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Antimicrobial Efficacy of Gandhagam (Raw Sulphur), Purified Gandhagam and Gandhaga Mezhugu - A Traditional Siddha Formulation

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Siddha system is one of the traditional systems of India, contributing towards the health care of human society. In the present work one of the herbo mineral preparations Gandhaga Mezhugu, especially used in skin diseases is evaluated for its antimicrobial potential. Antimicrobial efficacy of gandhagam (Raw sulphur) as well as, purified gandhagam and gandhaga mezhugu were evaluated against six pathogens *E. coli, P. vulgaris, K. pneumoniae, S. aureus, S. mutans, C. albicans* which are associated with various disease conditions. The agar well diffusion was used to determine the sensitivity of the samples, whilst the micro-dilution method was used for the determination of the minimal inhibition concentration (MIC). Of the samples assayed, the samples of gandhaga mezhugu were observed to be the more effective against all the tested pathogens. The results provided evidence that the studied samples might be potential sources of new antimicrobial drug.

Key words : Gandhagam, Sulphur , Agar well diffusion method, MIC, MMC.

Siddha system of medicine is a unique Indian system of medicine which is very potent and provides comforts to the body, mind and soul. This system was founded by 'Siddhars' who are known for their versatile abilities. They were the masters in natural chemistry and formulated medicinal preparations uti1lizing various natural resources such as Plants, animals and minerals. Gandhagam (sulphur) is a crystalline, non-metal used in the Siddha formulations, such as a Rasayanam (Semi dry powder), Mathirai (Pills), Mezhugu (Waxy medication), Parpam (Calcined powder) and Chenduram (Red powder). It possesses bitter and astringent taste.

Traditionally Sulphur in Tamil language, is synonymously known as Gandhagam, Kaarizhai Natham, Parai natham, Parai Veerayam, Atheetha prakasam, Beejam, Selvi vindhu, Sakthi, Sakthi peesam, Chendurathaathi, Theviuram, Natham, Naatram, Parai natham, Ponnvarni, Rasa sronitham[1]. Gandhagam (sulphur) before it is used in medicinal preparation has to be purified by different methods of purification which are available in the Siddha system. It is an invariable component of majority of Siddha formulations. There are 64 varieties of formulations of which 32 are internal medicines and 32 being used as external ones. Of those formulations, 'Mezhugu' is an internal medicine which is defined to have waxy consistency with Shelf life of 5years. It can be prepared by mechanical treatment (like rubbing), Phyto treatment, Photo treatment, Heat treatment, Acid treatment, salt treatment etc. In the present work, two such well known Siddha purification methods were adopted for the purification of Gandhagam . (Sulphur). Purification in Siddha system means not only eliminating the unwanted trace elements,

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but also priming the raw materials fit enough in the preparation of formulated drug. The appearances of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection[2-5].

In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is a need to search new infection fighting strategies to control microbial infections[6]. In the present study attempts were made to develop a good antimicrobial agent from naturally available sources. Based on the Siddha literature Gandhagam, purified Ganthagam and Ganthaga Mezhugu- Finished drug formulation of Ganthagam samples were selected and evaluated for their antimicrobial effect against various pathogens.

MATERIALS AND METHODS

Purification of Gandhagam

The Purification of Gandhagam involves two processes they are,

Process I

Gandhagam was melted with cow's butter and poured into juice of Banana rhizome and after cooling sulphur was collected .This process was repeated for ten times with fresh juice every time. At the end of the purification process, a total number of 11 samples were obtained, out of these 11 samples 1 of the sample was subjected to antimicrobial screening[7].

Process II

In this process the leaves of *Lawsonia inermis* (Maruthani) was ground in a stone mortar and mixed with cow's curd. The mixture was kept in a mud pot and covered with cotton cloth and sulphur was kept over the cloth. The pot was closed with the lid and covered with a cloth dipped in mud paste and sealed completely. The pot was buried in the earth up to the mouth level and cow dung cakes arranged over the mouth of the pot. Then the fire was set on cow dung cakes and the sulphur kept above the cloth melted due to heat and got collected at the bottom of the pot. The processed sulphur was taken out and this process was repeated for 7 times with fresh mixture each time. At the end of the purification process, a total number of 7 samples were obtained, out of these 7 samples 1 of the sample was tested against various microorganisms[7].

Preparation of Gandhaga Mezhugu

The selected purified samples for antimicrobial studies from the above two purification process were subjected to Mechanical treatment (rubbing). In the mechanical treatment, samples were taken and grounded separately in the electrically operated stone mortar (Kalvam) with the addition of white onion juice little by little for 5days. After five days ginger juice was also added and grounded for 5 days. Finally the Gandhaga mezhugu was obtained as a waxy consistency after the process of mechanical treatment, and then they are preserved in air tight bottles[8].

Determination of Antimicrobial activity

Microbial strains

The bacterial strains such as *E. coli, P. vulgaris, K .pneumoniae, S. aureus, S. mutans* were used for the antimicrobial screening were suspended in Nutrient broth and incubated for 24hrs at 37°C. The Yeast strain *C. albicans* was suspended in Potato dextrose broth and incubated for 3 days at room temperature.

Preparation of Samples

Sample 1 (Gandhagam),

Sample 2 (Gandhagam purified by process I),

Sample 3 (Gandhagam purified by process II) were dissolved using Carbon Disulphide (CS 2). Final concentrations of 50mg/ml, 100mg/ml, and 150mg/ml were used respectively for our studies.

Sample 4 (Gandhaga mezhugu prepared from purified gandhagam by process I) and

Sample 5 (Gandhaga mezhugu prepared from purified gandhagam by process II) were dissolved using water to make a final concentration of 50mg/ml, 100mg/ml, and 150mg/ml respectively.

Agar Well Diffusion Method

The Muller Hinton agar plates were prepared by pouring 15 mL of molten media into sterile Petri plates and allowed to solidify for 5min. Then 0.1% of inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 min. In each of these plates, wells were made using sterile cork borer. Different concentrations of samples (50mg/ml, 100mg/ml, and 150mg /ml) were loaded on to each well, simultaneously the

standard antibiotic discs, chloramphenicol was placed in each of the plates containing bacterial and yeast strain. The plates were allowed to diffuse at room temperature for 2hrs. The bacterial strain inoculated plates were incubated at 37°C for 18–24 h and yeast strain inoculated plates were incubated at room temperature for 3 days. After incubation, diameter of the zone of inhibition (ZOI) was measured with transparent ruler.

Minimum Inhibitory Concentration (MIC)

MIC of the test samples was determined according to the macro-broth dilution technique[9]. For this study standardized suspensions of the test organisms were inoculated in a series of sterile tubes containing Muller Hinton broth and twofold dilutions of samples. The bacterial strain inoculated tubes were incubated at 37oC for 24 h and yeast strain inoculated tubes were incubated at room temperature for 3 days.

RESULTS AND DISCUSSION

The antimicrobial efficacy of these samples was initially evaluated by the agar well diffusion method10, using six strains *E. coli, P. vulgaris, K. pneumoniae, S. aureus, S. mutans, C.albicans*. Table.1 shows the zones of inhibition (mm) of samples at different concentrations. It was found that the Sample 1 gave a zone of inhibition of around 2-11 mm, showing good activity against all organisms. Sample 2 gave a zone of inhibition of around 2-7mm, showing good activity against *C.albicans* and less activity to other all organisms. Sample 3 gave a zone of inhibition of around 2-10 mm, showing good activity to all organisms. Sample 4 gave a zone of inhibition of around 2-9 mm, showing good activity against all organisms except *E.coli*. Sample 5 gave a zone of inhibition of around 2-9mm, showing good activity against all organisms. All the tested sample reveals better activity except Sample 2.

Table.2 shows the Minimum inhibitory concentration (MIC) of the tested sample. The end result of the test gave a clear solution, i.e., no visual growth[11-12]. If samples displayed an MIC less than 100 µg/mL, the antimicrobial activity was considered as good; from 100 µg/mL to 500 µg/mL the antimicrobial activity was moderate; from 500µg/mL to 1000 µg/mL the antimicrobial activity was weak; over 1000 µg/mL the extract was

Table 1. Agar Well Diffusion of Gandhagam samples in the concentration of (mg/ml)

Microorganisms	Zone of Inhibition (ZOI) (mm)															
	Sample 1			Sample 2			Sample 3			Sample 4			Sample 5			Chloramp-henicol 10 µg
	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	
E. coli	2	5	6	2	3	3	4	6	10	3	4	4	2	6	8	16
P. vulgaris	3	8	9	3	4	4	3	3	6	3	7	8	5	9	9	11
K. pneumoniae	4	5	7	2	3	3	4	7	9	2	4	5	5	7	7	13
S. aureus	2	6	6	3	5	5	6	9	9	4	4	5	4	6	6	8
S. mutans	2	5	5	3	4	4	5	8	9	5	6	8	3	5	8	19
C. albicans	6	8	11	2	5	7	2	6	9	5	9	9	3	7	9	Nil

Table 2. Minimum inhibitory concentration (MIC) of Gandhagam samples in the concentration of (µg/ml)

Microorganism	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
E. coli	NA	400	800	600	600
P. vulgaris	400	600	600	200	200
K. pneumoniae	NA	>1000	400	600	600
S. aureus	>1000	800	600	800	NA
S. mutans	NA	NA	800	200	200
C. albicans	60	NA	30	NA	90

NA: no activity

considered inactive. Sample 1 presented a good activity against *C. albicans*, with MIC values of 60µg/mL, but was inactive against *S. aureus* (>1000 µg/mL). Sample 3 and 5 showed a good activity against C. *albicans,* with MIC values of 30µg/mL and 90µg/mL.

CONCLUSION

The study included the Siddha purification processes of Gandhagam (Sulphur) and antimicrobial efficacy of one sample of Ganthagam, two samples of Ganthagam purified by Siddha way and two samples of formulated Ganthaga Mezhugu drugs prepared from above purified samples (altogether 5 samples) against six pathogens namely *E. coli, P. vulgaris, K. pneumoniae, S.aureus, S. mutans, C. albicans* which are associated with various disease conditions. Reports of this current work concluded that, Gandhagam, purified Ganthagam intermediates and processed Ganthaga Mezhugu samples have a good antimicrobial efficacy against various pathogens. They can be suggested as potential sources of emerging antimicrobial agents. So far as our literature survey could confirm, a little scientific information was available about Gandhagam and it is the first time that purification process and antimicrobial efficacy of various pathogens were subjected to these studies.

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