

Evaluation of Antibacterial Activity of Combined Plant Extract of *Pyracantha crenulata* and *Zanthoxylum armatum*

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Abstract-Medicinal plants represent an essential source of drugs and have played an important role in healthcare system. *Pyracantha crenulata* and *Zanthoxylum armatum* have been used as traditional medicine. The main aim of the study was to find the antibacterial activity of the selected plants against bacterial species: *E.coli* and *Pseudomonas aeruginosa*. The solvents used for plant extraction were hexane, chloroform, ethanol and aqueous. The in vitro antimicrobial activity was performed by Agar disk diffusion method. The hexane and aqueous extracts showed moderate activity whereas the ethanolic extracts showed a significant antibacterial activity. In the study Tetracycline was used as standard. The combined ethanolic extract of both the selected plant showed the synergistic effect on the bacterial strain tested. This leads to the conclusion that the combined effect can have possible application in the development of products as antimicrobial.

Keywords: *Pyracantha crenulata*, *Zanthoxylum armatum*, Antibiotics, MIC, Antimicrobial activity

Introduction

Pioneer basins of natural or herbal medicines are procured by the Medicinal plants. The structural diversity of their phytoconstituents accomplishes them as an esteemed source of novel lead compounds for the quest of drugs to treat acute and chronic diseases. Because of rich biodiversity Indian subcontinent has richest plant based traditional medicinal system. Due to better recession and lesser side effects on human body these herbal medicines are primarily used for health care. The Indian Himalayan vicinity embrace about 1,748 different species of medicinal plants.¹ The Garhwal Himalaya is acknowledged for its affluent bio-resources and

ethano-culture diversity. The origin of drugs to the greatest extent depends on these natural products from plant, animal, microorganism and minerals, which are pre-owned in the therapy of human and animal disease. The extensive and lucrative use of antimicrobial medications are frequently engaged in the treatment of infectious diseases comes together with inadmissible side effects and augmentation of severe medical dilemma.^{2,3} This persuades medical chemist and pharmacist to come across new antimicrobial substances from natural sources. The plant extracts screening for antimicrobial activity has demonstrated that plants are inherent origin of peculiar antimicrobial substances.^{4,5} Going on the multiple drug resistance which is developed due to the extensive use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants.^{6,7} Antimicrobial drugs of the plant origin have immense therapeutic potential. These are virtuous in the treatment of infectious diseases while simultaneously mitigating many of the aftereffects that are much associated with synthetic antimicrobials. Growing bacterial resistance is exhorting restoration in research of the antimicrobial aspect of herbs in contrast to resistant strains.^{8,9} *Zanthoxylum armatum* belongs to the family Rutaceae, an important family from economic point of view. It is a wild species and locally known as Timur.¹⁰ It is extensively dispersed in the discrete countries like India, Nepal, China, Pakistan, Bhutan, Korea, Taiwan, Laos, Myanmar, Bangladesh, Japan,

Thailand, Vietnam and Indonesia.¹¹ Consistently, leaves and fruits of *Zanthoxylum armatum* are used for tooth care and mouth fresh, at the same time bark is used for exhilarating the fishes and also the leaves, fruits and barks are used as spice. Commonly, the plants essential oils is used as aromatic and flavouring agents for foods and beverages.¹² In Indian medicinal Literature, *Zanthoxylum armatum* is one of the most vital medicinal plants and about 10 species are grown. Almost every part of this plant are used in the Indian traditional system for the treatment of different ailments and the compelling medicinal properties were supplementary proclaimed through scientific investigation. Plants consist of effective and active ingredients are used in therapeutics of different diseases or in the relief of pain are called medicinal plants.¹³ Therapeutic and pharmaceutical role played by the plants in protecting human beings from the effects of diseases and other complications, are contemplated as to have a cogent role in the healthcare system. This is the main reason that large population of developing countries still count on the herbal medicines. Medicinal plants are taken care of in an unorganized manner and exploited for the future. Gradual increase in usage of medicinal plants has been documented over and over for both traditional users and pharmaceutical industry.¹⁴

Pyracantha crenulata belongs to the family Rosaceae. It is widely acknowledged as "Ghingaroo".¹⁵ The fruit of this plant has been used by Garhwal folk and in traditional medicines in the treatment of serious health conditions like heart disorders, hypertension, diabetes, blood pressure and circulation system especially in case of angina.¹⁶ The leaves of plants are found useful for antioxidant, immune-modulatory, anti-inflammatory activities and are also used as herbal tea. The pome fruit of *Pyracantha crenulata* is orange-red and rich in sugar.¹⁷ The food substances of the plant used as nutraceuticals, contain antioxidants, minerals, vitamins, prebiotics, probiotics, polyunsaturated fatty acids certain phytochemical and dietary fibers.¹⁸

Material and Methods

Plant materials: The leaves of *Pyracantha crenulata* and *Zanthoxylum armatum* were collected from the hills of Lohali village located in the Nainital District of Uttarakhand.

Preparation of extracts: *Pyracantha crenulata* and *Zanthoxylum armatum* leaves were collected in a cotton bag. Cleaned and shade dried the leaves were grounded to coarse powder.

***Pyracantha crenulata* leaf extract:** The coarse powder of leaf was subjected to successive hot continuous Soxhlet extraction with different solvents (*Hexane, Chloroform, Ethanol and Water*). The liquid extract with different solvents so obtained were evaporated to get concentrated extract and stored in a closed air tight containers.

***Zanthoxylum armatum* leaf extract:** The coarse powder of leaf was subjected to cold percolation process for 7 days to obtain the extract with different solvents (*Hexane, Chloroform, Ethanol and Water*). The extracts were concentrated by evaporation and stored in a close air tight containers. Two bacterial strains were used namely *Escherichia coli* and *Pseudomonas aeruginosa*. These were cultured in nutrient broth for 24 hours and the fresh in oculums were taken for the test and reconfirmed by gram staining and sub culturing in appropriate selective media.

Anti-Bacterial Assay: The antibacterial activity was assessed by agar well diffusion method. Muller Hinton agar medium was prepared by using 15g agar dissolved in 1L distilled water. Muller Hinton agar medium was poured into each Petri plate of 20 x 90mm and allowed to cool to 45°C to solidify. The freshly prepared in oculums were poured and spread with help of sterile glass rod all over the surface of the MHA plate. Wells of 8 mm diameter were made in the agar with a sterile cork borer. Hundred microliters of the working suspension/solution of different plant extracts were loaded in each well with the help of micropipette. In some MHA plates the well were poured with the combination of two plant extract of

similar solvent i.e. Hexane (*Pyracantha crenulata* + *Zanthoxylum armatum*), Chloroform (*Pyracantha crenulata* + *Zanthoxylum armatum*), Ethanol (*Pyracantha crenulata* + *Zanthoxylum armatum*), and Water (*Pyracantha Crenulata* + *Zanthoxylum Armatum*). Plates were left for some time till the

extracts diffused in the medium with the lid closed and incubated at 37°C for 24 hour. The tests were performed three times and the zones of inhibition were measured for each extract using a ruler and the results were recorded in Table-1

Table-1 Antimicrobial activity of plant extracts

Extract	Concentration	<i>Pyracantha crenulata</i>		<i>Zanthoxylum armatum</i>	
		Zone of Inhibition (Diameter in mm)			
		<i>E. coli</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
Hexane	10 mg/ml	-	12	12	11
	15 mg/ml	-	14	14	12
	20mg/ml	-	14	14	12
	25 mg/ml	-	11	14	14
Chloroform	10 mg/ml	16	12	14	16
	15 mg/ml	18	14	-	14
	20mg/ml	10	15	14	10
	25 mg/ml	14	16	12	14
Ethanol	10 mg/ml	14	22	16	16
	15 mg/ml	17	22	14	18
	20mg/ml	16	14	18	18
	25 mg/ml	18	12	18	18
Aqueous	10 mg/ml	16	16	12	11
	15 mg/ml	14	14	14	12
	20mg/ml	15	14	16	14
	25 mg/ml	17	15	14	12
Tetracycline		24	26	20	24

Results and Discussion

Antimicrobial activity of plant extracts:

Preliminary antibacterial studies were conducted on selected plant extract by using two bacterial strains *E. coli* (ATCC-11775) and *S. aureus* (ACC-132), at concentration of 10, 15, 20 and 25 µg/ml by agar plate method. The zone of inhibition of each strain recorded for comparison using Tetracycline as standard. From the antibacterial activity data, it was found that all the extracts have shown antibacterial activity against one or the other organisms except for hexane. Ethanolic extract has shown excellent activity against both the strain. Chloroform and aqueous extract show moderate activity against both

the bacterial strain in comparison to the standard Tetracycline. The antimicrobial activity of the selected plant species are summarized in Table -1.

Combinations of plant extract were performed based on the antibacterial activity of the single extracts summarized in Table-1. *Pyracantha crenulata* and *Zanthoxylum armatum* (50% v/v each) were mixed to evaluate in the antibacterial activity potency. The determination of the combination of *Pyracantha crenulata* and *Zanthoxylum armatum* ethanolic extracts is reported in Table-2. The result shows synergistic effect of using the combination of *Pyracantha crenulata* and *Zanthoxylum armatum* ethanolic extract.

Table-2 Antimicrobial activity of combined plant extracts

Plant Extract combination	Zone of Inhibition (Diameter in mm)	
	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
<i>Pyracantha crenulata</i> and <i>Zanthoxylum armatum</i>	24	26

Conclusion

In conclusion, the results of the present work provide additional information on the possible use of plant extracts, alone or in combination, in the treatment of infectious diseases. The unraveled synergistic effect of the mixture of *Pyracantha crenulata* and *Zanthoxylum armatum* ethanolic extracts is a proof of concept

that a combination may potentiate the antimicrobial activity of each single plant extracts. In the specific case, the demonstrated improved antibacterial activity of *Pyracantha crenulata* and *Zanthoxylum armatum* ethanolic extracts' combination may find a possible application in the development of products for infectious diseases.

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