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EVALUATION OF ANTI BACTERIAL AND ANTI FUNGAL ACTIVITY OF SIDDHA HERBO MINERAL FORMULATION - KUSTA GAJA KESARI

E. Saranyapriya[1*], R. Chandrasekar[1], B. Abarna[1], S. Murugesan Sannasi[2], R. Madhavan[3]

1. PG Scholar, Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai, Tamilnadu, India. saranyapriyabsms@gmail.com,chandru.sekar5696@gmail.com, abu.abarna96@gmail.com,
2. Associate Professor, Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai, Tamilnadu, India. ss.drss@gmail.com
3. Professor and HOD, Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai, Tamilnadu, India. drmadhavanji@gmail.com

Corresponding Author:
Dr. E. Saranyapriya,
PG Scholar, Department of Nanju Maruthuvam,
National Institute of Siddha,
Tambaram Sanatorium
Email: saranyapriyabsms@gmail.com.

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ABSTRACT:

Kusta Gaja Kesari is a herbo-mineral Siddha formulation consisting of *abraga parpam, kantha chenduram, Aya chenduram* and *rasa parpam* which has the indication for skin diseases.The aim of the study is to evaluate the anti-bacterial and anti-fungal activity of *Kusta Gaja Kesari.* The Anti-bacterial and Anti-fungal activity of *Kusta Gaja Kesari* is evaluated by determining minimum inhibitory concentration (MIC) using resazurin microtitre assay. It was observed that the *Kusta Gaja Kesari* reveals significant activity against the pathogen *E-coli (MIC 250*µg*)* and *Staphylococcus aureus (MIC 250*µg*)* with the lowest MIC values when compared to that of *Staphylococcus aureus (MIC 500*µg*)* and *Pseudomonas aeruginosa* with MIC value of 500µg. Further the sample found less effective against *Streptococcus pyogenes* which is provoked with highest MIC value of about 1000 µg. Similarly, the samples demonstrate consistent anti-fungal activity against *Aspergillus Niger and Candida albicans – CA* with the MIC value of 250 and 500µg respectively. The present investigation shows that the Siddha Herbo mineral formulation *Kusta Gaja Kesari* reveals convincing Anti-bacterial and Anti-fungal activity among all tested organisms.

Keywords: Kusta Gaja Kesari, anti-bacterial activity, anti-fungal activity, skin disease, Siddha.

INTRODUCTION:

Siddha medicine is a traditional medicine has its roots in southern India. It treats the body as well as the mind and soul. Siddha medicine has its origin from plants, animals and minerals. As per Siddha literature skin diseases are classified into 18 types which are referred as *kuttam, kuttam* is also mentioned as *thol pini, thol noi* and *sarma noi*. *Kuttam* affects 7 *thathus* of the body such *as saram, senneer, oon, kozhupu, enbu, moolai and sukilam/sronitham* (Indian Tamil Language) [1]. Symptoms of *kuttam* includes hardening of skin, increased sweating, itching, hyper or hypopigmentation of skin, blister, *thinavu, thimir* or *moodam* (Indian Tamil Language) and irritation. In the Siddha medicinal system, there are many formulations for the treatment of skin conditions. *Kusta Gaja Kesari* is one of the formulations described in *Siddha Vaidhya Thirattu* for skin diseases [2].

Kusta Gaja Kesari is a Herbomineral Siddha formulation consisting of *abraga parpam, kantha chenduram, Aya chenduram* and *rasa parpam* triturated with the fruit juice of *Savuri pazham* (*Trichosanthes tricuspidate*) and pilled as *Payir alavu mathirai* indicated along with the vehicle sugar/honey for the treatment of skin diseases *(kuttam)* Diabetes and urogenital diseases (*megam)*. Most of the skin disease involves microorganism, So this study is about to evaluate the anti-fungal and anti-bacterial activity of Herbo mineral drug *Kusta Gaja Kesari* by determining minimum inhibitory concentration of *Kusta Gaja Kesari* using resazurin microtitre assay [3]. Anti-bacterial activity of *Kusta Gaja Kesari* against the bacteria, *Staphylococcus aureus, Streptococcus pyogenes, E. coli* and *Pseudomonas aeruginosa* are studied. Anti-fungal activity of *Kusta Gaja Kesari* against the fungi, *Aspergillus Niger* and *Candida albicans* are studied.

The drug *Kusta Gaja Kesari* has already proven for its Anti-oxidant [4], Anti-inflammatory activity [5] and Anti-diabetic activity [6]. Ingredients of the Herbo mineral formulation *Kusta Gaja Kesari* has been extensively studied in previous researches in recent years which are Instrumental standardization of the Herbo mineral drug- *abraga parpam* [7], Clinical evaluation of *paling abraga parpam* in management of diabetes mellitus [8] Standardization of *Kantha chenduram*[9], Toxicity study on *Kantha chenduram*[10], Evaluation of toxicity profiles of Siddha metallic preparation of *Aya chenduram* in laboratory animal [11], and scientific validation of potent anti-cervical cancer, anti-tumor and anti-oxidant activities of Siddha Herbo mineral formulation “rasa parpam” in-vitro studies [12]. *Trichosanthes tricuspidate* has proven to exhibit antioxidant, anti-inflammatory and anti-microbial activity[13, 14, 15, 16]. This research is evaluated to assess the Siddha formulation *Kusta Gaja Kesari* against various microbes using resazurin microtitre assay. Resazurin-based viability staining is commonly used to assess the viability of biofilm cells cultivated in microtiter plates (MTP). Resazurin, which is not fluorescent, is converted by metabolically active cells to resorufin, a fluorescent compound. The quantity of living cells present is correlated with the amount of fluorescence produced. This study is conducted to prove that this drug also has the anti-microbial activity.

MATERIALS AND METHODS

INGREDIENTS:

The formulation *KGK* consist of following ingredients

- *Abraga parpam*
- *Kantha chenduram*
- *Aya chenduram*
- *Rasa parpam*
- *Savuri pazham* (*Trichosanthes tricuspidate*)

PROCUREMENT OF THE DRUG:

The drug *Kusta Gaja Kesari mathirai* was procured from Ayothidoss Pandithar Hospital OPD-National Institute of Siddha.

PREPARATION OF SAMPLE:

After crushing the tablets, it is mixed with DMSO (Dimethyl Sulfoxide), it is a widely used solvent due to its ability to penetrate biological tissues and enhance the absorption of certain substances then this mixture is used further in this study.

RESAZURIN MICROTITRE ASSAY:

The resazurin microtiter assay is widely used method for determining the minimum inhibitory concentration (MIC) of antimicrobial agents against bacterial and fungal species. It is a colorimetric assay that relies on the reduction of resazurin, a blue dye, to its pink and colorless form by metabolically active microorganisms. The change in color indicates microbial growth and is used to assess the sensitivity of microorganisms to various substances.

A sterile 96-well plate was used as the experimental platform. The test was conducted under aseptic conditions. A sample in DMSO was prepared, and this sample is presumably a solution of the substance being tested for its antimicrobial activity.

METHODS:

A volume of 1000µl of the sample in DMSO was pipetted into the first well of the 96-well plate. Subsequently, 50% of the volume from the first well was transferred to each of the following wells, creating a serial dilution gradient of the sample across the plate. This dilution series allows for testing the substance at different concentrations. In parallel, other wells in the plate received 50µl of a nutritional broth that had been serially diluted. Nutritional broth serves as a growth medium for microorganisms and is used to assess microbial growth in the absence of the test substances. Following the addition of the sample and control substances, each well received 10µl of a resazurin indicator solution. To promote microbial growth, the 96-well plate was then incubated at 37°c for a period ranging from 24 to 48 hours. This incubation period allows microorganisms to grow and potentially interact with the test substance or control substances, After the incubation period, the color of each well was visually evaluated. Any change in color from purple to pink or colorless were recorded as positive value indicates microbial growth and suggests that the microorganisms in the well were not inhibited by the test substances at that particular concentration.

The MIC value, or the minimum inhibitory concentration, was determined to be the lowest concentration of the test substance at which a color change (from purple to pink or colorless) occurred. This concentration signifies the minimum amount of the test drug required to inhibit microbial growth. In this assay, common reference standards were used for assessment. Chloramphenicol, a well-known antibiotic was employed as a positive reference standard to assess the sensitivity of bacterial species. Fluconazole, a common antifungal medicine, was used to assess the sensitivity of fungal species.

RESULTS:

The *Kusta Gaja Kesari* reveals significant activity against the pathogen *E-coli* and *Staphylococcus aureus* with the lowest zone of inhibition values compared to that of *Staphylococcus aureus (MIC 500*µg*)* and *Pseudomonas aeruginosa* with MIC value of 500µg. Further the sample found less effective against *Streptococcus pyogenes* which is provoked with highest MIC value of about 1000 µg. Similarly, the samples demonstrate consistent anti-fungal activity against *Aspergillus Niger and Candida albicans – CA* with the MIC value of 250 and 500µg respectively. The results of the antibacterial and antifungal activities are presented in Tables 1 & 2**.**

Table 1 - Anti-Bacterial Activity- KGK - Growth of inhibition Chart for the Sample and Standard Drug

S. no	Sample/ Microorganisms	Growth of inhibition										
		W-1 1000 µg	**W-2** 500 µg	**W-3** 250 µg	**W-4** 125 µg	**W-5** 62.5 µg	**W-6** 31.2 µg	**W-7** 15.6 µg	**W-8** 7.8 µg	STD Chloramphenicol (10µg)	DMSO	Culture
1	***Staphylococcus aureus – SA***	-	-	+	+	+	+	+	+	-	+	+
2	***Streptococcus pyogenes – SP***	-	+	+	+	+	+	+	+	-	+	+
3	***E-coli - EC***	-	-	-	+	+	+	+	+	-	+	+
4	***Pseudomonas aeruginosa- PA***	-	-	+	+	+	+	+	+	-	+	+

Table - 2 Anti-Fungal Activities - Growth of inhibition Chart for the Sample and Standard Drug

S. no	Sample/ Microorganisms	Growth of inhibition										
		W-1 1000 µg	**W-2** 500 µg	**W-3** 250 µg	**W-4** 125 µg	**W-5** 62.5 µg	**W-6** 31.2 µg	**W-7** 15.6 µg	**W-8** 7.8 µg	STD Fluconazole (20µg)	DMSO	Culture
1	***Aspergillus Niger – AN***	-	-	-	+	+	+	+	+	-	+	+
2	***Candida albicans – CA***	-	-	+	+	+	+	+	+	-	+	+

Figure – 1 - 96 Well plate- Anti-Microbial Spectrum Image

Figure – 1 shows the 96 Well plate- Anti-Microbial Spectrum Image

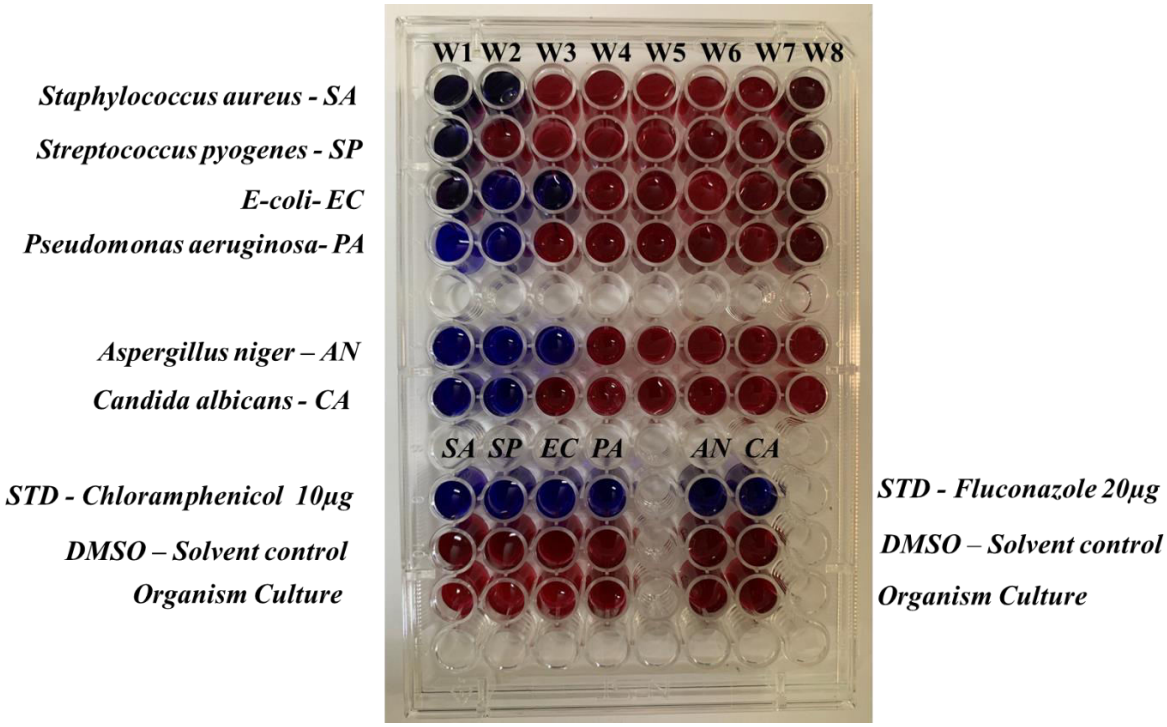
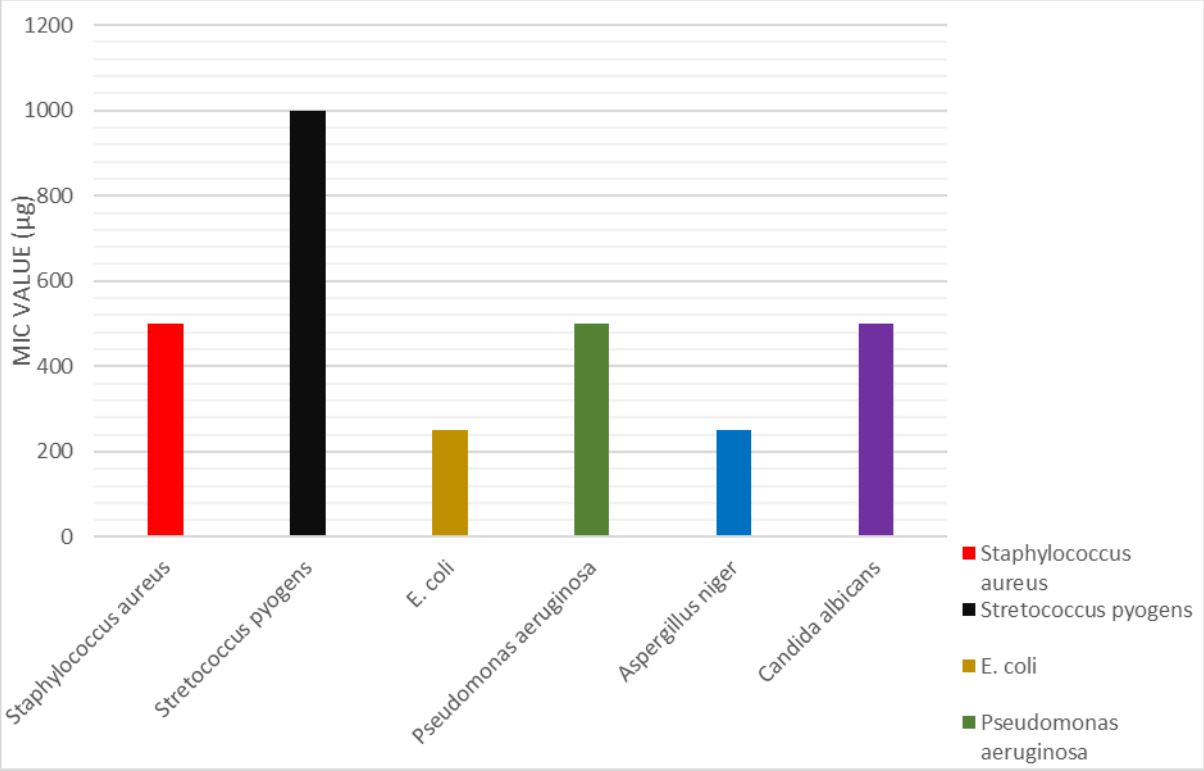


Table – 3 Report on MIC (Minimum inhibitory concentration) value of the Sample – KGK

S.no	Name of the Organism	MIC Value (µg)
1	***Staphylococcus aureus – SA***	500 µg
2	***Streptococcus pyogenes – SP***	1000 µg
3	***E-coli - EC***	250 µg
4	***Pseudomonas aeruginosa- PA***	500 µg
5	***Aspergillus Niger – AN***	250 µg
6	***Candida albicans – CA***	500 µg

Figure – 2 Graphical Representation of MIC of KGK on Micro-organisms



DISCUSSION

The above study investigates the antimicrobial activity of a drug KGK, against a variety of pathogenic microorganisms, both bacterial viz, *Staphylococcus aureus, Streptococcus Pyogenes, E. coli* and fungal viz, *Pseudomonas aeruginosa, candida albicans and aspergillus Niger* which are responsible for numerous human infections.The drug chloramphenicol is taken as standard drug for bacteria involved in this study; it shows anti-bacterial activity at the dosage of 10µg. The drug fluconazole is taken as standard drug for fungi involved in this study; it shows anti-fungal activity at the dosage of 20µg.

Staphylococcus aureus is a gram- positive bacteria it causes boils, skin sepsis, postoperative wound infection, scalded skin syndrome catheter- associated infection, food-borne infection, septicaemia, endocarditis, toxic shock syndrome, osteomyelitis and pneumonia [18,19]. The drug KGK kills *Staphylococcus aureus* at the dosage of 500µg. ***Streptococcus pyogene*s** is a gram-positive coccus which infects upper respiratory tract, skin and soft tissue (e. g. pharyngitis, cellulitis, erysipelas, lymphadenitis) [18, 20]. The MIC value of the drug KGK for *Streptococcus Pyogenes* is 1000µg. ***Escherichia coli*** is a gram-negative bacteria, it causes urinary tract infection, diarrheal diseases, neonatal meningitis and septicemia [18, 21]. The MIC value for E. coli is 250µg. ***Pseudomonas aeruginosa*** is a gram-negative rod-shaped bacteria, it causes infections of skin and burns, it is major lung pathogen in cystic fibrosis and can cause pneumonia in intubated patients. It can also cause urinary tract infection, septicemia, osteomyelitis and endocarditis [18,22]. The MIC value observed for *pseudomonas aeruginosa* is 500µg. As represented in Table 3 and Figure 2.

Candida albicans is a dimorphic fungus produces opportunistic infections in stresses, suppressed and antibiotic treated individuals. It causes candidiasis and thrush [18, 23]. The observed MIC value for candida albicans is 200µg. ***Aspergillus Niger*** is a filamentous fungi causing opportunistic infections in immune-compromised patients, invade lungs and blood

vessels, causes aspergilosis [18, 24]. The MIC value for aspergillus is 500µg. As represented in Table 3 and Figure 2.

Previously several medicines have been tested for antimicrobial activity against the above mentioned pathogens. Anitha akilan et al, studied the antimicrobial activity of kungiliya parpam, silasathu parpam, vengara parpam and padigara parpam at 0.1mg against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.* The study results showed resistance of Kungiliya parpam and silasathu parpam against all the pathogens were as vengara parpam and padigara parpam showed 11-14mm MIC [25]. Kokila et al, assessed the antimicrobial activity of *pattai chooranam* against *Escherichia coli, Staphylococcus aureus, Candida albicans* which showed resistance of the drug against the pathogens [26].

The finding of this study shows that *KGK* has significant antimicrobial activity against a range of pathogenic microorganisms, both bacteria and fungi. From the above results it is evident that the drug is more effective against streptococcus pyogenes. Further more studies could be conducted for evaluating the potential effects. These microorganisms are responsible for a variety of infections, including skin infections, respiratory tract infections, urinary tract infections and fungal diseases. The MIC values observed in this research are notably lower than the dose (100mg) prescribed in the Ayothidoss Pandithar Hospital, National Institute of Siddha Outpatient Department (NIS-OPD). This finding suggests that the current prescribed dosage of *KGK* may be higher that what is necessary to treat these infections. It implies that lower doses of *KGK* may be equally effective, which could be of significance in terms of reducing the treatment cost. While the results are promising, it's essential to conduct further research, including clinical trials and toxicity studies, to confirm safety and efficacy of *KGK* in treating these infections. The study underscores the potential of *KGK* as a significant antimicrobial agent against a various pathogens. Its efficacy at lower doses than currently prescribed suggests a re-evaluation of dosing strategies could be beneficial, potentially leading to cost-effective treatments. However, comprehensive clinical and safety evaluations are essential to confirm the role of *KGK* in treating these infections effectively.

CONCLUSION

It was observed from the results of the present investigation that the Siddha Herbo mineral formulation *Kusta Gaja Kesari* reveals significant antimicrobial activity against a broad range of pathogens, with varying MIC values indicating different levels of sensitivity. While it is particularly effective against *Streptococcus pyogenes* at higher concentrations, its lower MIC values for *E. coli* and *A. Niger* indicate strong potential as an antimicrobial agent. Further research is essential to fully understand its capabilities and optimize its use in clinical settings.

The drug *KGK* is already indicated for *Kutta noigal* in the Siddha literature S*iddha Vaidhya Thirattu.* From this study we scientifically proven that *KGK* inhibits the microbial activity and it will be useful in treating various skin diseases.

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Nil

CONFLICT OF INTEREST

All the authors declare that there is no conflict of interest.

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