



In-vitro* antibacterial effect of medicinal plants against *Neisseria gonorrhoeae

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Abstract

The bark of *Ficus religiosa* L., *Ficus benghalensis* L., *Ficus gloomerata* L., fruits of *Tribulus terrestris* L. and roots of *Saussurea lappa* Clarke. were investigated for *in vitro* antibacterial activity. The various solvent extracts like petroleum ether, chloroform, aqueous and methanol of plants were screened against *Neisseria gonorrhoeae* isolated from the patients suffering from vaginitis. The extracts were subjected for antibacterial activity against the pathogen at 200mg/ml concentration by agar well diffusion and agar disc diffusion method. The results of antibacterial activity revealed that methanol extracts of all the plants exhibits good activity as compared to petroleum ether, chloroform and aqueous extracts. The antibacterial activities of extracts were compared with standard antibiotic cefotaxime. The MIC of the methanol extract of all the plants was also calculated against the pathogen.

Keywords:- Agar disc diffusion method, Agar well diffusion method, Medicinal plants, *N. gonorrhoeae*, Vaginitis

Introduction

Humans are the only known host of *Neisseria gonorrhoeae*, an organism that is commonly called the gonococcus and is the cause of gonorrhea and other types of disease i.e. vaginitis. *N. gonorrhoeae* is a gram negative bacteria, aerobic but may grow anaerobically also but it is essential to provide 5-10% CO₂. Vaginitis is an inflammation of the vagina that can result in discharge, itching and pain. Excessive vaginal discharge which is purulent in character is a common complaint, especially in sexually active women.

In India, the use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times (Bhattacharjee, 1998). Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of human kind. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Iwu *et al.*, 1999). India has a treasure of medicinal plants and a number of herbs are traditionally

used for the treatment of many diseases. Thus, in recent years there has been a phenomenal rise in the interest of scientific community to explore the pharmacological activities of medicinal plants (Chah *et al.*, 2006).

Ficus religiosa (Pipal), *F. benghalensis* (Bargad) and *F. gloomerata* (Gular), belongs to the family Moraceae. Their bark have been used for diarrhoea, dysentery, leucorrhoea, menorrhagia, for vaginal and other urogenital disorders. *F. benghalensis* is used in Ayurveda for treatment of diarrhoea, piles, teeth and skin disorders (Warrier *et al.*, 1995). All the *Ficus* species are distributed throughout India. *Tribulus terrestris* (Gokhru) is a flowering plant belongs to family zygophyllaceae. Its fruits are used in folk medicine as tonic, analgesic, astringent, stomachic, anti-hypertensive, diuretic and urinary anti-infective (Ody, 2000). *Saussurea lappa* (Kustha) belongs to family Asteraceae. The root has a pungent taste and a peculiar fragrant aromatic odour. Root has been used in cough, asthma, chronic rheumatism and skin diseases, fever and dyspepsia and used as an ingredient in stimulating mixtures for cholera. The present paper deals with the antimicrobial activity of *Ficus religiosa*, *Ficus benghalensis*, *Ficus gloomerata*, *Tribulus terrestris*, *Saussurea lappa* against *Neisseria gonorrhoeae*.

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Materials and Method

Plant Material

The bark of the three *Ficus* sp. were collected from the plants growing wild in Hardwar (U.K.), the fruits of *Tribulus terrestris* were collected from the plants growing in the botanical garden of Patangali Yog Peeth, Hardwar and the roots of *Saussurea lappa* were collected from Shivalik range of Himalayas and identified at Botanical Survey of India, Dehradun, Uttarakhand. Fresh plant materials were washed under running tap water, shade dried at room temperature and then homogenized to get a coarse powder and used for further successive extraction.

Preparation of Plant Extracts

The plant extracts were prepared by immersing 200 gm of dried powder in 600ml of solvents i.e. petroleum ether, chloroform, methanol and water by Soxlet assembly. At the end of extraction each extract was passed through Whatmann Filter Paper No 1. The extracts were concentrated by using vacuum evaporator at 30°C and stored in sterile bottles at 4°C until further use.

Microorganism

The pathogenic organism was selected for the study on the basis of its clinical pharmaceutical importance as well as for its potential to cause infection. *Neisseria gonorrhoeae* was isolated from infected females suffering from vaginal infections in Subharati Institute of Medical Sciences, Meerut and was identified according to the published guidelines by Burnet *et al.* (1994).

Agar well diffusion method

This method was given by Perez *et al.*, 1990. It depends upon the diffusion of the tested material to such an extent that growth of the added microorganism is prevented entirely in a zone around the hole containing a solution of tested material (Ahmad *et al.*, 1998). Chocolate agar media was inoculated with 10⁵ cfu/ml of 24 hours old culture of test organisms and shaken. Wells of 8mm diameter were punched into the agar medium and filled with 45 µl (200 mg/ml) of plant extract, solvent blanks and antimicrobial drug. The antibiotic Cefotaxime was used. The plates were incubated for 18-24 hours at 37 °C in the presence of 5-10% CO₂. The results

were obtained by measuring the diameter of zone of inhibition in millimeters (mm). All the tests were done in triplicate.

Agar disc diffusion method

This method was given by Bauer and Kirby, in 1966. The disc of 6mm was saturated with 45 µl (200 mg/ml) of the plant extract, solvent blank and antimicrobial drug, allowed to dry and was introduced on the upper layer of the seeded agar plate using a flamed forcep and gently pressed down to ensure contact. All the solvents served as negative control. The plates were then incubated for 18 to 24 hours at 37 °C and microbial growth was determined by measuring the diameter of zone of inhibition in millimeters (mm). All the tests were done in triplicate.

Minimum Inhibitory Concentration (MIC)

The MIC of the methanolic extract was determined according to the broth dilution test (NCCLS, 1992). Standardized suspensions of the test organism was inoculated into a series of sterile, disposable 24 well polystyrene microtitre plate containing two fold dilutions of the extract and incubated for 18 to 24 hours at 37 °C. The MIC was read as the lowest concentration of the plant material inhibiting the development of visible growth after a period of time.

Results and Discussion

Depending on the nature of the infecting microorganism, the condition may vary from minor ailments to major health concern. The results indicated differential activities of the plant extracts against the growth of bacteria (Table-1). All the plant extracts showed significant antimicrobial activity against the microorganism at a concentration of 200 mg/ml. Considering the need of an eco- friendly approach to control the plant pathogens, it was considered worth while to screen the antimicrobial effects of locally available flora. The need of the hour is to screen the plants for promising biological activity or the treatment of diseases and ailments. In the present work different extracts of five traditionally used Indian medicinal plants have been tested against *Neisseria gonorrhoeae*. Methanol



Table-1 : Antibacterial effect of *Ficus religiosa*, *Ficus benghalensis*, *Ficus gloomerata*, *Tribulus terrestris*, *Saussurea lappa* extracts (mm) against *Neisseria gonorrhoeae*

Plants	Agar well diffusion method (Zone of inhibition in mm)				Agar disc diffusion method (Zone of inhibition in mm)			
	P	C	M	W	P	C	M	W
<i>Ficus religiosa</i>	12	14	15	13	09	11	13	10
<i>Ficus benghalensis</i>	12	13	16	12	09	10	12	12
<i>Ficus gloomerata</i>	13	15	17	14	11	13	13	12
<i>Tribulus terrestris</i>	13	16	18	15	09	13	14	12
<i>Saussurea lappa</i>	12	13	13	11	08	11	11	10

Note:-P : Petroleum ether ; C : Chloroform ; M : Methanol ; W : Water and Average of three replicates

extract of all the plants showed good antibacterial activity against *Neisseria gonorrhoeae* followed by aqueous, chloroform and petroleum ether extract in both the methods. In agar well diffusion method maximum activity was shown by *Tribulus terrestris* (18 mm) followed by *Ficus gloomerata* (17 mm), *Ficus benghalensis* (16mm), *Ficus religiosa* (15 mm), and *Saussurea lappa* (13 mm) while in agar disc diffusion method maximum activity was shown by *Tribulus terrestris* (14mm) followed by *Ficus gloomerata* and *Ficus religiosa* (13mm), *Ficus benghalensis* (12 mm), and *Saussurea lappa* (11 mm). Among all the plants *Tribulus terrestris* was found to be the best plant showing significant antibacterial activity against the pathogen because more chemical compounds were found to be present in it and least activity was shown by *Saussurea lappa*. The antibiotic Cefotaxime showed 28 mm zone of inhibition when performed by agar well diffusion method and 22 mm when done by agar disc diffusion method. MIC of the methanol extract was also calculated of all the plants. *N.gonorrhoeae* was more susceptible to the methanol extract of *T.terrestris*, MIC (1.56 mg/ml) followed by *F. benghalensis* and *F. religiosa* (3.125 mg/ml) and *F. gloomerata* and *Saussurea lappa* (6.125 mg/ml). Seeds of *Saussurea lappa* have considerable

antifungal activity especially against pathogenic fungi (Ray and Majumdar, 1976). The aqueous extract of *Saussurea lappa* did not show any antimicrobial activity against *B. cereus*, *S. epidermidis*, *E. aerogenes*, *P. vulgaris*, *S. typhimurium* (Parekh and Chanda, 2007). The aqueous extract of *Ficus benghalensis* did not show activity against the *P.aeruginosa* ATCC 27853 and *P. mirabilis* NCIM 2241 (Nair and Chanda, 2007). The aqueous extract of *Ficus religiosa* showed low activity against *B. cereus* ATCC 11778 and high activity was shown in ethanol extract against it. The aqueous extract did not show any activity against *A. fecalis* ATCC 8750 whereas low activity was shown in ethanol extract against it. The aqueous extract showed no activity against *S. typhimurium* ATCC 23564 and low activity in ethanol extract (Nair and Chanda, 2007).

In the present work the antimicrobial activity of the extracts was quantitatively assessed by the presence or absence of the zone of inhibition and the plants selected are potentially a rich source of antimicrobial agents. Medicinal plants possess antimicrobial properties that support their value in herbal medicine for the treatment of diseases and ailments. Our preliminary findings on these medicinal plants indicate a promising antimicrobial activity against



N. gonorrhoeae. Lastly to conclude the extracts were found to inhibit the growth of microorganisms and the methanolic extract was comparably more effective to inhibit the growth of microbes than petroleum ether, chloroform and aqueous extracts.

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