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IN VITRO ANTI INFLAMMATORY ACTIVITY OF STEM OF *ZANTHOXYLUM RHETSA* (ROXB.) DC.

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ABSTRACT

Zanthoxylum rhetsa (Roxb.) DC. is a valuable medicinal plant which has been valuable for centuries in ayurvedic medicine. Phytochemical analysis of *Z.rhetsa* (Roxb.) DC. stem extracts revealed the presence of various bio chemical compounds such as flavonoids, glycosides, alkaloids, aleurone, starch, amino acids, lignin, volatile oil, fats and fixed oils, mucilage, pectins, proteins, steroids and triterpenoids. Since glycoside and flavonoids have remarkable anti-inflammatory activity. Our present work aims at evaluating the in *vitro* anti in-flammatory activity of *Zanthoxylum rhetsa* (Roxb.) DC. by protein denaturation and HRBC method. Denaturation of protein is a well-documented cause of inflammation and rheumatoid arthritis. The data of our studies suggests that *Zanthoxylum rhetsa* (Roxb) DC. of stem extract showed significant anti- inflammatory activity.

KEYWORDS: Zanthoxylum rhetsa, anti-inflammatory, HRBC method, flavonoid.

INTRODUCTION

The *Zanthoxylum rhetsa* (Roxb.) DC is under the the family of Rutaceae. A genus of herbs is well distributed in India, Bangladesh, bhutan, china etc. This plant contains a terpenoid, xanthyletin and sesamin, alkaloids, flavonoids and sabinene.[1] The different parts of this

species have been used in traditional medicine. The bark is having diuretic properties and used to management of dental caries.[2] In India traditional medicine, the bark has been used to treat cardiac, respiratory diseases, tooth infection, stomach infection and rheumatism.[3,4] The fruits are used as spice and the essential oil extracted from the fruits is known as "Mullilam oil" used as anti-inflammatory, antiseptic, anticholera, diarrhoea, hypo-cholesterolemic, mosquito repellent and soothing agent for dental caries.[5,6] The Kanikkars family prepares a paste of hard spines prepared by rubbing them against rock with water and applies the extract to the breast of a nursing mother to relief pain and also to increase milk supply.[7] In the Phillippines, the powdered bark mixed with oil is a best formula to treat stomach ache. In addition, the bark decoction is also taken to treat chest pain and chewed bark applied as antidote for snake bites. The present work was aimed to study the anti-inflammatory activity of stem of the plant *Zanthoxylum rhetsa* (Roxb.) To achieve the objectives the plan is executed to extraction of the stem of *Zanthoxylum rhetsa* (Roxb.) and *in vitro* study of anti-inflammatory activity.

MATERIALS AND METHODS

Plant material

The stem of *Zanthoxylum rhesta* (Roxb.) DC was collected from the area of Kollimalai hills in Erode district, Tamilnadu, India. The stem of *Zanthoxylum rhetsa* (Roxb.) DC. The plant material was authenticated by Dr. P. Jayaraman, Ph.D., Director, Plant Anatomy Research Centre, Chennai, Tamilnadu, India and a voucher specimen no: PARC/2014/2089 was deposited at the museum SSM College of Pharmacy, Erode (638312) Tamilnadu, India.

METHODS

Extraction

Fresh stem of *Zanthoxyllum rhetsa* (Roxb.) DC. were collected cut into small pieces and dried under shade morning time for 10 days. The dried parts were passed through sieve (coarse 10/40) this powder was used for the preparation of solvent extraction and 500 gram of powder was extracted by maceration technique.

Extraction of Plant Material

The stem material was dried in the shade for two month. Then shade dried plant was subjected to get coarse powder. The coarse powders were subjected to Soxhlet apparatus by using various solvent according to their polarity.

Benzene Extract

The marc left after petroleum ether extraction was dried and extracted with 2-3 liters of benzene of (79-81^{o}C) by continuous hot percolation using Soxhlet apparatus. After completion of extraction it was filtered and the solvent was removed by distillation under reduced pressure. The extract was then stored in a desiccators. A brown colour residue was obtained.

Alcohol Extract

The marc left acetone extraction was dried and extracted with 2-3 liters of alcohol 95% by continuous hot percolation using Soxhlet apparatus. After completion of extraction, it was filtered and the solvent was removed by distillation under reduced pressure. The extract was stored in desiccators. A light brown color residue was obtained.

Aqueous Extract

The marc left after alcohol extraction was dried and macerated with 2-3 liters of chloroform water (0.25%) in mouthed bottle for three days. After completion of extraction it was filtered and the solvent was removed by distillation under reduced pressure. The extract was then store in desiccators. A black colour residue was obtained.

All the above extracts were used for identification of constituents by phytochemical tests. From the weight of drug, the extract content was calculated.[8]

$$\text{Extractive Value (\%)} = \frac{\text{Wt. of Extractive}}{\text{Wt. of Drug}} \times 100$$

Phytochemical Test

The powder and various extracts of the plant were subjected to chemical tests for identification of its active constituents.[11,12]

Table No.1: Preliminary Phtyo-chemical Test of Zanthoxylum rhetsa (Roxb.) DC.

S.N0	CHEMICAL TEST	EXTRACT'S			
		BENZENE	ETHANOL (ALCOHOLIC EXTRACT)	AQUEOUS	*Zanthoxylum rhetsa* (Roxb.) DC.
1.	ACIDIC COMPOUNDS	-	+	-	+
2.	ALEURONE GRAINS	+	+	+	+
3..	TEST FOR ALKALOIDS	+	+	_	+
4.	AMMINO ACIDS	-	+	+	+
5.	TEST FOR CARBOHYDRATES	–	–	–	–
6.	CELLULOSE	_	_	_	_
7.	LIGNIN	+	_	_	+
8.	VOLATILE OIL	+	+	+	+
9.	FATS &FIXED OILS	+	+	+	+
10.	FLAVONOIDS	+	+	+	+
11.	GLYCOSIDES	_	+	_	+
12.	INULIN	-	-	-	-
13.	MUCILAGE	+	+	+	+
14.	PECTINS	+	-	-	+
15.	TANNINS	-	+	-	+
16.	PROTEINS	+	+	+	+
17.	STARCH	-	-	+	+
18.	STEROIDS & TRITERPENOIDS	+	+	-	+
19.	NAPTHOQINONES	-	-	-	-

PHARMACOLOGICAL SCREENING

Evaluation of *in vitro* anti-inflammatory activity

Human Red Blood Cell (HRBC) Membrane Stabilization Method

The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of alsever Solution (2% dextrose,0.8% sodium citrate,0.5% citric acid and 0.42% NaCl) and centrifuged at 3000rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various Concentrations of extract were prepared (20, 60,80 & 100µg/ml) using distilled water and to each concentration 1ml of phosphate buffer, 2ml hyposaline and 0.5ml of HRBC suspension were added. It was incubated at 37^0c for 30min and centrifuged at 3000rpm for 20min and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560nm Aspirin (100µg/ml) was used as reference standard and a control was prepared by omitting the extract.

The percentage of HRBC membrane stabilization or protection was calculated by using the following formula,[9]

% inhibition = (Abs $_{control}$ – Abs $_{sample}$) x 100 /Abs $_{control}$

$Abs_{control}$ =Absorbance of control.

Abs_{sample} = Absorbance of sample.

Membrane stabilization test

Preparation of RBC Suspension

Fresh whole human blood (10ml) was collect and transferred to the haparinized centrifuged tube. The tubes were centrifuged at 3000 rpm for 10 min and were washed three time with equal volume of normal saline. The volume of the blood was measured and reconstituted at 10% v/v suspension with normal saline.

Heat Induced Hemolysis

The reaction mixture (2ml) consisted of 1ml of test drug solution and 1ml of 10% HRBC suspension. Instead of drug only saline was added to the control test tube. Aspirin was taken as a standard drug. All the centrifuge tubes containing mixture were incubated in a water bath at 56^{o}C for 30 mints. At the end of the incubation, the tubes were cooled under running tap water.

The reaction mixture was centrifuged at 2500 rpm for 5 mints and the absorbance of the supernatants was taken at 560nm.The experiment was performed in triplicates. Percent membrane stabilization activity was calculated by using the formula.[10]

100 x (V_t / V_c – 1)

Where,

V_t = Absorbance of test sample

V_c = Absorbance of control

RESULT

Phyto-Chemical Investigation

- The dried powder of the stemswas extracted by continuous hot percolation (**Soxhlet** apparatus) with different solvents of increasing polarity and percentages of extracts were calculated.
- The various extracts were subjected to Phyto-chemical staining the extracts answered Positively for Aleurone grains, Alkaloids, Amino Acids, Lignin, Volatile oils, Fats &

Fixed Oils, Mucilage's, Protein, Glycosides, Pectins, Starch, Steroids & Triterpenoids, Flavonoid's.

- It negatively answered for Carbohydrates, Cellulose, Inulin and Napthoquinones

Table 2: In-vitro anti-inflammatory activity of stem of *Zanthoxylum rhetsa* (Roxb.) DC. On inhibition of Protein denaturation.

Treatment	Concentration	Absorbance at 660 nm	% Inhibition of Protein Denaturation
Control	-	0.37	-
Ethanol	20	0.28	24
	60	0.26	29
	80	0.19	48
	100	0.16	56
Aqueous	20	0.30	18
	60	0.29	21
	80	0.22	40
	100	0.18	51
Aspirin	100	0.10	72

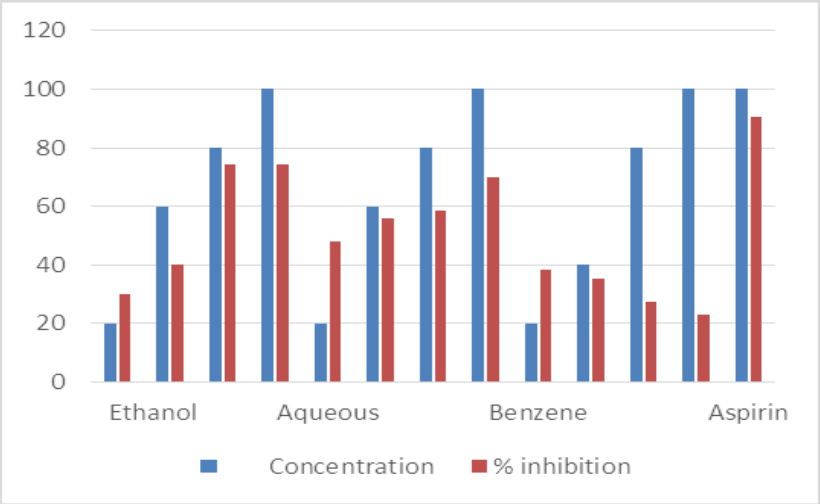

Fig.No:2. In-vitro anti-inflammatory activity of stem of *Zanthoxylum rhetsa* (Roxb.) DC. on inhibition of Protein denaturation.

1. Inhibition of protein denaturation

The *in vitro* anti-inflammatory effect of ***Zanthoxylum rhetsa* (Roxb.) DC.** was evaluated by denaturation of bovine albumin. The results are summarized in Table: 2 and Fig No: 2. The *in vitro* anti-inflammatory effect of ***Zanthoxylum rhetsa* (Roxb.) DC.**was performed by inhibition of protein denaturation method. ***Zanthoxylum rhetsa* (Roxb.) DC.** showed significant anti-inflammatory activity in a concentration dependant manner.

Ethanol extract of ***Zanthoxylum rhetsa* (Roxb.) DC.** at concentration of 20, 60, 80 and 100 µg/ml showed 24%, 29%, 48% and 56% inhibition, Aqueous extract of ***Zanthoxylum rhetsa* (Roxb.) DC.** at concentration of 20, 60, 80 and 100 µg/ml showed 18%, 21%, 40% and 51% inhibition, All the results were compared with standard drug Aspirin at 100µg/ml which showed 72% of protein denaturation respectively.

Table.3: In-vitro anti-inflammatory activity of stem of *Zanthoxylum rhetsa* (Roxb.) DC. on human red blood cell membrane.

Treatment	Concentration	Absorbance at 660 nm	% Inhibition
Control	-	1.412	-
Ethanol	20	0.9894	29.92
	60	0.8482	39.92
	80	0.6195	56.26
	100	0.3654	74.12
Aqueous	20	0.7359	47.88
	60	0.6248	55.75
	80	0.5891	58.27
	100	0.4256	69.85
Benzene	20	0.8727	38.19
	40	0.9143	35.24
	80	1.024	27.47
	100	1.089	22.87
Aspirin	100	0.1322	90.63

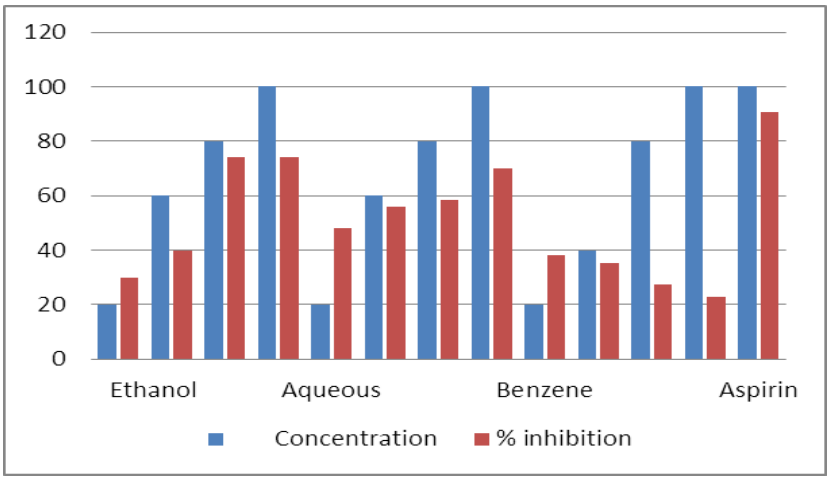

Fig.No.3: *In-vitro* anti-inflammatory activity of stem of *Zanthoxylum rhetsa* (Roxb.) DC. On human red blood cell membrane.

Human red blood cell membrane

The result of *in vitro* anti-inflammatory activity of *Zanthoxylum rhetsa* (Roxb.) DC. on human red blood cell membrane were given in Table:3 and Fig. No: 3. The in vitro anti-

inflammatory activity of *Zanthoxylum rhetsa* (Roxb.) DC. was performed by using human red blood cell membrane stabilization method. *Zanthoxylum rhetsa* (Roxb.) DC. Showed significant anti-inflammatory activity in a concentration dependant manner.

Benzene extract of *Zanthoxylum rhetsa* (Roxb.) DC. at concentration of 20, 60, 80 and 100 µg/ml showed 38%, 35%, 27% and 23% inhibition.

Ethanol extract of *Zanthoxylum rhetsa* (Roxb.) DC. at concentration of 20, 60, 80 and 100 µg/ml showed 30%, 40%, 56% and 74% inhibition.

Aqueous extract of *Zanthoxylum rhetsa* (Roxb.) DC. at concentration of 20, 60, 80 and 100 µg/ml showed 48%, 56%, 58% and 70% inhibition.

All the results were compared with standard drug Aspirin at 100 µg/ml which showed 91% inhibitionof Human red blood cell membrane.

DISCUSSION

The various concentration of compound *Zanthoxylum rhetsa* (Roxb.) DC. ranging from 20µg/ml to 100µg/ml were tested for its protein denaturation and HRBC method. The results were clearly demonstrated that the compound *Zanthoxylum rhetsa* (Roxb.) DC. at different concentration have anti denaturation activity.

Maximum percentage of inhibition 56% was observed from ethanol extracts followed by aqueous 51% at the maximum concentration of 100 µg/ml. Aspirin, a standard anti-inflammatory drug showed the maximum inhibition 72% at the concentration of 100µg/ml. In HRBC method ethanol extract of maximum concentration100µg/ml shows 74%, aqueous extract 69% followed by benzene shows 22%. Aspirin, a standard drug showed the maximum inhibition of 90% at the concentration of 100µg/ml.

Literature suggest that, the anti-denaturation property of BSA was due to the presence of two interesting binding sites in the aromatic tyrosine and aliphatic threonine and lysine residue regions of the BSA. They have also reported that therapeutic molecules could be activating the tyrosine motif rich receptor dually with threonine that regulates signal transduction biological pathways for their overall biological action.[13-15]

Compounds interacting with the aliphatic region around the lysine residue on the BSA could be interesting as anti-oxidant with anticancer activity such as polyphenols, phenylpropanoids and the disulphides. However the isolated compound ***Zanthoxylum rhetsa* (Roxb.) DC.** is phenolic in nature; hence they may be the region for its possible anti-denaturation activity.[16]

CONCLUSION

***Zanthoxylum rhetsa* (Roxb.) DC.** is a valuable medicinal plant which has been valuable for centuries in ayurvedic medicine. Phytochemical analysis of ***Z.rhetsa* (Roxb.) DC.** stem extracts revealed the presence of various bio chemical compounds such as flavonoids, glycosides, alkaloids, aleurone, starch, amino acids, lignin, volatile oil, fats and fixed oils, mucilage, pectins, proteins, steroids and triterpenoids. Since glycoside and flavonoids have remarkable anti-inflammatory activity. Our present work aims at evaluating the *in vitro* anti in-flammatory activity of ***Zanthoxylum rhetsa* (Roxb.) DC.** by protein denaturation and HRBC method. Denaturation of protein is a well-documented cause of inflammation and rheumatoid arthritis. The data of our studies suggests that ***Zanthoxylum rhetsa* (Roxb) DC**. of stem extract showed significant anti- inflammatory activity.

Therefore our studies support the isolation and use of active constituents of ***Zanthoxylum rhetsa* (Roxb.) DC**. in treating inflammation.

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REFERENCES

1. Vidyamadhavi K, Chandrashekhar G Joshi, Manjunath Hullikere M, Nivya MT, Anand D, Raju NG. Evaluation of In-Vitro Antioxidant, Anti-inflammatory Properties of Aerial Parts of Zanthoxylum rhesta. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2014; 5(5): 997-1003.
2. Nadkarni, KM. Indian Materia Medica, Vol II. Bombay: Popular Prakashan, 1982-968.
3. Das NG, Baruah II, Talukdar PK, Das SC. Evaluation of botanicals as repellents against mosquitoes. J Vect Borne Dis, 2003; 40: 49-53.

4. Moura NF, Giacomelli SR, Machado EC, Morel AF, Silveira CFS, Bitterncourt CF. Antibacterial activity of Zanthoxylum rhoifolium. Fitoterapia, 1998; 3: 271-272.
5. Dhar M, Dhar MM, Dhawan BN, Mehrothra BN, Srimal RC, Tandaon JS. Screening of Indian plants for biological activity. Part IV. Indian J Exp Biol, 1973; 11: 43-54.
6. Trongtokit Y, Rongsriyam Y, Komalamisra N, Apiwathnasorn C. Comparative repellency of thirtyeight essential oils against mosquito bites. Phytother Res, 2005; 19: 303-309.
7. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry, IInd Edition. Vol-I, IVth Edition; 1997; CBS publishers, Delhi, 297-298.
8. Sachin S. Sakart, Archana R Juvekar, Manoj N Gambhira. *In-vitro* antioxidant and anti-inflammatory activity of methanol extract oxalis corniculata linn. International journal of Pharmacy and Pharmaceutical sciences, 2010; l2(1): 2249-3379.
9. Sangita Chandra, Pretapaditya Dey, Sanjib Bhattacharya. Preliminary in vitro assasment of anti-inflammatory property of mikanra scandens flower extract. Journal of advanced pharmacy education and research, 2012; 2(1): 25-31.
10. Ramadevi M, Sivssubramaniyan N, TamilSelvan A, Sree Ari B, Prasad, S. Screening of invitro anti-inflammatory activity of Ficus Virens Bark. Journal of global trends in Pharmaceutical sciences, 2014; 5(4): 2034-2036.
11. Nagaraja TG, Patil V. Lakshimikant, Aramo Padhye N.G. In-Vitro antibacterial properties of *Zanthoxylum rhetsa* (Roxb.) DC. Bioinfolet-A Quactecly Journal of life science, 2001; 8(1): 85-86.
12. Lalitharani S, Kalpana Devi V, Mohan VR. Pharmacognostic Studies on the spine of *Zanthoxylum rhetsa* (Roxb.) DC. ISSN, 2013; 4(1): 05-11.
13. Williams LAD, Rosner H, Conard J, Moller W, Berfuss U, Chiba K. Selected secondary metabolites from Phytolaccaccae and Their Biological Pharmaceutical Significance. Research Signpost. In: Recent Res navel in Phytochem, 2002; 6: 13-68.
14. Ringer S, Whittaker JA, Conard J, Vosgter B, Rosner H, Krans W. The in vitro anti denaturation effects induced by natural products and non steroidal compounds in heat treated (immunogenic) bovine serum albumin. West Indian Med J, 2008; 57: 327-331.
15. Rosner H, Williams LAD, Jung A, Krans W. Disassembly of micro tubules and Inhibition of neunte out growth, Neuroblastoma cell Proliferation and MAP Kinse Dyrosine Dephosphorylation by Dibenzyl Trisulphide. Biochem Biophys Acta, 2001; 1540: 166-77.
16. Kawabata T, Packer L. Lipoate can protect against glycation of serum Albumins but not low Density Lipoproteins. Biochem Biophys Res Commun, 1994; 203: 99-104.