Antibacterial activity of stem bark extract of traditionally used *Terminalia bellirica* (Gaerth.) Roxb.

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Abstract- Medicinal Plants have been practised for hundreds of centuries by tribes all over the world. From the earliest times until the end of nineteenth century plants are still the common source of medicinal treatment yet. Using natural, plant-derived medicines that are "healthier" than prescription drugs derived from synthesized products is something that appeals to consumers. In this study, the plant *Terminalia bellirica* was taken for study due to the mesmerizing medicinal properties of the plant. The antibacterial activities of acetone, ethyl acetate, benzene and methanol extracts of *Terminalia bellirica* stem bark were tested for four pathogenic bacterial strains by agar disc diffusion method. Among the various extract, acetone extracts showed good antibacterial activity and maximum zone of inhibition was obtained for *Staphylococus aureus* (zone size 8mm) followed by *Pseudomonas aurogenosa* (10mm). The results are given in **Table-1**. Methanol extract showed antibacterial activity against *E. coli* (zone size 8mm). On the other hand ethyl acetate and benzene extract was found to be totally unaffected against these bacterial strains.

Keywords: *Terminalia bellirica,* agar disc diffusion method, antibacterial activity, *Pseudomonas aurogenosa.*

Introduction

Infectious diseases caused by bacteria, fungi, viruses and parasites are still major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to the relative unavailability of medicines and emergence of widespread drug resistance.[1] Research on new antimicrobial substances must therefore be continued and all possible strategies should be explored. Besides small molecules from medicinal chemistry, natural products are still major source of innovative therapeutic agents for various conditions, including infectious diseases.[2] *Terminalia bellirica* commonly known as belliric myrobalan belongs to the family combretaceae. It is routinely used as traditional medicine by tribal folk of district

Tehri Garhwal, Uttarakhand, India which lies in between $30^0$10' - $30^0$17' north latitude and $78^0$18' - $78^0$30' longitude. It is beneficial for the eyes, hair and cure jaundice, voice disorders, nasal problem, blood disorders, throat and breathing problems. Anthelmints seed of the fruit help to cure eyes problem. The fruit of *Terminalia bellirica* is one of the constituents of "Triphala", an Ayurvedic medicine.[3-4] The bark is useful in anemia and leucorrhoea Fruit of *Terminalia bellirica* causes fall in blood pressure of rats with its concentration of 70mg/kg body weight[5]. Chemical constituents, β-sitosterol, gallic acid, ethyl gallate, galloyl glucose, a new triterpene the belleric acid and chebulagic acid have been isolated from many species of *Terminalia* genus[6]. This prompted us to carry out the ethanobotanical study of the plant and antibacterial investigation of stem bark extract of traditionally used *Terminalia bellirica*.

Material and Methods

Clean and healthy bark of *Terminalia bellirica* were collected from District Tehri Garhwal Uttarakhand, India authenticated by Dr. J. K. Tiwari, Taxonomist, Botany department, HNB Garhwal University Campus Srinagar (Garhwal). Bacterial strains *Escherichia coli, Staphylococus aureus, Bacillus subtilis, Pseudomonas aerogenosa* were obtained from department of microbiology, Hill Campus Ranichauri and checked for purity by conventional biochemical method. Barks obtained were dried in tray drier for 7 days at 40-50^0 C and powdered.

Extraction

Powdered thus obtained was extracted with various solvent like, acetone, ethyl acetate, benzene and methanol using Soxhlet apparatus. The extract was concentration under reduced pressure to yield reddish waxy mass 80gm, 100gm, 90gm and 110gm and screened for their antibacterial efficacy against various pathogenic cultures by agar disc diffusion methods.

Antibacterial Activity Studies (Agar Disc Diffusion Methods)

The extract of stem bark of *Terminalia bellirica* was tested for antimicrobial activity using agar disc diffusion method on solid media[7-9]. Luria agar was used as basal medium for *Escherchia coli* and *Bacillus subtilis*; and nutrient agar was used as basal medium for *Pseudomonas aeroginosa, Staphylococcus aureaus*. 5 g of luria broth and 4 g of agar powder; 3.25 g of nutrient broth and 4 g of agar powder was weighed and 250 ml of water was added separately. The mixture was heated to dissolve the components. Luria agar and nutrient agar was sterilized in an autoclave[10]. Luria agar and nutrient agar was poured in the sterile Petri plates mother cultures of each organism were set up 24 h before the assays in order to reach stationary phase of growth[11]. The tests were assessed by inoculating Petri dishes from the mother cultures which had been surface spread with 0.1 ml of each bacteria, with the aim of obtaining microorganism concentration of 10^5 colony forming units (CFU/ml)[12]. An aliquot of Dimethysulphoxide (DMSO) was added to the extract in order to obtain 5mg/ml concentration range[13]. Sterile dilutions of essential oil were deposited on the sterile Whatmann filter paper No.1 discs (5mm disc diameter), which were

subsequent placed in inoculated Petri plates Therefore the Petri plates were than incubated at 37° C for 24 h. The antibacterial activity was determined by measuring the diameter of zone of inhibition surrounding bacterial growth[14].

Results and Discussion

The shade dried and powdered stem bark were exhaustively extracted with acetone, ethyl acetate, benzene and ethanol. A reddish waxy mass 80gm, 100gm, 90gm and 110gm obtained after removing the solvent from acetone, ethyl acetate, benzene and ethanol extract respectively. Under reduced pressure the above extracts were subjected for their antimicrobial activity. The stem bark extracts were found to be quite effective in inhibiting the growth of various bacterial strains as indicated by zone of inhibition. Among the various extracts, acetone extract showed good antibacterial activity and maximum zone of inhibition was obtained for *Staphylococus aureus* (zone size 8mm) followed by *Pseudomonas aurogenosa* (10mm), results are given in **(Table-1).** Ethanol extract showed antibacterial activity against *E. coli* (zone size 8mm). On the other hand ethyl acetate and benzene extract was found to be totally unaffected against these bacterial strains. Results of activity with zone of inhibition are shown in **Figure-1, 2, 3, 4.** Acetone extract is being subjected to column chromatography for isolation of the bioactive compounds for further studies.

Figure-1 Methanol extract against *Escherichia coli*

Figure-2 Acetone extract against *Pseudomonas aeruginosa*

Figure-3 Benzene & Ethyl Acetate extract totally in affected

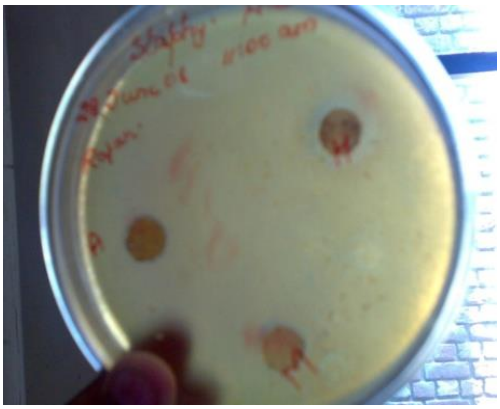

Figure- 4: Acetone extract against *Staphylococcus aureus*

Conclusion

It is concluded that the acetone extracts of stem bark of *Terminalia bellirica* are much more active against *Stephylococus aureus* and *Pseudomonas aerugenosa* (zone of inhibition 8mm, 10mm respectively), and methanol extract is active against *Escherichia coli* (zone of inhibition 8mm). Ethyl acetate & benzene extracts are totally unaffected for all the bacterial strains.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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Table-1 Antibacterial activity of Different extract of *Terminalia bellirica* stems bark

S. No.	Bacterial strain	Group	Zone of inhibition			
			Acetone Ext.	**Acetate Ext.**	**C_6H_6 Ext.**	**MeOH Ext.**
1.	*Bacillus subtilis*	Gram (+)	-ve	-ve	-ve	-ve
2.	*Staphylococcus aureus*	Gram (+)	8mm	-ve	-ve	-ve
3.	*Escherichia coli*	Gram (-)	-ve	-ve	-ve	8mm
4.	*Pseudomonas aerugenosa*	Gram (-)	10mm	-ve	-ve	-ve

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