STUDIES ON EFFECT OF ROOT EXTRACTS OF Withania somnifera ON SOME CLINICALLY ISOLATED BACTERIAL PATHOGENS

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ABSTRACT

Withania somnifera commonly called as Ashwagandha is the major Indian medicinal plant used in Ayurveda. The present study evaluates the antibacterial activity of ethanolic, chloroform and aqueous extracts of Withania somnifera roots against three clinically isolated bacterial pathogens viz. Staphylococcus aureus, Salmonella typhi and Shigella dysenteriae causing infections in human beings. The study revealed that the crude ethanol root extract was more effective in inhibiting pathogens as compared to chloroform and aqueous root extracts as determined by agar diffusion method using respective solvents as negative controls. The results obtained in the study indicates that the ethanol extract of Withania somnifera roots might be exploited as natural drug for the treatment of several infectious diseases caused by these pathogens.

Key Words: Antibacterial activity, Withania somnifera, Agar diffusion, Pathogens, Clinically isolated bacterial

INTRODUCTION

Diseases and drugs are the two equally important aspects and to know one without the other is meaningless. Ours is the age of synthetic drugs, antibiotics and radioactive substances which are used to treat the ailments which is possibly due to recent developments and achievements in the chemical field.1

Multidrug resistance is now a worldwide problem attributed to the extensive use of antibiotics, selection pressure on bacterial strains and lack of new drugs, vaccines and diagnostic aids.

With the rapid strides in knowledge of natural sciences the crude drugs have come to be studied more exhaustively at present than at any other time in the past. These crude drugs are mainly obtained from medicinal plants and consists of either entire plants or their parts such as roots, stem, leaves, flower and seeds which have curative properties due to presence of various chemical substances of different compositions.2

Withania somnifera commonly known as Ashwagandha or Indian ginseng is the major Indian medicinal plant used in Ayurveda, Siddha and Unani systems of medicine. It belongs to family Solanaceae.3 The major biochemical constituents of Ashwagandha roots are steroidal alkaloids and steroidal lactones in a class of constituents called withanolides.4-6 Withaferin A and Withanolide D are the two main Withanolides that contribute to most of the biological activity of Withania somnifera.7,8 The total alkaloid content of Indian root vary between 0.13% to 0.31%. Withania somnifera has been used as an anti-oxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent, astringent and more recently as antibacterial agent.9

Several reports have demonstrated the immunomodulator and anti-tumor activity of root extract of Withania somnifera.10-12 Ethanolic root extract alone or in combination with other herbal material is used in many commercial preparations and found to be effective in alcoholism, emphysema and pulmonary tuberculosis. Ashwagandha has been found useful in treating hypothyroidism.13

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AIMS AND OBJECTIVES

The present work was carried out to study the antibacterial activity of root extracts of *Withania somnifera* in different solvents against some common pathogens.

MATERIAL AND METHODS

Collection and processing of plant material

Roots of *Withania somnifera* are light brown from outside, white from inside and have peculiar sweet smell.14 Roots of *Withania somnifera* were collected from Botanical Garden, College campus of Y. C. College, Karad. They were washed with distilled water thoroughly and kept for drying in oven at temperature of 45°C for 7 days. The dried roots were then crushed in mixer grinder into powdered form.

Preparation of root extract

Aqueous extraction

For this extraction 10g of root powder was dissolved in 100ml of distilled water in a conical flask and boiled at 100°C in water bath for 6 hrs and then filtered through Whatmann no.1 filter paper. The filtrate was then stored at room temperature for further study.15

Ethanol extraction

For preparation of ethanol extract 10g of root powder was dissolved in 100ml of ethanol and kept at 27°C for two days and filtered through Whatmann no. 1 filter paper. The filtrate was then allowed to evaporate to get concentrated filtrate which was again reconstituted in small volumes of same solvent.

Chloroform extraction

10% chloroform extract was also prepared by similar method as described for ethanol extraction.

All these three extracts of *Withania somnifera* roots were used in two different forms as crude extracts and membrane filtered extracts for further study of antibacterial activity.

Bacterial cultures used

For the study of antibacterial activity the bacterial cultures tested were three different bacterial pathogens isolated from clinical samples

Collection of clinical samples

A pus sample of patient was collected from Shalini laboratory. The stool samples of patients suspected to be suffering from bacillary dysenteriae and typhoid fever were collected from Hardikar laboratory.

Isolation and identification of isolates

The pus sample was streak inoculated on sterile mannitol salt agar plate while the stool samples were streak inoculated on sterile Mac Conkeys agar plates aseptically by four quadrant method of inoculation. After inoculation plates were incubated at 37°C for 24 hrs. After incubation isolated colonies on the plates were selected and studied for their morphological characteristic such as colony characteristics, Gram nature by Hucker and Conn’s modified Gram staining method (1923) and motility by hanging drop method. The biochemical characteristics of the isolates such as carbohydrate fermentation and enzyme activity were also studied.

Study of antibacterial activity

The antibacterial activity study was carried out by agar diffusion method.16-19 Bacterial suspensions in the quantity of 0.1 ml were aseptically introduced and spread on sterile nutrient agar plates. Three wells of about 6 mm diameter were prepared aseptically on each agar plate. 1 ml of each aqueous, ethanol and chloroform root extracts of *Withania somnifera* were introduced into the wells in the plates. The negative control was also kept with 1 ml of the respective solvents viz. ethanol, chloroform and water. Plates were kept in refrigerator for half an hour for diffusion of extract and then incubated at 37°C for 24 hrs. Observations were recorded in the form of zone of inhibition measured in millimeter. Negative control showed no zone of inhibition against any test pathogen.

RESULTS AND DISCUSSION

Table 1 : Clinical samples and coding of isolates

<table>
<thead>
<tr>
<th>S/N</th>
<th>Clinical sample</th>
<th>Isolate code</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pus sample</td>
<td>Sh-1</td>
</tr>
<tr>
<td>2</td>
<td>Stool sample</td>
<td>HI-1</td>
</tr>
<tr>
<td>3</td>
<td>Stool sample</td>
<td>HI-2</td>
</tr>
</tbody>
</table>
Table 1 represents the coding of isolates obtained from pathological laboratories from Karad city namely Shalini laboratory and Hardikar laboratory. The isolates obtained were coded as Sh-1 from pus sample and HI-1 and HI-2 from stool samples.

After studying the colony characters, morphological and biochemical characteristics of the three isolates they were identified as, The isolate Sh-1 was identified as Staphylococcus aureus, HI-1 was identified as Salmonella typhi and HI-2 as Shigella dysenteriae.

The crude ethanol root extract of Withania somnifera showed strong antibacterial activity against two of the test pathogens i.e. Staphylococcus aureus and Salmonella typhi compared to chloroform root extract while aqueous root extract showed no inhibitory effect as indicated in Fig. 1 The effect of membrane filtered root extract of Withania somnifera is indicated in Fig. 2 which showed that the ethanol extract inhibited the growth of Staphylococcus aureus and Salmonella typhi but did not inhibited the growth of Shigella dysenteriae. The chloroform extract showed antibacterial activity against only Staphylococcus aureus. It was observed that none of the test bacterial pathogens were inhibited by aqueous extract applied. None of the extract inhibited the growth of Shigella dysenteriae.

![Fig. 1](image1.png)  
**Fig. 1**: Effect of crude root extracts of Withania somnifera

![Fig. 2](image2.png)  
**Fig. 2**: Effect of membrane filtered root extracts of Withania somnifera
CONCLUSION

Thus the present study strongly demonstrated that the crude root extracts of *Withania somnifera* were found to be more effective in inhibiting the test pathogens compared to membrane filtered root extracts. It concludes that the crude ethanol root extract of *Withania somnifera* hold an excellent potential as an antibacterial agent and ascertains the value of medicinal plants used in ayurveda which could be of considerable interest to the development of new drug.

REFERENCES