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Phytochemical Screening and Antimicrobial Activity of Leaf Extracts of *Zanthoxylum armatum* against some Bacteria and Fungi DC: An Important Medicinal Plant

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Abstract: ***The medicinally active substance were isolated from dried leaves of Zanthoxylum armatum DC by using Soxhlet apparatus and identified by photochemical tests. The soxhlet extraction in powdered form was performed using Petroleum ether, chloroform, n-hexane, acetone, ethanol and methanol. The result indicated that alkaloids were present in methanol and ethanol extracts. Flavanoids were present in acetone,chloroform, methanol and ethanol extract, Carbohydrates were present in acetone, petroleum ether, methanol, ethanol and n-hexane extract. Proteins and triterpenoids were not present in any of the extracts. The most Pseudomonas aerugenosa while most resistant bacteria wasE.coli.Maximum zone of Staphylococcus aureusat 100% concentration.Among fungal strains Aspergillus fumigateswas the most susceptible whileAspergillusniger, Candida albicans and Saccharomyces cereviseae are the resistant ones. n-hexane and acetone extract did not show any antimicrobial activity against all the tested organism. None of the extracts showed any inhibitory potential against bacteria E.coli and Candida albicans, Saccharomyces cereviseae and Aspergillus niger***

Keywords: antimicrobial**,**alkaloids, flavanoids,photochemical activity *Zanthoxylum armatum*

1. Intoduction

Finding healing powers in plants is an ancient idea. The use of medicinal plants as a source of relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind. Neanderthals living 60,000 years ago in present day Iraq used plants such as hollyback, these plants are still widely used in ethno medicine around the world (Stockwell, 1988). However, scientific data on their efficacy, pharmacological properties and action mechanism as well as on their chemical constituents have so far been lacking. Among the estimated 2,50,000-5,00,000 plant species only a small percentage has been investigated phytochemically and the fraction submitted to pharmacological screening is even smaller. Thus, any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Phytochemical screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics (Kroschwitz and Howe-Grant, 1992). Even now, drugs from higher plants continue to occupy an important position in modern medicine. On a global basis, at least 130 drugs, all single chemical entities extracted from higher plants, or modified further synthetically, are currently in use, though some of them are now being made synthetically for economic reasons (Newman et al., 2000). Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava et al., 1996). A wide range of medicinal plant parts is used for extract as (Elamathi et al., 2011) raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of the raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries (Uniyal et al., 2006). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of them not been adequately evaluated (Balandrin et al., 1985). In the present study we have selected an Indian plant to be screened for various phtochemicals and antimicrobial activities. The selection of medicinal plants is based on their traditional uses in India.

2. Material and Methods

Preparation of plant extracts

Leaves of fresh plants of *Zanthoxylum armatum* was collected from Tehri Garhwal range of Uttarakhand, India and the plant were identified and authenticated at the Department of Biotechnology SBS PGI, Deheradun .

Extraction of plant material

Various extracts of the study plant was prepared according to the methodology of Indian Pharmacopoeia (Anonymous, 1966). The leaves were shade dried and ground into small particles to get coarse powder.The coarse powder was subjected to Soxhlet extraction separately and successively withPetroleum ether, chloroform, n-hexane, acetone, ethanol and methanol.These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled

temperature (40-500C). Both the extracts were stored in a refrigerator in air tight containers. All the extracts were analyzed for phytochemical screening of compounds and antimicrobial activities.

Qualitative phytochemical studies
Qualitative phytochemical analyses were done by using the procedures of Kokate et al. (1995). Alkaloids, carbohydrates, flavonoids,triterpenoides and proteins were qualitatively analyzed.

Test organisms
The stored culture of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,Bacillussubtilis*andthe pathogenic fungal strains *Aspergillusniger, Aspergillus fumigates, Candida albicans* and *Saccharomyces cereviseae* were collected from the Biotechnology Lab, SBS PGI, Deheradun,Uttarakhand.

Antibacterial Studies Bacterial Media
Twenty grams of Nutrient Agar Media (NAM) was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into petridishes.

Antifungal studies: Fungal media
Fifty eight gram per litre CzapecdoxAgar media were boiled with distilled water and autoclaved.The sterilized media were poured into petridishes(Imran and Al Rubai2015).

Disk diffusion method
Antibacterial and antifungal activity of the plant extract was tested usingthedisk diffusion method (Barry and Thornbarry 1991). NAM and CzapecDox Agar plates were inoculated with different bacteria and fungus(Imran and Al-Karrem2016).50µl of extract was taken in a disc and disc were placed yasceptically on a solid agar media. The plates were incubated at 37°Cfor 24 hours for bacterial activity and 48 hours for fungal activity(Imranet al., (2016).The plates were observed for the zone formation around the wells. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the disc(in mm). The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated.

3. Results

The result of phytochemical screening showed the presence of different compounds in different extracts and were given in Table 1.Alkaloids were present in methanol and ethanol extracts. Proteins were not present in any of the extracts. Flavanoids were present in acetone, chloroform, methanol and ethanol extracts. Triterpenoides were not present in any of the extracts. Carbohydrates were present in acetone, petroleum ether,n-hexane, methanol and ethanol extracts.

The leaf extracts of *Zanthoxylumarmatum* were tested for antibacterial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis* and antifungal activity against *Aspergillusniger, Aspergillusfumigates, Candida albicans* and *Saccharomyces cereviseae.* The result were presented in Table 2 the most susceptible bacteria were *Bacillus subtilis* following by *Staphylococcus aureus* and *Psudomonas. aerugenosa* while most resistant bacteria was E.coli. The *Aspergillusfumigatus* strain examined was the most susceptible to inhibition while *Aspergillus niger, Candida albicans*and *Saccharomyce scereviseae* are the resistant ones.Inhibition was dose dependent in efficacy against bacteria and fungi with most of the extracts showing maximum inhibition at 100% concentration.Maximum ZOI (18mm) was obtained for Chloroform extracts against *Bacillus subtillus* and *S. aureus* at 100%. The growth of fungal strain *Aspergillus fumigates* was also inhibited by the extract.Petroleum ether inhibited the growth of three bacterial strains namely *S.aureus,P.aerugenosa* and *B. subtilis*. Maximum inhibition (ZOI-15mm) at 100% was in case of *B.subtilis*. It also inhibit the growth of *Aspergillusfumigates.*Ethanol and methanol fraction inhibited the growth of three bacterial strains namely *P.aerugenosa, B.subtilis, S.aureus*. Maximum inhibition ZOI (11mm) at 100% was inthe case of *P.*. aerugenosa.n-hexane and Acetone extract did not show any antimicrobial activity against all the tested organism. None of the extracts showed any inhibitory potential against the one bacterial strain *E.coli, C.albicans, Aspergillus niger* and *S. cerevisiae*.

The ethanol extractswas found to have maximum inhibitory potential at 25% concentration, therefore it was used for determination of minimum inhibitory concentration (MIC). The results of MIC test was presented in Table 3. The Ethanol extract with bacterial strain*Pseudomonas aeruginosa* has MIC value at 3.12% concentration with zone of inhibition measuring 6mm. The MIC for *S.aureus* was 6.25% with ZOI measuring 7mm. The MIC for *Bacillus subtilis* was 12.5% with ZOI measuring 7mm.

4. Discussion

Moderate antimicrobial activity was found to be present in fractions extracted from leaves of Zanthoxylumarmatum. The extracts showed inhibitory potential against three bacterial and one fungal strain. The most potent extract is ethanol, which showed maximum inhibitory potential at 25% concentration, therefore it is used for determination of minimum inhibitory concentration.

5. Conclusion

The results of the present study support the traditional usage of the studied plants and suggest that the plant extracts possess compounds with antibacterial and antifungal properties that can be further explored. In future the plants could serve as useful sources for new antimicrobial agents and overcome the side effects caused by synthetic drugs and increase efficacy and provide effective herbal formulation that is cheap, reproducible and stable in suitable condition.

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Table 1: Preliminary phytochemical screening of leaf extracts of *Zanthoxylumarmatum*

S.No	Test	Petroleum ether Extract	Chloroform Extract	n-hexane	Acetone Extract	Methanolic Extract	Ethanolic Extract
I	Alkaloids						
	1. Hager's test	-	-	-	-	+	+
	2.Dragendroff's test	-	-	-	-	+	+
	3. Wagners test	-	-	-	-	+	+
	4. Mayer's test	-	-	-	-	+	+
II	Flavonoids						
	1. $Fecl_3$ test	-	+	-	+	+	+
	2. Zn-HCL test	-	-	-	-	+	+
	3. Alkaline test	-	-	-	-	+	+
III	Triterpenoids						
	1.Salwkowski test	-	-	-	-	-	-
	2. Libbermann- Burchards test	-	-	-	-	-	-
IV	Carbohydrate						
	1. Molish's test	+	-	-	+	+	+
	2. Barfoed test	-	-	+	+	-	-
	3. Anthrone test	-	-	-	-	-	-
V	Protein						
	1. Millon's test	-	-	-	-	-	-
	2. Biuret test	-	-	-	-	-	-
	3. Ninhydrin test	_-	_-	_-	_-	-_	_-

+ Present; - Absent of photochemical compound.

Table 2: Antibacterial and Antifungal activity of *Zanthoxylumarmatum* extracts

Test organism	Petroleum Ether fraction			Chloroform fraction			Methanol fraction			Ethanol fraction			n-hexane fraction			Acetone fraction		
	%			%			%			%			%			%		
	100	50	25	100	50	25	100	50	25	100	50	25	100	50	25	100	50	25
B.subtilis	15	12	6	18	9	6	11	9	7	12	8	8	-	-	-	-	-	-
S.aureus	13	10	7	18	10	7	10	5	4	12	9	8	-	-	-	-	-	-
E.coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P.aerogenosa	10	7	4	15	10	9	11	8	6	11	8	10	-	-	-	-	-	-
Fungi																		
A. fumigatus	10	6	4	12	9	7	9	8	6	9	7	6	-	-	-	-	-	-
A. niger	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. albicans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. cerevisiae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

` Value of zone inhibition diameter(mm) , - no inhibition , Tested concentration: 50 µl/discs , < 10mm - low activity, 10-20 - Moderate activity , >20mm -High activity.

Table 3: Determination of MIC of Ethanol fraction of leaves of *Zanthoxylumarmatum*

S.No	Test organism	Cons.Conc*(IZD) mm				
		50%	20%	12.5%	6.25%	3.12%
1	*E.coli*	_	_	_	_	_
2	*Bacillus. Subtilis*	11	9	7	_	_
3	*P. aerogenosa*	15	10	9	7	6
4	*S.aureus*	11	10	9	7	_

***IZD**:Inhibition zone diameter