

Local Anaesthetic And Anti Microbial Activity Of Stem Bark Of *Zanthoxylum Tetraspermum* Wight & Arn.

V.R.Ravikumar^{*1}, V.Gopal², N. Ravichandran³, T.Sudha⁴, and A. Muthukumar⁵

^{*}Research Scholar, Sastra University, Thanjavur

^{*1}Department of Pharmacognosy, ⁴ Department of Pharmaceutical analysis ⁵ Department of Pharmacology The Erode College of Pharmacy and Research, Erode-638112. Tamil Nadu.

² Motherthersa Post graduate Research Institute of Health sciences, Puducherry.

³ CARSIM Sastra University, Thanjavur.

Email – ravisrkumar@yahoo.com

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Abstract

Ethanollic and aqueous extracts of stem bark of *Zanthoxylum tetraspermum wight & Arn* were evaluated for local anaesthetic activity by surface anesthesia method using, rabbits, Nerve block anaesthesia method using frogs, Infiltration anaesthesia method using guinea pig and anti-microbial activity by disc diffusion method using microbial stains respectively. The result indicated that crude extracts exhibited significant local anesthetic and Anti-microbial activity when compared to standard drugs. The ethanolic extracts shows moderately significant activity compare to Aqueous extract of *Zanthoxylum tetraspermum wight & Arn*.

Key Words: *Zanthoxylum tetraspermum wight & Arn*, local anaesthetic, Anti-microbial

Introduction

Zanthoxylum tetraspermum wight & arn (Rutaceae) is a thorny, stout, aromatic, climbing shrub, with brown bark having short recurved prickles, found in the western ghats in the nilgiri and annaimalai hills and in kollis hills (namakal district, TN) at attitudes of 1,200 1,800m and in kerala and Karnataka. The wood is yellowish and soft. The plant is credited in srilanka with stimulant, astringent and digestive properties and is prescribed in dyspepsia and diarrhoes [1-3]. 8-acetyonyldihydroneitidine, 8-acetyonyldihydrovicine, Liriodenine, sesamin, Lichexanthone, (+) - piperitol-gamma-dimethylallaylether have been reported [4]. The present investigation was undertaken to study the phytochemicals present in the stem bark extracts of this plant by GC-MS method.

Material and Methods

Plant material:

The stem bark of *Zanthoxylum tetraspermum wight & arn* were collected from cholakkadu, a village of kolli hills, 60km away from Namakkal (TN) in the month of August (2007). The plant was identified by local people of that village and authenticated by G.V.S. Murthy, Join Director, botanical survey of India, southern circle, Coimbatore, (No. Bsi/sc/5/23/07-08/ Tech 715). A herbarium specimen of the plant was preserved in the department of pharmacognosy of our institute for further references.

Chemicals:

All the reagents used were of analytical grade obtained from S.D. fine chemicals Ltd, Mumbai.

Preparation of stem bark extracts:

The stem barks of *Zanthoxylum tetraspermum* were washed with water, air- dried at room temperature and then reduced to coarse powder. The dried powder

(250mg) was subjected to Soxhlet extraction with petroleum ether (40-60), chloroform, ethyl acetate, ethanol and aqueous (in order of increasing polarity) for continuous hot extraction. The extracts were filtered and the filtrates were concentrated under reduced pressure to obtain the extracts as solid residues. The percentage extraction values (% w/w) were 1.25, 1.37, 1.96, 3.75 and 4.06% respectively. The freshly prepared extracts were chemically tested for the presence of different constituents using standard methods. [5- 8]

Local Anesthetic Activity

Surface Anaesthesia:

The local Anaesthetic activity of bark of *Zanthoxylum Tetraspermum wight & Arn* was determined by surface anaesthesia method by using rabbits [9]. The rabbits were divided into three Groups. Group I were treated with standard drug lignocaine (2%). Group II were treated with ethanolic extract of bark of *Zanthoxylum Tetraspermum wight & Arn* (20mg/ml) and Group III are treated with Aqueous extract of bark of *Zanthoxylum Tetraspermum wight & Arn* (20mg/ml). The rabbits were placed in rabbit box by keeping the head outside and the corneal reflex was recorded by touching the side of cornea with cotton piece. The extract samples were instilled into right eye and control sample (normal saline) was instilled into left eye. The corneal reflexes were recorded at 5 minutes interval up to one hour. The results are recorded in Table no. 1.

Nerve Block Anaesthesia:

Three frogs (150 gm) were decerebrated and the upper part of the spinal cord was destroyed with the help of pithing needle.[10] Abdomen was cut opened and all the abdominal organs were removed, to get a pouch(sac) made of abdominal walls. The sciatic nerve was exposed to each test drug(ethanolic and aqueous extract of bark of *Zanthoxylum Tetraspermum wight & Arn* 20mg / ml) separately. Piece of cotton was immersed in 0.25%w/v of xylocaine (lignocaine) solution and places in the abdominal pouch of 1st frog. Similarly, separate pieces of cotton was immersed in ethanolic and aqueous extract of *Zanthoxylum Tetraspermum wight & Arn* (20 mg/ml) and then placed in abdominal pouch of 2nd and 3rd frogs respectively. The frog board as placed vertically so that the hind legs of frog hang freely. Right and Left hind legs were immersed in the beakers containing 0.1N HCl and normal. Sudden leg withdrawal action was noted before and

after administration of drug. The results are recorded in table no. 2

Infiltration Anaesthesia:

A healthy adult male guinea pig(CPCSEA regd no. ECP/IAEC-A/2010/06/05) was taken and fur on its back was removed by using depilating agents.[10] The depilated area was washed with normal saline and allowed to dry. Then the standard and test drug(ethanolic and aqueous extract of *Zanthoxylum Tetraspermum wight & Arn* 20 mg/ml) was injected intradermally to three different guinea pigs. Squeak or twitch response of animal upon touching the injection site with sharp pin was marked as(+) and (-) if the animal does not show any response to pin prick. The results are recorded in table no. 3.

Screening of Anti Microbial Activity:

Determination of anti-bacterial activity

The ethanolic and aqueous extracts of bark of "*Zanthoxylum tetraspermum wight & arn*" were tested for anti-bacterial activity by the disc diffusion method [11,12,13]. Different concentration of extract (10, 20, 50µg/ml) were prepared by reconstituting with dimethyl formamide (DMF). The test micro organisms (10µg/ml) were seeded into respective medium by spread plate method with the 24h culture of bacteria growth in nutrient broth. After solidification the filter paper discs (whatman no.1, 6 mm in diameter) impregnated with the extract were placed on test organism seeded plates. Ampicilline (10 µg/ml), was used as positive control while dimethyl formamide (DMF) solvent (100µg/ml) was used as negative control. The antibacterial assay plates were incubated at 37°C for 24h, the diameter of each zone of inhibition was measured in mm.

Antifungal activity:

The antifungal activity of ethanolic and aqueous extracts of bark of "*Zanthoxylum tetraspermum wight & arn*" was tested by disc diffusion method. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper disc (whatman no.1, 6mm in diameter) impregnated with (10, 20, 50µg/ml) concentration of the extracts was placed on test organism seeded plates. A blank disc impregnated with dimethyl formamide (DMF) was used as negative control and ketoconazole 10 µg disc was used as positive control. The activity was determined after 72h of incubating

at 28°C. The diameter of inhibition zones was measured in mm.

Standard antibiotics:

Ampicilline and ketoconazole were used as reference antibiotics against bacteria and fungus respectively.

Microbial stains:

Two gram-positive bacteria, one gram-negative bacteria and two fungi were used in the study. Two gram-positive bacteria where *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 9144), gram-negative bacteria *Escherichia coli* (ATCC 25922) and the two fungi were *Candida albicans* (ATCC 2091) and *Aspergillus niger* (ATCC9029). The bacterial strains were maintained in nutrient agar and the fungal species in potato dextrose agar slants and stored at 4°C.

Results and Discussion

Local anaesthetic:

The local anesthetic activity of the ethanolic and aqueous extracts of bark of "*Zanthoxylum tetraspermum wight & arn*" (20mg/ml) was evaluated by surface anesthesia method using rabbits. Both extracts showed significant local anesthetic activity when compared to standard drug lignocaine (2%). The activity of ethanolic extract (up to 10 min) is comparably more than the activity produced by aqueous extract (up to 15 min). 0.1N Hcl in beaker was used to find out the anaesthetic activity of extract of bark of "*Zanthoxylum tetraspermum wight & arn*". in Nerve block anesthesia method, before administration of drug, when the acid has been conducted with the hind leg there was a sudden with drawal of leg from the beaker in which the 0.1N Hcl was taken. After administration of the drug, when tested with Hcl, there was absence of withdrawal of leg from the acid. Hence it was proved that the extract of bark of "*Zanthoxylum tetraspermum wight & arn*", had local anaesthetic activity. The ethanolic extract of "*Zanthoxylum tetraspermum wight & arn*" exhibited equal activity like that of the standard drug lignocaine (xylocaine) when compared to aqueous extract of bark of "*Zanthoxylum tetraspermum wight & arn*". In the Infiltration method, before administration of the drug, there was response for pin

prick up to 1 minute. After administration of the test drug of ethanolic extract of bark of "*Zanthoxylum tetraspermum wight & arn*", there was no response for pin prick up to 30 minutes. The ethanolic extract of bark of "*Zanthoxylum tetraspermum wight & arn*" exhibited good local anaesthetic activity when compared to aqueous extract (20 mg/ml) of bark of *Zanthoxylum tetraspermum wight & arn*,. So, the duration of local anaesthetic activity was found to be 30 minutes.

Anti – microbial activity:

Results obtained present study exhibits that the tested plant extract possess potential antibacterial and anti fungal activity against the microorganisms studied. (Table no - 4) when tested by disc diffusion method. The ethanolic extracts of bark of "*Zanthoxylum tetraspermum wight & arn*" at 50 µg/ml showed significant effect against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* (around 20 - 21mm) and aqueous extract at 50 µg/ml showed significant effect against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* (around 18 - 19mm) zone of inhibition and standard ampicilline shows around 22-23 mm zone of inhibition. The antifungal activity the ethanolic extract at 50 µg/ml showed around 20-21 mm zone of inhibition against *Candida albicans* and *Aspergillus niger*. In aqueous extract at 50 (µg/ml showed around 17-18 mm zone of inhibition against *Candida albicans* and *Aspergillus niger* and standard ketoconazole shows around 23-24 mm zone of inhibition. The both ethanolic and aqueous extract showed almost similar activity when compared with the ampicilline and ketoconazole for antimicrobial and anti fungal activity respectively. In summary ethanolic extract exhibited more than aqueous extract.

Conclusion

From the above observation the both ethanolic and aqueous extract of bark of "*Zanthoxylum tetraspermum wight & arn*" has significant local anaesthetic and antimicrobial activity. The ethanolic extract moderately significant activity when compared to that of aqueous extract of bark of *zanthoxylum tetraspermum wight & arn*. In future further studies will be performed for isolation of particular phytoconstituent which is responsible for local anathesthetic activity.

Table No.1

Local Anaesthetic Activity Of Ethanolic & Aqueous Extract Of Bark Of “*Zanthoxylum Tetraspermum Wight&Arn*” By Surface Anaesthesia Method In Rabbit

S. No	Group	Corneal Reflux in minutes												
		0	5	10	15	20	25	30	35	40	45	50	55	60
1.	Control (normal saline) in left eye	+	+	+	+	+	+	+	+	+	+	+	+	+
2.	Lignocaine 2 %	+	-	-	-	-	-	-	-	-	-	-	-	-
3.	Ethanolic extract (20mg/ml)	+	+	+	-	-	-	-	-	-	-	-	-	-
4.	Aqueous extract (20mg/ml)	+	+	+	+	-	-	-	-	-	-	-	-	-

No of animals in each group-4 (n=4)

(+) Presence of corneal reflux.

(-) Absence of corneal reflux.

Table No. 2

Local anaesthetic activity of ethanolic & aqueous extract of bark of “*Zanthoxylum tetraspermum wight&arn*” by nerve block anaesthesia method in frog.

Standard vs Test(extract)	Response	
	Left leg	Right leg
Before administration of drug	+	+
standard drug lignocaine		
0 minute		
1 minute	+	+
2 minute	-	-
3 minute	-	-
4 minute	-	-
5 minute	-	-
6 minute		
Ethanolic extract(20mg/ml)		
0 minute		
1 minute	+	+
2 minute	+	+
3 minute	-	-
4 minute	-	-
5 minute	-	-
6 minute	-	-
Aqueous extract(20mg/ml)		
0 minute		
1 minute	+	+
2 minute	+	+
3 minute	+	+
4 minute	-	-
5 minute	-	-
6 minute	-	-

Table No. 3

Local anaesthetic activity of ethanolic & aqueous extract of bark of “*Zanthoxylum tetraspermum* Wight & Arn ” by Infiltration anaesthesia method in guinea pig.

Group	Time in minutes	Response
Before administration of the drug	0	+
NS(reproduced)	1	+
	5	+
	10	+
Ethanolic extract (20mg/ml) (After administration of drug)	15	-
	20	-
	25	-
	30	-
	35	+
	NS(reproduced)	1
Aqueous extract(20mg/ml) (After administration of drug)	5	+
	10	+
	15	+
	20	-
	25	-
	30	-
	35	+

Table No. 4

Data showing Anti Microbial Activity of ethanolic and aqueous extract of bark of "*Zanthoxylum tetraspermum wight & arn*"

Extracts	Antibacterial activity			Antifungal activity	
	Zone of Inhibition in (mm)			Zone of Inhibition in (mm)	
	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli mutant</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>
Ethanolic extract					
10 µg/ml	10	11	10	12	11
20 µg/ml	17	18	18	16	18
50 (µg/ml)	20	21	20	21	20
Aqueous extract					
10 µg/ml	08	07	07	08	08
20 µg/ml	14	14	15	15	14
50 µg/ml	19	18	19	18	17
Standard *	22	23	22	23	24
Control DMF	-	-	-	-	-

*Standard Antibiotics ampicilline for bacteria, ketoconazole for fungus (10µg/ml)

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