Research Paper

Green Farming Vol. **11** (2 & 3) **:** 267-270 ; March-June, 2020

ISSN Print : 0974-0775
Online : 2582-4198

Evaluation of chemical constituents of *Zanthoxylum armatum* and its medicinal effects

HIMANKI DABRAL[a1*] and RAMESH SINGH[b2]

[a]*Department of Agriculture, Doon Business School, Dehradun - 248 001 (Uttarakhand)*

[b]*Samvetan Society for Social and Scientific Research, Dehradun - 248 001 (Uttarakhand)*

Received : 02 May 2020 ; **Revised accepted :** 30 May 2020

ABSTRACT

The aim of this study was the analysis of antifungal activity and phytochemical screening of leave extracts of *Zanthoxylum armatum* for the evaluation of active ingredients. *Zanthoxylum armatum* belongs to the family of deciduous evergreen shrub and tree, Rutaceae. In the traditional system of medicine various parts of *Zanthoxylum armatum* are used for the treatment of fevers, dyspepsia, cholera, dental caries, rheumatism, diuretic and stomach ache. The preliminary screening of phytochemicals using soxhlet apparatus for the presence of secondary metabolites from leave extracts have shown the presence of alkaloids, flavanoids, carbohydrate and proteins. The crude extract showed antifungal activity (Disc diffusion method) against *Aspergillus fumigatus.* None of the extracts showed any inhibitory potential against Candida albicans, *Saccharomyces cereviseae* and Aspergillus niger. The generated data has provided the basis for its medicinal value and can be used as a therapeutant.

Key words : *Alkaloids, Antifungal activity, Flvanoids and Phytochemicals, Zanthoxylum armatum.*

INTRODUCTION

Zanthoxylum armatum D.C. is a small xerophytic tree or shrub. Leaflet blades usually with prickles. Leaves are compound, imparipinnate with 3-7 foliolate and pellucid punctate. Petiole and rachis are winged. Leaflets are sessile, elliptic to ovate-lanceolate with crenate or entire margins. Flowers are born axiliary, minute and polygamous. Calyx is 6-8-acute lobed, while petals are absent. Male flowers have 6-8 stamens with rudimentary ovary. Female flowers possess 1-3 carpels and 1-3 locular ovary. Fruit is drupe and having round seed. *Z. armatum* prefers semi shady environment or no shade for growth. It grows wild in foothills starting from about 800m up to 1500m in Malak and,Swat, Dir, Hazara, Buner, Muree hills, and Rawalpindi (Shinwari *et al*., 2006). It is known as Dambrary, Tamur (Urdu) and Dambara (Pashtu). The plant is used for treatment of pneumonia and tick infestation (Iqbal *et al*.,2010). Young shoots are used for curing gum diseases. Fruit is used for toothache, dyspepsia, stomachache, and as a carminative. Seeds are used as condiment and flavoring agent. Powdered fruit, mixed with Menthaspp and table salt is eaten with boiled egg for chest infection and other digestive problems (Islam *et al*., 2009).The leaves and fruits of this plant is used for various pharmacological activities including antipyretic action (Barkatullah *et al*., 2011). They are also used to ward off houseflies (Gaur *et al.,* 1999).

Therefore, the present study, has been focused on the preliminary screening of phytochemicals and quantitative estimation of secondary metabolites for antifungal activity from the leaves of *Zanthoxylum armatum.*

MATERIALS AND METHODS

Sample collection

Leaves of *Zanthoxylum armatum* was collected from Tehri Garhwal range of Uttarakhand, India and the plant were identified and authenticated at the Department of Bio-technology SBS PGI, Dehradun.

Extraction of plant material

Various extracts of *Z. armatum* were prepared as per the method given by (Anonymous, 1966). The leaves were shade dried and grounded into coarse powder. The coarse powder was subjected to Soxhlet extraction separately and successively with Petroleum ether, chloroform, n-hexane, acetone, ethanol and methanol. These solvents differ in their boiling Table 1. The extraction chamber is suspended above the round bottomed flask containing the solvent. The flask is heated and the solvent evaporated and moves up into the condencer where it gets converted into liquid that trickles into the extraction chamber containing the sample. At the end of the extraction

[1]Assistant Professor *(dabralhimanki@gmail.com), [2]Scientist

process, the flask containing the solvent is removed.

Percentage yield

After extraction, percentage yield of Chloroform, Petroleum ether, Acetone, n-hexane, methanol and ethanol were determined by using the following formula :

$$\text{Yield (\%)} = \frac{\text{Weight of extract recovered}}{\text{Weight of powdered plant part used}} \times 100$$

Preliminary Screening of phytochemicals

These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-500C) and were analyzed for phyto-chemical screening of compounds using standard procedure (Kokate, 2000 ; Sowmya *et al.,* 2011 and Arunkumar *et al.,* 2013).

Estimation of Phytochemicals

A.) Test for Alkaloids : To the aqueous leaf extract dilute HCl was added, shaken well and filtered. With the filterate following test were performed.

(i) Dragendorff's test : To 2 mg of the extract, 5 ml of distilled water was added, 2M Hydrochloric acid was added until an acid reaction occurs. To this 1 ml of Dragendorff's reagent was added. Formation of orange red precipitate indicated the presence of alkaloids.

(ii) Hager's Test : In a test tube add 2-3 ml of filtrate and hager's reagent was added. Formation of yellow ppt showed the presence of alkaloids.

(iii) Mayer's Test : To 2-3 ml of filtrate hager's reagent was added. Formation of yellow ppt showed the presence of alkaloids.

(iv) Wagner's Test : 2 mg of the extract was acidified with 1.5% v/v of hydrochloric acid and a few drops of Wagner's reagent were added. A yellow or brown precipitate indicated the presence of alkaloids (Memelink *et al.,* 2001).

B.) Test for Terpenoids

(i) Salkowski,s Test : 5 ml leaf extract was mixed with 2 ml chloroform then 3 ml concentrated sulphuric acid was added to form a layer A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

(ii) Liebermann Burchard Test : 2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated H_2SO_4 was added along the sides of the test tube. Formation of a violet colored ring indicated the presence of triterpenoids.

C.) Tests for flavanoids

(i) Alkaline Reagent Test : A few drops of dilute NaOH solution was added to 1 ml of test solution. An intense yellow colour was appeared in the test tube which became colourless on the addition of few drops of dilute acid that indicated the presence of flavanoids.

(ii) Ferric chloride Test : The extract is dissolved in distilled water. To this, few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compounds.

(iii) Zn-HCL Test : The extract is dissolved in alcohol and few fragments of magnesium ribbon and concentrated hydrochloric acid (drop wise) are added. If any pink to crimson colour develops, presence of flavanol glycosides is inferred by the development of pink to crimson colour.

D.) Tests for Proteins

(i) Millon's Test : A few drops of the millons reagent added to 1 ml of the extract solution, which is then heated gently. A reddish brown precipitate indicates the presence of tyrosine residue which occurs in nearly all proteins.

(ii) Biuret Test : Add 1ml of 40% NaOH solution and 2 drops of 1% $CuSO_4$ solution till a blue colour develops, then add 1ml of the extract. Formation of pinkish or purple violet colour indicates the presence of proteins.

(iii) Ninhydrin Test : Add two drops of freshly prepared 0.2% ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heat. Development of blue colour reveals the presence of proteins, peptides or amino acids.

E.) Test for carbohydrates

(i) Molisch's Test : To 2ml of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid along the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates.

(ii) Barfoed 's Test : Add 2 ml of Barfoed reagent to 1 ml of leave extract. Keep the test tube in water bath. The formation of brick red precipitate indicate the presence of Carbohydrates.

(iii) Anthrone Test : Add 2 ml of Anthrone reagent to 1 ml of leave extract. The formation of bluish green colour indicate the presence of Carbohydrates.

Antifungal activity

For various extracts antifungal activity were tested against the selected fungal pathogens was given in Table 2. The fungal strain include in the study were *Aspergillus fumigates, Aspergillus niger, Candida albicans* and *Saccharomyces cereviseae* . Antifungal activity of the plant extract was tested using disk diffusion method (Barry and Thornbarry, 1991). Czapec Dox Agar plates were inoculated with different fungus. 50µl of extract was taken in a disc and disc were placed asceptically on a solid agar media. The plates were incubated at 37° for48 hours for fungal activity. 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.

Determination of Minimum Inhibitory Concentration (MIC)

Microbial growth inhibition measurement

Microbial growth inhibition was determined as the diameter of inhibition around the well. Zone of inhibition (ZOI) were measured in millimeter with the help of scale.

MIC Testing

Minimum inhibitory conc level of any antifungal substance is the lowest conc of drug inhibiting the fungal growth. The MIC value of microorganism against a particular fraction is considered which exhibit maximum activity in preliminary screening process by disc diffusion method. MIC was determined by making further dilution (12.5% ,6.25%, 3.12% of extract solution and screening them for antifungal activity.

RESULTS AND DISCUSSION

The present study carried out on *Zanthoxylum armatum* revealed the presence of medicinal active constituents. The phytochemical active compound of this species were analyzed and result are presented in **Table 1**. In these screening process 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 medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, alkaloids protect against chronic diseases. Triterpenoids show the analgesic for central nervous system activities. The leaf extract were also tested for antifungal activity, among fungal strain *Aspergillus fumigatus* strain examined was the most susceptible to inhibition while *Aspergillus niger, Candida albicans* and *Saccharomyces cereviseae* are the resistant ones. The growth of fungal strain *Aspergillus fumigatus* was inhibited by the extract. Maximum inhibition (ZOI-10mm) at 100% was in case of *Aspergillus fumigatus*. None of the extracts showed any inhibitory potential against *C. albicans*, *A. niger* and *S. cerevisiae*. Antifungal activity have been previously reported in several other species of Zanthoxylum against a range of strains.(Islam *et al.,* 2001 ; Yeboa *et al.,* 2005 and Steenkamp *et al.,* 2007). The previous reports suggest that the presence of alkaloids in plant may possibly responsible for antifungal activity (Thouvenel *et al.,* 2003 and Yang *et al.,* 2008). The chloroform extracts was 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 chloroform extract with fungal strain has MIC value at 3.12% concentration with zone of inhibition measuring 5mm.

The quantitative analysis of *Z. armatum* showed that this plant might be a rich source of bioactive constituents for future uses in pharmaceutical preparations.

Table 1. Preliminary phytochemical screening of leaf extracts of *Zanthoxylum armatum*

Sr.No.	Test	Petroleum ether Extract	Chloroform Extract	n-hexane	Acetone Extract	Methanol Extract	Ethanol Extract
1.	Alkaloids						
	i. Hager's test	–	–	–	–	+	+
	ii .Dragendroff's test	–	–	–	–	+	+
	iii. Wagners test	–	–	–	–	+	+
	iv. Mayer's test	–	–	–	–	+	+
2.	Flavonoids						
	i. $Fecl_3$ test	–	+	–	+	+	+
	ii. Zn-HCL test	–	–	–	–	+	+
	iii. Alkaline test	–	–	–	–	+	+
3.	Triterpenoids						
	i. Salwkowski test	–	–	–	–	–	–
	ii. Libbermann- Burchards test	–	–	–	–	–	–
4.	Carbohydrate						
	i. Molish's test	+	–	–	+	+	+
	ii. Barfoed test	–	–	+	+	–	–
	iii. Anthrone test	–	–	–	–	–	–
5.	Protein						
	i. Millon's test	–	–	–	–	–	–
	ii. Biuret test	–	–	–	–	–	–
	iii. Ninhydrin test	–	–	–	–	–	–

'+' Present; '-' Absent of photochemical compound.

Table 2. Antifungal activity of *Zanthoxylum armatum* 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
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 Chloroform fraction of leaves of *Zanthoxylum armatum*

Sr.No.	Test organism	Conc (ZOI) mm				
		50%	20%	12.5%	6.25%	3.12%
1	*A. fumigatus*	9	7	6	4	5
2	*A. niger*	_	_	_	_	_
3	*C. albicans*	_	_	_	_	_
4	*S. cerevisiae*	_	_	_	_	_

CONCLUSION

In conclusion it can be stated that tested extracts of *Z. armatum* proved to be good and safe antifungal agent which could also be used in future for valuable pharmaceutical preparations from this plant. Further studies are required to explore this plant phytochemically and pharmacologically, which could prove this plant as cheaper and more easily accessible source of valuable drugs.

ACKNOWLEDGMENT

I pay my immense thanks to Department of Biotechnology Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences and Research, Dehradun for providing the necessary facilities for the completion of this project work.

REFERENCES

Anonymous. 1966. Pharmacopoeia of India (The Indian Pharmacopoeia) 2nd Edition, Manager of Publications, New Delhi, India. pp. 947-48.

Arunkumar S. 2013. Preliminary phytochemical analysis, hptlc finger printing and in vitro anti cancer screening of extracts of aerial parts of *Cardiospermum helicacabum. IJPPS.* **5** (3) **:** 301-304.

Barry A L and Thornberry C. 1991. Susceptibility test. Diffusion test procedures. In manual of Clinical Microbiology Edited by Ballow A, *et al. American Society for Microbiology.* pp. 1117-1125.

Bafi-Yeboa N, Arnason J, Baker J and Smith M. 2005. Antifungal constituents of Northern prickly ash, *Zanthoxylum americanum* Mill *Phytomedicine.* **12** (5) **:** 370-377

Barkatullah, Ibrar M and Muhammad N 2011.Evaluation of *Zanthoxylum armatum* D.C. for *in vitro* and *in vivo* pharmacological screening. *Afr. J. Pharm. Pharmacol.* **5** (14) **:** 1718-1723.

Gaur R D. 1999. Flora of District Garhwal, North Western Himalayas, Transmedia Srinagar. pp. 811.

Islam A , Sayeed A, Bhuiyan M, Mosaddik M and Astaq Mondal Khan G 2001. Antimicrobial activity and cytotoxicity of *Zanthoxylum budrunga* Fitoterapia. **72** (4) **:** 428-430.

Islam M S, Akhtar M, Rahman M M, Rahman M A, Sarker K K and Alam M F. 2009. Antitumor and phytotoxicactivities of leaf methanol extract of *Oldenlandia diffusa* (Willd.) *Roxb. Global J. Pharmacol.* **3** (2) **:** 99-106.

Iqbal Z, Khan M, Jonsson N and Siddique M 2010. Documentation of ethno veterinary practices used for treatment of different ailments in a selected hilly area of Pakistan. *Int. J. Agric. Biol.* **12** (3) **:** 353-358.

Kokate C K. 2003. *Practical Pharmacognosy*. Pune : Vallabh Prakashan. pp. 222.

Memelink J, Verpoort R and Kigine J W. 2001. Organization of jasmonate responsive gene expression in alkaloid metabolism. *Trends in Plant Sci.* **6** (5) **:** 212-219.

Shinwari Z, Watanabe T, Rehman M and Youshikawa T. 2006. A Pictorial Guide to Medicinal Plants of Pakistan. *Kohat University of Science & Technology, Pakistan.* pp. 247.

Sowmya G. Shetty, Vinayachandra, Syed Hidayathulla and Chandrasheka K R. 2011. Antimicrobial activity and phytochemical screening of *Pterospermum reticulatum* wight & arn. *IJPPS.* **3** (5) **:** 35-37. 20.

Steenkamp V, Fernandes AC and Van Rensburg CE.2007 Screening of venda medicinal plants for antifungal activity against *Candida albicans . S. Afr. J. Bot.* **73** (2) **:** 256-258.

Thouvenel, Gantier J C and Duret. 2003. Antifungal compounds from *Zanthoxylum chiloperone var. angustifolium. Phytother Res.* **17** (6) **:** 678-680

Yang C H, Cheng M J, Chiang M Y, Kuo Y H, Wang C J and Chen I S. 2008. Dihydrobenzo [c] phenanthridine alkaloids from stem bark of *Zanthoxylum nitidum. J. Nat. Prod.* **71** (4) **:** 669-673.

Citation :

HIMANKI DABRAL and RAMESH SINGH. 2020. Evaluation of chemical constituents of *Zanthoxylum armatum* and its medicinal effects. ***Green Farming*** Vol. **11** (2 & 3) **:** 267-270 ; March-June, 2020.