



## Research Article

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### ANTIMICROBIAL STUDY OF GANDHAK TAILA: AN AYURVEDIC WOUND HEALER' ON *STAPHYLOCOCCUS AUREUS*, *ESCHERICHIA COLI* AND *CANDIDA ALBICANS*

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Received on: 23/02/19 Accepted on: 15/05/19

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DOI: 10.7897/2277-4343.100361

#### ABSTRACT

Gandhak has been used as a potent medicine in Ayurvedic system of treatment for various kinds of skin manifestations of different fungal as well as bacterial origin. This miraculous medicines is used both internally or/and topically, in different dosage form as suitable for the patient as well as diseases. In the current scientific work, effect of Gandhak in taila form is assessed for antimicrobial effect on *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, in, in-vitro study. In the culture medium, growth of strains of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* such as *Staphylococcus aureus* [ATCC6538], and *Escherichia coli* [ATCC8739] and *Candida albicans* [ATCC1023] as well as in broth nutrient are found negative when inoculated with Gandhak taila.

**KEY WORDS:** Gandhak taila, Antimicrobials, Ayurvedic wound healer

#### INTRODUCTION

Ayurveda the most ancient healing heritage of the world is serving the ailing society with the indigenous medicines of herbal, mineral and metallic origin. The medicines used in Ayurveda for various diseases are time tested with respect to their efficacy and safety. Now in traditional medical system, in the way of reverse pharmacology, medicines in the clinics are taken back to the laboratory for their scientific validations and for facilitating their rational therapeutic use. Also, it is needed to re-access the said effect of many thousand years old practices of medicines, for their current effectiveness, as the socio-economic and geographical scenario of the Globe has been changed.

Suddha Gandhak is a potent wound healer of various types of wound of different origin. It is used in most of the skin diseases in traditional healing of Ayurveda, in different dose form. Gandhak taila is a very peculiar medicine prepared from suddha gandhak and is used against many infections topically as well as internally.

*Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* are the most commonly involved microbes causing the skin diseases at different body parts. As gandhak is used as a broad spectrum medicine for curing the skin diseases at any body parts irrespective of origin of microbes hence gandhak taila prepared from suddha gandhak by following SOP is accessed for potency as antimicrobial property against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The study is conducted in culture media and in broth nutrient to validate the effect of gandhak taila in current language, in current period of medical practice.

#### Preparation of Gandhak Taila

For the current study Gandhak Taila is prepared by following two methods mostly practiced by Ayurvedic physicians described in two different literatures namely Rasa Ratna Samuchhaya and Rasendra Chintamani.

#### First Method: <sup>1</sup>

By following Rasa Ratna Samuchhaya Gandhak taila was prepared by following method:

Coarse powder of Shuddha Gandhaka was mixed with butter and triturated. After this the mixture obtained was applied to the cotton cloth, which was coated by *Arka* and *Snuhi ksheer*, in a thick layer. Then the cotton cloth was rolled and tied by a thread around it. The holding the roll at clamp at one end the other end was ignited. As the fire goes up then drops of gandhak taila was emerged and collected in a clean glass container. This is called Gandhak taila-1 as research drug candidate for microbiological study.

#### Second Method: <sup>2</sup>

By following Rasendra Chintamani gandhak taila was prepared by following method.

Coarse powder of measured quantity *Suddha Gandhak* was added to boiling milk followed by further boiling till all the gandhak gets melted. Then curd is prepared out of this milk. After this, ghee is prepared called as Gandhak taila-2 as research drug candidate for microbial study.

Antimicrobial study of Gandhak taila prepared by both the method: <sup>3-5</sup>

Microbial study of both the samples of *Gandhaka Taila* method I and II were studied against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* in the Bio Lab, Bhilai, Chhatisgarh.

#### MATERIALS AND METHODS

##### Drugs

Gandhak taila-1  
Gandhak taila-2

##### Microorganisms

*Staphylococcus aureus*, ATCC6538  
*Escherichia coli*, ATCC8739  
*Candida albicans* 20°C/48hrs, ATCC10231

**Equipments:**

Test tubes, oven, water bath apparatus, dropper, microscope, nutrient agar, sterile saline etc.

**Method**

For the microbial study of both the samples of *Gandhaka Taila* standard cultures of these microorganisms were taken. As per following steps the study was conducted.

Antimicrobial Activity Response was studied against:

- a. *Staphylococcus aureus*, ATCC6538
- b. *Escherichia coli*, ATCC8739
- c. *Candida albicans* 20°C/48hrs, ATCC10231

**Inoculums Preparation:**

Sub-culturing of above stated organisms with Nutrient Agar was done and then these were incubated at 30°C to 37°C for 24hrs.

Young culture suspension of above stated organisms was prepared with nutrient broth and again incubated.

**Preparation of cell suspension**

Preparation of cell suspension of about, 100orgs/ml with the help of Neplometer Standard chart was carried out. A cell suspension of 24 hrs old culture of specified micro-organisms was prepared with sterile saline.

The cell density was matched to the density of suspension prepared as in Tube 3 of Neplometer Standard chart. Then the cell density was adjusted using sterile saline. As given in the chart, the cell suspension thus formed had approximate cell density of  $1.4 \times 10^9$  cells /ml.

Above cell suspension was used to prepare a cell suspension of  $1.4 \times 10^2$  cells /ml approx. This dilution was confirmed by plate count method.

**Antibacterial Response Test:**

**Sample Preparation:**

Nutrient Broth was prepared and sterilized at 121°C in mini autoclave for 15 min. Sample was heated in water bath to 42°C to melt it. 1 ml of the melted sample i.e. *Gandhaka Taila* (Method I) was added to the nutrient broth at 40°C aseptically; and then it was shaken vigorously; after that it was cooled to 30°C and then 1 ml of cell suspension having approximate count  $1.4 \times 10^2$  cells/ml was added as mentioned in the following table and this tubes are incubated and readings were taken after 24hrs, 48 hrs and 72hrs and readings were taken.

After 60 hrs of initial inoculation sub culturing from all the above suspensions is carried out with 0.1 ml of above suspension into freshly prepared nutrient broth (without culture in pre-sterilized nutrient broth) media tube containing 10 ml of broth and reading were taken after 24hrs, 48hrs and 72hrs.

**Table 1: Initial inoculation of Gandhaka Taila (Method I)**

Cultures	Observations		
	24 hrs	48 hrs	72 hrs
<i>Staphylococcus aureus</i> ATCC 6538	Negative At 30/37° C	Negative At 37° C	-
<i>Escherichia coli</i> ATCC 8739	Negative At 30/37° C	Negative At 37° C	-
<i>Candida albicans</i> ATCC 10231	Negative At 20°/25° C	Negative At 20°/25° C	Negative At 20°/25° C
REMARK	No growth found in all the above cultures after 60 hrs.		

**Table 2: Secondary inoculation of Gandhaka Taila (Method I)**

Media	Observations		
	24 hrs	48 hrs	72 hrs
Nutrient Broth – 01	Negative At 30 to 37° C	Negative At 37° C	-
Nutrient Broth – 02	Negative At 30 to 37° C	Negative At 37° C	-
Nutrient Broth – 03	Negative At 20°/25° C	Negative At 20°/25° C	Negative At 20°/25° C
REMARK	No growth found in all the above cultures after 60 hrs.		

**Table 3: Initial inoculation of Gandhaka Taila (Method II)**

Cultures	Observations		
	24 hrs	48 hrs	72 hrs
<i>Staphylococcus aureus</i> ATCC 6538	Negative At 30/37° C	Negative At 37° C	-
<i>Escherichia coli</i> ATCC 8739	Negative At 30/37° C	Negative At 37° C	-
<i>Candida albicans</i> ATCC 10231	Negative At 20°/25° C	Negative At 20°/25° C	Negative At 20°/25° C
REMARK	No growth found in all the above cultures after 60 hrs.		

**Table 4: Secondary inoculation of Gandhaka Taila (Method II)**

Media	Observations		
	24 hrs	48 hrs	72 hrs
Nutrient Broth – 01	Negative At 30 to 37° C	Negative At 37° C	-
Nutrient Broth – 02	Negative At 30 to 37° C	Negative At 37° C	-
Nutrient Broth – 03	Negative At 20°/25° C	Negative At 20°/25° C	Negative At 20°/25° C
REMARK	No growth found in all the above cultures after 60 hrs.		

**OBSERVATIONS**

**Observations for Gandhak taila-1**

From the table it is noted that the preparation under study i.e. *Gandhaka Taila* (Method I) is having antibacterial activity

against with inoculums of  $1.4 \times 10^2$  cells/ml under the above said condition. (Table 1 & 2)

*Staphylococcus aureus*, ATCC 6538

*Escherichia coli*, ATCC 8739

*Candida albicans* 20°C/48hrs, ATCC10231

Procedure for assessing antimicrobial effect of Gandhak taila-2 was followed as like the process followed in case of Gandhak taila-1

#### Observations of Gandhak taila-2

From the table it is noted that the preparation under study i.e. Gandhaka Taila (Method II) is having antibacterial activity against with inoculum of  $1.4 \times 10^2$  cells/ml under the above said condition. (Table 3 & 4)

*Staphylococcus aureus*, ATCC 6538

*Escherichia coli*, ATCC 8739

*Candida albicans* 20°C/48hrs, ATCC10231

#### DISCUSSIONS

To evaluate the microbial activity of *Gandhaka Taila* prepared by two methods, this study was performed. For the said study basic microbiological techniques mentioned for evaluating microbial activity in the current practice of anti microbial were followed. *Gandhaka Taila* is an oil base sample, which is used mostly in locally or to the skin diseases. Therefore for this study, three common pathogenic microorganisms which are common in skin diseases i.e. *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* were procured. In Vitro studies were undertaken to assess the antimicrobial activity at Bio Lab, Bhilai, Chhatisgarh. For the Antimicrobial study the American culture type organisms chosen are *Staphylococcus aureus*, ATCC 6538, *Escherichia coli*, ATCC 8739 and *Candida albicans* 20°C/48hrs, ATCC10231 because these are mostly found organisms causing skin infestations.

Then sub-culturing of these organisms with Nutrient Agar was done and then these were incubated at 30° to 37° for 24 hrs. After incubation for 24 hrs the young culture suspension was prepared with Nutrient Broth and again incubated. Preparation of cell suspension of about, 100 orgs/ml with the help of Neplometer Standard chart was carried out. A cell suspension of 24 hrs old culture of specified micro-organisms was prepared with sterile saline.

The cell density was matched to the density of suspension prepared. Then the cell density was adjusted using sterile saline. As given in the chart, the cell suspension thus formed will had approximate cell density of  $1.4 \times 10^9$  cells /ml.

Above cell suspension was used to prepare a cell suspension of  $1.4 \times 10^2$  cells /ml approx. This dilution was confirmed by plate count method. Antibacterial Response Test:

Anti microbial study of both sample of gandhak tail undertaken through vitro studies. *Staphylococcus*-there are gram positive cocci, non sporing bacteria responsible for minor skin disease to severe life threatening infections. *Escherichia coli*-gram negative bacteria, non-sporing causes of frequent opportunistic infections in wounds etc. *Candida albicans* - yeast species. Infections of the skin commonly involve the moist area may involve the oral cavity, the vagina, trachea, bronchi, the webs of the finger, toes, arm pits etc.

The study shows the three common pathogenic microorganisms which are common skin disease were procured. Therefore, the sample "Gandhaktail" can be used for skin disease.

The fresh cultures were taken and a standard suspension of organism is inoculated with one microbial agent, incubated at 35-37.c after 24 hours, 48hours and 72 hours. It was observed that the microbial growth in all the cases were very negligible. Hence Gandhak taila prepared in both the references are able enough to prohibit the bacterial growth and can be used as tropical applications for various types of skin diseases caused by said microbes.

#### CONCLUSION

Gandhaka taila is a herbo-mineral preparation which is applied tropically for healing various types of skin diseases. In Rasa literatures two types of gandhaka taila are described one is described in Rasa Ratna Samuchaya and another is described in Rasendra Chintamani by following different manufacturing technique. After preparing the two samples of gandhak oil by following both the two methods in a environment suit fit for standard operative procedures for respective oils. After validating the final product both the gandhak taila were evaluated for their anti-microbial effect. In nutrient agar method of in-vitro study it was observed that both the oils are potentially effective to resist the microbe's growth. Also it is observed that there is no any significant difference between two types of gandhak oil for inhibiting growth of microbes i.e. both the oil have similar potency for inhibiting growth of microbes in test culture. Hence it can be concluded that gandhak taila is an effective anti microbial oil against *Staphylococcus aureus*, ATCC 6538, *Escherichia coli*, ATCC 8739 and *Candida albicans* 20°C/48hrs, ATCC10231.

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#### Cite this article as:

Himsiksha Verma et al. Antimicrobial study of gandhak taila: An Ayurvedic wound healer' on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Int. J. Res. Ayurveda Pharm. 2019;10(3):52-54 <http://dx.doi.org/10.7897/2277-4343.100361>

Source of support: Nil, Conflict of interest: None Declared

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