pseudomonas aeruginosa.* From the investigation of DPPH radical scavenging assay of DLD it was concluded that the test drug has shown promising antioxidant activity ns exhibit significant percentage inhibition against DPPH radicals when compared to that of standard BHT. Hence by considering the potential of DLD in near future the *Dhasalavana dhravagam* may be explored for effective control and clinical management of several infectious and stress related disease in humans.

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