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Research Article

ANTIMICROBIAL STUDY OF GANDHAKA TAILA

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ABSTRACT

Microbiology is developed as an established science since last two centuries. Earlier the investigations were confined to observation of the effect of various drugs on the whole body. With the development of microbiological techniques, it has now become possible to investigate the action of the drug on isolated micro-organisms i.e. in vitro antimicrobial susceptibility testing. Since long, good number of Ayurvedic classical preparations were being used in cases of infection and they were found to be effective clinically. Gandhaka taila is one such a herbo-mineral oil based formulation, which is mentioned in the treatment of Karnasrava as a topical agent. In this study, antimicrobial study of Gandhaka taila was conducted against various micro-organisms which are responsible for chronic suppurative otitis media (CSOM). Except Haemophillus infiuenza and Klebsiella pneumonae other microorganisms had shown good sensitivity to Gandhaka taila.

Keywords: Microbiology, Gandhaka taila, Antimicrobial study, CSOM

INTRODUCTION

The history of infection and infectious diseases is as old as mankind. The references of microorganisms are available in oldest manuscripts of Ayurveda and Vedas as well. In Athervaveda, references are available regarding microbes and infectious diseases in the name of krimi and krimirogas. According to Shabdhakalpadruma, the meaning of krimi is derived as "Kramatitikrimi" means that which moves or roams about. There are lot of descriptions of microbes found in Ayurvedic texts and mentioned as krimi, jantu, bhuta, rakshas, pishacha, which effects the human beings and cause serious diseases[1]. The term collectively means those organisms which produces toxins. These toxins are supposed to cause diseases in different ways. While describing Vishama jwara Acharya Charaka seems except this theory[2].Acharya Sushruta has accepted the involvement of agantuja nidana for the occurrence of Vishama jwara[3]. The mode of spread of infectious diseases has been described clearly by Acharya Sushruta as through breath, touching the body, sexual intercourse, eating together and even by sharing the same bed and sharing clothing etc. Acharya Charaka has given very good explanation of mode of spread in case of Rajahakshma, that seems to similar to that we are observing today by means of droplet infection[4]. It is evident from the above description that the great Indian sages had the knowledge of mode of spread, fate of microorganisms and how to combat the diseases since long back. Many scholars have successfully tried to correlate these krimi's with some strains of pathogenic microbes. But without experimental research validation of claims of Ayurveda is not possible. Because many concepts in Ayurveda are not clear, some are implicit, some mentioned as pointer or passing remark and some raise doubts.

Infectious diseases are the consequences of host and microorganisms interaction. These microorganisms are present everywhere, even though they are present, only few of the thousands invade, multiply and produce diseases in the human beings. Severity of the disease depends on the virulence of the invader and resistance offered by the host. Antibiotic resistance has become a global concern. The clinical efficacy of many existing antibiotics is threatened by the emergence of multi drug resistant pathogens. There is a continuous and urgent need to discover new anti-microbial compounds with diverse chemical structure and novel mechanisms of action for new and emerging infectious diseases. Therefore researches are increasingly turning their attention to fold medicine, looking for new leads to develop better drug against microbial infection. The increase in failure of chemotherapeutic agents, their hazardous effect and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several herbal or herbo-mineral formulations for their potential anti-microbial activity.

Chronic supportive Otitis media (CSOM) is an infective disorder, is the result of initial episode of acute Otitis media and characterised by a persistent ear discharge from the middle ear through a tympanic perforation. It is an important cause of preventable hearing loss. The commonly found organisms responsible for CSOM are *Pseudomonas aeruginose, Escherichia coli, Staphylococcus aureus, Streptococcus pyogens, Streptococcus mutants, Haemophillus influenza, Kelbsiella pneumonia*[5]. These microorganisms exhibit great strain variations in susceptibility to antimicrobial agents. Therefore it is essential to determine the susceptibility of isolates of pathogenic organisms to anti-microbial agents that are likely to be used in the treatment.

Gandhaka taila is a herbo-mineral oil based medicine specified by Yogaratnakara[6]as a topical agent in Karnasrava. This comprises of purified Manhshila, purified Gandhaka, Haridra, juice of Dattura leaves and Sarshapataila, prepared with the procedure of snehapaka. Being a formulation of drugs having anti-microbial properties in combination because of synergism it may act as an effective anti-microbial agent in CSOM. Hence the anti-microbial study of Gandhaka taila was conducted against various microorganisms which are responsible for CSOM. Prolonged installation of oil into the ear may lead to the growth of fungus in the ear, hence along with antibacterial study antifungal study also had been carried out on fungus named *Aspergillus niger* the one which is commonly seen in ear.

MATERIALS AND METHODS

Source of data

Raw drugs required for the murchana of Sarshapataila and preparation of Gandhaka taila were purchased from the Udupi market. Procedures like purification of Gandhaka as well as Manhashila, extraction of Datturapatra swarasa and preparation of Gandhaka taila were carried out at Rasashastra and Bhaishajya Kalpana practical laboratory, SDMCA, Udupi. Anti-microbial study was carried out at SDM Research centre of Ayurveda and Allied sciences, Udupi, Karnataka.

Objectives of the study

- To evaluate the anti-bacterial activity of Gandhaka taila
- To evaluate the anti-fungal activity of Gandhaka taila

Method

Broth dilution method

Micro-organisms tested

Both gram positive and gram negative bacteria and fungus were used as test organisms for the study.

Gram positive organisms – 1.*Staphylococcus aureus*
2. *Streptococcus mutants*
3. *Streptococcus pyogens*

Gram negative organisms –1. *Pseudomonas aeruginosa*
2. *Escherichia coli*
3. *Haemophillus influenza*
4. *Klebsiella pneumonia*

Fungus - 1. *Aspergillus Niger*

Standards used for the study

1. Ampicillin 100 µl (1000µl/ml) was used for antibacterial study against *Staphylococcus aureus, Streptococcus mutants, Streptococcus pyogens, Escherichia coli, Haemophillus influenza, Klebsiella pneumonia*.
2. Gentamycin 100 µl (1000µl/ml) was used for antibacterial study against *Pseudomonas aeruginosa*.
3. Fluconozole100 µl (1000µl/ml) was used for antifungal study.

Antibacterial study: Working area was cleaned in luminary air flow using 70% ethyl alcohol and switching on UV for 20minutes. Sterile nutrient broth media was prepared in conical flask. One loop of microbial culture was diluted in 4ml of nutrient broth and after mixing incubated at 37° C for 24 hours. Next day 4ml of the broth containing the organism was transferred in to respective test tubes labelled as positive control, negative control and test. 100 µl of 0.9% saline was added in to the test tube labelled as negative control and 100 µl standard drug (1000 µg/ml) into positive control. Different volumes of test drugs (25µl, 50µl, 100µl, 150µl) were added into respective labelled test tubes. After proper mixing incubated in BOD incubator at 37°C for 24 hours. From each test tube 200µl of culture was loaded into the respective well of the 96 well plates and optical density was measured at 630 nm using ELISA reader. Same experiment was carried out in triplicate.

Antifungal study: Working area was cleaned in luminary air flow using 70% ethyl alcohol and switching on UV for 20minutes. Sterile nutrient broth media was prepared in conical flask. One loop of Aspergillus niger from culture was diluted in 2ml of nutrient broth and after mixing incubated at 30°C for 24hours. Next day 2ml of the broth containing the organism was transferred in to respective test tubes labelled as positive control, negative control and test. 100 µl of 0.9% saline was added in to the test tube labelled as negative control and 100 µl standard drug (1000 µg/ml) into positive control. Different volumes of test drugs (25µl, 50µl, 100µl, 150µl) were added into respective labelled test tubes. After proper mixing incubated in BOD incubator at 30°C for 6 days. After completion of incubation time, the mycelium was taken from each tube and dried. The dry weight of mycelium corresponds to the growth of the fungus which is determined separately and difference in the weights from that of control was recorded. Same experiment was carried out in triplicate.

RESULTS

Inhibition of bacterial growth was calculated by comparing the optical densities of the negative control with the positive control and test drug tubes. Dry weight of mycelium was taken as a measure of growth of Aspergillus niger and inhibition of growth was calculated by comparing the weight of the negative control with the positive control and test drug tubes. Formula of calculation of percentage of inhibition is

% inhibition = (negative control – treated / negative control) X 100

From the results in Table 1, it is found that the Gandhaka taila at dosage level 25µl has higher and at dosage level 50µl has less antibacterial activity when compared to the other dosage levels tried.

From the optical density values it is inferred that Gandhaka taila at 25µl, 50µl, 100 µl dosage levels has better activity against *Streptococcus mutans* when compared to positive control. (Table 2)

From the results in Table 3, it is inferred that Gandhaka taila at 50µl, 100 µl, 150µl dosage levels has higher antibacterial activity than positive control drug Ampicillin. And at 25µl dosage level has mild antibacterial activity.

From the results in Table 4, it is inferred that Gandhaka taila at different dosage levels has higher antibacterial activity when compared to positive control. Antibacterial activity is increased with increased quantity till 100 µl and decreased in 150µl.

From the results in Table 5, it is found that Gandhaka taila with different dosage levels tried having better activity against *Escherichia coli* when compared to the positive control.

From the results in Table 6, it is inferred that Gandhaka taila at different dosage levels does not have antibacterial activity against *Haemophillus influenza*.

From the results in Table 7, it is inferred that Gandhaka taila at different dosage levels does not have antibacterial activity against *Klebsiella pneumonia*.

It is observed that the growth of *Aspergillus niger* was inhibited by different quantities Gandhaka taila and inhibition is quantity dependent. The maximum percentage of inhibition was observed when 150µl of oil was used. (Table 8)

Table 1: Antibacterial sensitivity test against *Staphylococcus aureus*

Sl.No.	Woking sample concentration	OD at 630nm			Mean	Mean±SD
1	- Control	0.5495	0.5123	0.5111	0.524	0.524±0.02183
2	+ Control	0.2284	0.2333	0.2304	0.230	0.230±0.00246
3	25µl	0.2141	0.2405	0.2334	0.229	0.229±0.01366
4	50µl	0.2203	0.2583	0.2986	0.259	0.259±0.03916
5	100 µl	0.2495	0.2375	0.2406	0.242	0.242±0.00622
6	150µl	0.2353	0.2675	0.2133	0.239	0.239±0.02726

Table 2: Antibacterial sensitivity test against *Streptococcus mutans*

Sl.No.	Woking sample concentration	OD at 630nm			Mean	Mean±SD
1	- Control	0.3795	0.3649	0.3611	0.368	0.368±0.00971
2	+ Control	0.3528	0.3558	0.3581	0.355	0.355±0.00265
3	25µl	0.3086	0.3032	0.2947	0.302	0.302±0.00700
4	50µl	0.3087	0.3146	0.3123	0.311	0.311±0.00297
5	100 µl	0.3484	0.3436	0.3413	0.344	0.344±0.00362
6	150µl	0.3945	0.4020	0.4245	0.407	0.407±0.01561

Table 3: Antibacterial sensitivity test against *Streptococcus pyogenes*

Sl.No.	Woking sample concentration	OD at 630nm			Mean	Mean±SD
1	- Control	0.3687	0.3590	0.3596	0.362	0.362±0.00543
2	+ Control	0.3523	0.3541	0.3341	0.346	0.346±0.01106
3	25µl	0.3512	0.3502	0.3628	0.355	0.355±0.00700
4	50µl	0.3094	0.3359	0.3844	0.343	0.343±0.03803
5	100 µl	0.3284	0.3138	0.3055	0.316	0.316±0.01159
6	150µl	0.3148	0.3144	0.3039	0.311	0.311±0.00618

Table 4: Antibacterial sensitivity test against *Pseudomonas aeruginosa*

Sl.No.	Woking sample concentration	OD at 630nm			Mean	Mean±SD
1	- Control	0.3029	0.3207	0.3118	0.311	0.311±0.00890
2	+ Control	0.2139	0.2297	0.2237	0.222	0.222±0.00797
3	25µl	0.2075	0.2351	0.2133	0.219	0.219±0.01455
4	50µl	0.2131	0.2213	0.2197	0.218	0.218±0.00434
5	100 µl	0.2195	0.2204	0.2041	0.215	0.215±0.00916
6	150µl	0.2150	0.2161	0.2274	0.220	0.220±0.00686

Table 5: Antibacterial sensitivity test against *Escherichia coli*

Sl.No.	Woking sample concentration	OD at 630nm			Mean	Mean±SD
1	- Control	0.4082	0.4080	0.4043	0.406	0.406±0.00219
2	+ Control	0.3841	0.3749	0.3681	0.374	0.374±0.00665
3	25µl	0.3970	0.3902	0.3083	0.365	0.365±0.04937
4	50µl	0.3310	0.3046	0.3895	0.341	0.341±0.04345
5	100 µl	0.3020	0.3057	0.4586	0.355	0.355±0.08936
6	150µl	0.3066	0.3567	0.4075	0.356	0.356±0.05045

Table 6: Antibacterial sensitivity test against *Haemophillus influenza*

Sl.No.	Woking sample concentration	OD at 630nm			Mean	Mean±SD
1	- Control	0.1287	0.1258	0.1264	0.127	0.127±0.00153
2	+ Control	0.1148	0.1233	0.1221	0.120	0.120±0.00460
3	25µl	0.4049	0.3541	0.4092	0.389	0.389±0.03065
4	50µl	0.6156	0.6743	0.6934	0.661	0.661±0.04054
5	100 µl	0.7466	0.7538	0.7165	0.739	0.739±0.01979
6	150µl	0.7367	0.8692	0.8709	0.825	0.825±0.07699

Table 7: Antibacterial sensitivity test against *Klebsiella pneumonia*

Sl.No.	Woking sample concentration	OD at 630nm			Mean	Mean±SD
1	- Control	0.3853	0.3701	0.3474	0.367	0.367±0.01907
2	+ Control	0.2523	0.2500	0.2674	0.256	0.256±0.00945
3	25µl	0.4177	0.4269	0.4062	0.417	0.417±0.01037
4	50µl	0.4406	0.4452	0.4212	0.435	0.435±0.01274
5	100 µl	0.4396	0.4309	0.4492	0.439	0.439±0.00915
6	150µl	0.3946	0.4387	0.4476	0.427	0.427±0.02838

Table 8: Antifungal sensitivity test against *Aspergillus Niger*

Sl.No.	Woking sample concentration	Dry weight of mycelium (gm)			Average	% of inhibition
1	- Control	0.018	0.021	0.022	0.020	-
2	+ Control	0.011	0.013	0.012	0.012	40
3	25µl	0.013	0.017	0.018	0.016	20
4	50µl	0.018	0.017	0.022	0.019	05
5	100 µl	0.011	0.011	0.014	0.012	40
6	150µl	0.006	0.011	0.012	0.010	50

Table 9: Antibacterial and antifungal study

No.	Strains	Percentage inhibition				
		Positive	**25** µl	**50** µl	**100** µl	**150** µl
1.	*St. aureus*	56.106	56.297	50.572	53.816	54.389
2.	*Str. mutans*	3.532	17.934	15.489	6.521	-10.597
3.	*St. pyogenes*	4.419	1.933	5.248	12.707	14.088
4.	*Pseudomonas*	28.617	29.581	29.903	30.868	29.260
5.	*E.coli*	7.881	10.098	16.009	12.561	12.315
6.	*H. influenza*	5.511	-206.29	-420.472	-481.889	-540.606
7.	*K. pneumonia*	30.245	-13.623	-29.427	-19.618	-16.348
8.	*Aspergillus*	40	20	05	40	50

DISCUSSION

Nowadays, infections continue to be one of the most common causes of diseases and death. In order to avoid different infections there are lots of antibiotics which are derived from the microbial sources in synthetic manner. However all the synthetic microbial agents are local irritants and are responsible for hyper sensitivity reactions. Second important thing is, antibiotics from the microbial sources have become ineffective and the infectious organism develops resistant against them. The quest for newer treatments continues as the realm of research for modern diseases expanding to herbal and herbo-mineral Ayurvedic products. This has led to an increased demand in Ayurvedic sector leading to the research for several newer formulations of newer applications of the already mentioned classical formulations.

Chronic supportive Otitis media mainly characterised by ear discharge is caused by the invasion of pyogenic organisms, results in the inflammation of middle ear cleft. Chronic supportive Otitis media is still prevalent around the world despite of advances in public health and medical care. It is most common in developing countries, especially among the poor because of poor socio-economic standards, poor nutrition and lack of health education. Most of the treatment have been unsatisfactory or very expensive and difficult.
As per recent study on CSOM, antibiotic ear drops were found more effective in resolving otorrhoea and eradicating middle ear bacteria[7].

Gandhaka taila is one such a formulation mentioned to treat ear discharge, is prepared by using Sarshapataila as a base. This Sarshapataila was processed with other drugs like purified Manhashila, purified Gandhaka, Haridra and juice of Dattura leaves. While explaining the properties of Sarshapataila it is mentioned as karnya, krimighna, and kothagna which means Sarshapataila alone is sufficient to cure the ear infection. When such oil mixed with other krimighna drugs definitely better efficacy can be expected. According to Ayurveda, the disease Karnasrava is mainly caused by vitiation of kapha and vatadosha, hence kaphavatahara properties of all these ingredients helps in resolving Karnasrava. Tikta, Katy, kashaya rasa, ushnaveerya, rukshaguna of all the ingredients facilitated in getting positive result in Karnasrava[8].

There are a number of antimicrobial susceptibility testing methods available to determine the bacterial susceptibility to antimicrobials. Two tests that are most commonly used are Diffusion method and Broth dilution method[9]. Though the Diffusion method is relatively economical and simple, it provides only qualitative or semi qualitative information on the susceptibility of a given microorganism to a given antimicrobial agent. Whereas Broth dilution method provides both qualitative and quantitative information and it can be applied to wide range of isolates than the Diffusion method.

As Gandhaka taila is an oil based formulation, because of lack of proper diffusion through agar media, Broth dilution method was selected for the study.Broth dilution method is based on inhibition of microbial growth as indicated by measurement of transmittance of suspensions of a suitable microorganism in a fluid medium, to which have been added graded amounts of the test compound. Changes in the transmittance produced by the test compound are compared with those produced by known concentrations of standard[10].

In vitro antimicrobial study of Gandhaka taila has shown antimicrobial activity against gram positive organisms such as *Staphylococcus aureus*, *Streptococcus pyogens*, *Streptococcus mutans* and gram negative organisms such as *Pseudomonas aeruginosa*, *Escherichia coli* which are responsible for Chronic supportive Otitis media. Compared with the positive control

Gandhaka taila has shown significant percentage of inhibition against these organisms. Percentage inhibition of bacterial growth varied with quantity of oil (concentration). Maximum percentage inhibition was observed against streptococcus aureus and *Pseudomonas arruginosa*. But in case of organisms like Haemophillus influenza, *Klebsiella pneumonia* Gandhaka taila didn't show inhibitory action.

According to modern science, long term installation of antibiotic drops or oil into the ear leads to the growth of fungus especially *Aspergillus niger*. But Gandhaka taila has shown significant antifungal activity against *Aspergillus niger*. Inhibition of fungal growth by Gandhaka taila was found to be quantity dependent. Compared to positive control, Gandhaka taila has shown significant inhibition against the growth of *Aspergillus niger*.

CONCLUSION

Gandhaka taila has got significant anti-bacterial activity against gram positive and gram negative organisms which are responsible for Chronic supportive otitis media exception is *Haemophillus influenza, Klebsiella pneumonia*. Installation of Gandhaka taila into the ear does not lead to the growth of fungus named *Aspergillus niger*, as it has also got significant antifungal activity.

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