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EVALUATION OF WOUND HEALING EFFECT OF AQUEOUS STEM EXTRACT OF *ZANTHOXYLUM RHETSA* (ROXB.) DC ON WISTAR RAT

Abarnadevika A.*, Parthiban S. and Dr. Manisenthil Kumar K. T.

Department of Pharmacology, KMCH College of Pharmacy, Coimbatore.

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***Corresponding Author**
Abarnadevika A.
Department of Pharmacology, KMCH College of Pharmacy, Coimbatore.

ABSTRACT

The present investigation has been undertaken to study the wound healing properties of aqueous extract of *Zanthoxylum rhetsa (AQZR).* The plant *Zanthoxylum rhetsa* has a long history in herbal medicine in many countries. Experiments were conducted following standard procedures. The extract was evaluated for their *invitro* antioxidant, antimicrobial and total phenol and flavonoid content. The AQZR ointment were administered topically, for evaluating the wound healing potential in excision wound model for twenty one days. Povidone iodine ointment was the standard for excision wound model. Extracts treated group showed *in vitro* antioxidant, anti microbial properties as compared with standard and control group. AQZR exhibited similar wound healing activity that of the standard but with lesser magnitude. The result may be attributed to the phytoconstituents such as flavonoids and phenolics present in it which may be due to their individual or cumulative effect that enhanced wound healing and provided scientific evidence to the ethnomedicinal futures of *Zanthoxylum rhetsa*. These findings could justify the inclusion of this plant in the management of wound healing.

KEYWORDS: AQZR, Wound Healing Potential, Phytoconstituents.

1. INTRODUCTION[1,2]

Inflammation is a protective response that involves immune cells, blood vessels, and molecular mediators. The purpose of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and to initiate tissue repair. Wounds are inescapable events in life. Wounds may arise due to physical, chemical or microbial agents. Wound healing involves a

complex series of interactions between different cell types, cytokine mediators, and the extracellular matrix. The phases of normal wound healing include haemostasis, inflammation, proliferation and remodeling. Each phase of wound healing is distinct, although the wound healing process is continuous, with each phase overlapping the next. Because successful wound healing requires adequate blood and nutrients to be supplied to the site of damaged tissue.

2. METHODOLOGY[3]

2.1 Plant Collection and Authentication

The stem part of the plant *Zanthoxylum rhetsa* (Roxb) DC was collected from Paalakkad District of Kerala and authenticated from the Plant Anatomy Research Center, Tambaram, Chennai, TamilNadu. The authentication certificate number is PARC/2016/3324. Soon after collection, the stems were cleaned and shade dried. After drying, these stems were crushed to a coarse powder, stored in air tight plastic containers for further use.

2.2 Extraction of the Plant Material: The dried stem was coarsely powdered. The powdered stem (200gm) was taken in a round bottom flask and was extracted with water for 48hours at room temperature. After extraction the extracts were evaporated or concentrated by using rotary evaporator and dried at room temperature. The obtained crude extracts were weighed and stored at 4^0C for the further analysis and study.

2.3 *Invitro* Antioxidant Study of aqzr

DPPH Free Radical Scavenging Assay[4]

The method adopted here is Blois method. Where by using the stable DPPH radical, the antioxidant capacity of the extract was measured in terms of hydrogen donating or radical scavenging ability. 1mL of 0.3mM solution of DPPH in ethanol was added to various concentrations of sample (10, 20, 40, 60, 80, 100 µg/mL) and the reference compound (5, 10, 15, 20, 25 and 30 µg/mL), shaken vigorously, and left to stand in the dark at room temperature. After 30 min absorbance was measured at 517nm against a blank. Quercetin was used as reference compound. A control reaction was also carried out without the test sample. All the tests were performed in triplicate in order to get the mean values. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. Free radical scavenging activity was expressed as percentage inhibition (I%) and calculated using the following equation.

Percentage inhibition (I%) = (Abs control- Abs sample /Abs control) X 100

ABTS Assay[5,6]

ABTS radical scavenging activity of the extract was measured by Rice-Evans method. ABTS was dissolved in water to a 7mM concentration. ABTS radical cation (ABTS+) was produced by reacting ABTS stock solution with 2.45mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12- 16h before use. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. For the study, ABTS solution was diluted with phosphate buffer saline pH 7.4 (PBS) to an absorbance of 0.70 (± 0.02) at 734nm and equilibrated at 30^0C. After addition of 1mL of diluted ABTS solution to various concentrations of sample or reference compound (Quercetin), the reaction mixture was incubated for 6min and then absorbance was measured at 734nm against a blank. A control reaction was carried out without the sample. All the tests were performed in triplicate in order to get the mean values. The percentage inhibition of ABTS+ by the sample was calculated according to the formula.

Percentage inhibition (I %) = (Abs control- Abs sample /Abs control) X 100

NITRIC OXIDE Assay[7,8]

The nitric oxide scavenging activity was conducted based on the Greiss assay method. 2.0 mL of 10 mM sodium nitroprusside and 0.5mL of phosphate buffer were mixed with 0.5mL of different concentration of the plant extract and incubated at 25ºC for 150 min. The sample was run as above but the blank was replaced with the same amount of water. After the incubation period, 2mL of the above incubated solution was added to 2mL of Griess reagent and incubated at room temperature for a period of 30mins. The absorbance of the chromophore formed was read at 540nm.

Percentage inhibition (I%) = (Abs control- Abs sample /Abs control) X 100

2.4 ANTIMICROBIAL ACTIVITY OF AQZR[9]

Antibacterial study of AQZR

The standardized inoculums is inoculated in the sterilized plates prepared earlier (aseptically) by dipping a sterile in the inoculums removing the excess of inoculums by passing and rotating the swab firmly against the side of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60º after each application. Finally pass the swab round the edge of the agar surface. Leave the inoculums to dry at room temperature with the lid closed. The sterile discs are soaked overnight in sample solutions, AQZR. Each Petri dish is divided into 3 parts.

First and second compartment were loaded with sample disc such as AQZR (100µg) and standard Ciprofloxacin disc (10µg), is placed on the fourth compartment of the plate with the help of sterile forceps. After that Petri dishes are placed in the refrigerator at 4ºC or at room temperature for 1hour for diffusion. Incubate at 37ºC for 24hours. Observe the zone of inhibition produced by different samples. Measure it using a scale and record the average of two diameters of each zone of inhibition.

Anti fungal activity of AQZR

The standardized inoculums is inoculated in the sterilized plates prepared earlier (aseptically) by dipping a sterile in the inoculums removing the excess of inoculums by passing and rotating the swab firmly against the side of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60º after each application. Finally pass the swab round the edge of the agar surface. Leave the inoculums to dry at room temperature with the lid closed. The sterile discs are soaked overnight in sample solutions, AQZR. Each Petri dish is divided into 3 parts. First and second compartment were loaded with sample disc such as AQZR (100µg) and standard Clotrimazole disc (10µg), is placed on the fourth compartment of the plate with the help of sterile forceps. After that Petri dishes are placed in the refrigerator at 4ºC or at room temperature for 1hour for diffusion. Incubate at 37ºC for 24hours. Observe the zone of inhibition produced by different samples. Measure it using a scale and record the average of two diameters of each zone of inhibition.

2.5 Determination of Minimum Inhibitory Concentration of Aqzr[10,11]

Preparation of test drug: The selected test drugs were prepared in DMSO at a concentration 1000µg/Ml.

Preparation of inoculums: *Bacillus subtilis, Staphylococcus aureus, E-coli, Pseudomonas auregenosa, A.niger,* and *M.purpureas* were the strains of organisms selected for the study. Overnight culture are grown at 37^0C Kirby- Bauer procedure and diluted to Muller Hinton Broth and Sabouraud Dextrose Broth for bacterial and fungal strains respectively. This overnight culture was diluted to 10^{-2}.

Inoculation

The sterile tubes were labeled 1-8 and 8^{th} tube was taken as control. 1mL of Muller Hinton Broth was transferred to all tube. 1mL of drug solution was added to 1^{st} tube and mixed well.

From the 1st tube transfer 1mL of solution to the 2nd tube and was repeated up to 7th tube. From the 7th tube 1mL of solution was pipette out and discarded. 0.01mL of culture was added to all the test tubes. All the tubes were incubated at 37^0C for 18-24hrs. After incubation observe the turbidity by visually. The highest dilution without growth is the minimal inhibitory concentration.

2.6 Pharmacological Evaluation of Aqzr

Acute Toxicity Study[12]

Based on previously conducted study, the dose was selected.

Experiment

Povidone Iodine Ointment was used as standard drug. The ointments were applied topically over the wound area. AQZR were formulated to ointment (1%) and it is applied topically over the wound area.

Preparation of Simple Ointment base[13]

Procedure: Hard paraffin and cetostearyl alcohol taken in a china dish kept on water-bath at 70^0C. Wool fat and white soft paraffin are added to this mixture and stirred until all the ingredients are melted. If required decanted or strained and stirred until cold and packed in suitable container.

1% of AQZR were separately mixed with the above prepared simple ointment base.

Experimental Model

Evaluation of Wound Healing Effect of Aqzr in Excision Wound Model

Rats were divided into 4 groups each containing 5 animals as follows.

Table. 1: Experimental design for excision wound model.

Group	Group Specification	Intervention
Group I	Control	Untreated
Group II	Simple base ointment	Only with simple base ointment
Group III	Standard	Povidone iodine ointment 5%
Group IV	Test 1	AQZR ointment 1%

Induction of Wound[14]

On wounding day the rats were anaesthetized prior to creation of the wounds, by ether anaesthesia. The dorsal fur of the animal was shaved with an electric clipper and the area of

the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of 1.5cm in width (circular area 2.25cm^2) created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound left open. All the surgical interventions were carried out under sterile condition. After 24h of wound creation, the ointment was applied gently to cover the wounded area once daily until complete healing. Wound area and wound contraction, epithelializtion period and hydroxyproline content were monitored.

2.7 Estimation of Parameters

Measurement of wound contraction[15]

The progression of wound healing was judged by the periodic assessment of the contraction of excision wounds. Wound contraction was monitored by tracing the outline of the wound on tracing sheet and then using graph sheet to calculate the area of the wound size. All animals in each group were monitored until complete healing of wounds occurred and the day at which each wound healed was recorded. Mean of all healed wounds was determined.

$$\text{Percent wound contraction} = \frac{\text{healed area} \times 100}{\text{Total area}}$$

Estimation of hydroxyproline[16,17]

Known amount of tissue (50mg) was taken in glass tubes and 4mL of 6N HCl was added to each tube to hydrolyse the tissue sample. The glass tubes were sealed and were incubated for 22hrs. The tubes are then opened and the contents are decanted into a china dish. HCl was then removed by evaporation and the residue was dissolved in water and made up to known volume (10mL) using a standard flask. A series of standards were prepared containing 20-200µg of hydroxyproline with a final volume of 2mL. 1mL of the hydrolysed tissue samples was used to estimate the contents of hydroxyproline. Hydroxyproline oxidation was initiated by adding 1mL of chloramine-T to each tube in a predetermined sequence. The tube content were mixed by shaking a few minutes and allowed to stand for 20min at room temperature. Chloramine-T was then destroyed by adding 1mL of perchloric acid to each tube in the same order as before. The contents were mixed and allowed to stand for 5min. Finally 1mL of p-Dimethyl amino benzaldehyde solution was added and the mixture was shaken well. Tubes were placed in a 60°C water bath for 20min and then cooled in tap water for 5min. The colour developed was read spectrophotometrically at 557nm. Hydroxyproline value was determined from the standard curve.

Estimation of Hexosamine[18]

Procedure

Tissue samples (50mg) were hydrolysed with 2NHCL (5mL) at 100°C for 6 hrs. Hydrochloric acid was then removed by evaporation, then the residue was dissolved in water and made up to a known volume (10mL) using a standard flask. Aliquots containing 10-50mg hexosamine were treated with 1mL of freshly prepared 2% acetyl acetone in 0.5M sodium carbonate in capped tubes and kept in boiling water bath for 15min. After cooling in tap water, 5mL of 95% ethanol and 1mL of Ehrlich's reagent (1.33% Dimethyl amino benzaldehyde in 1:1 ethanol: concentrated hydrochloric acid mixture) were added and mixed thoroughly. The purple red colour developed was read after 30 min at 530 nm. Water blank and standard glucosamine solution of various concentrations were also treated similarly to get a standard curve.

Estimation of uronic acid[19]

A buffered copper solution was prepared by adding 23.2g NaCl, 3.2g sodium acetate, and 1.0mL glacial acetic acid to 80mL water. Once dissolved, 0.5 g CuSO4 is added, the pH adjusted to 4.8 with NaOH, and the final volume brought to 100mL. This solution is stable for weeks at room temperature. For the assay, equal volumes of this solution and the sample are mixed, giving final reagent concentrations in the assay of 2M NaCl, 0.2M acetate, and 10mM CuSO4. In our standard assay, sample and assay solution volumes of 0.1mL each are mixed in test tubes, then the tubes are covered with glass marbles and placed in an aluminum heating block at 100°C. A diluted Folin–Ciocalteau reagent is then prepared by mixing 1mL of 2N Folin–Ciocalteau with 39mL of water. After 40min, the samples are removed from the heating block and 8 volumes (0.8mL in our standard assay) of the 40-fold diluted Folin–Ciocalteau reagent is added. A colored product forms immediately; absorbance was measured at 750nm. Where the BCA reagent was used the procedure was the same, except instead of adding the diluted Folin– Ciocalteau reagent, 0.8mL of solution ‘‘A” from the procedure described in Waffenschmidt and Jaenicke, containing 5.0mM BCA in pH 10.1 carbonate buffer, was added. Absorbance was measured at 560nm.

Histological Assessment

Histological studies of wounded tissues provide accurate diagnosis of level of healing of the wound. Histopathology is the microscopical study of tissues for pathological alterations. This involves collection of morbid tissues from biopsy or necropsy, fixation, preparation of

sections, staining and microscopical examination. Thin pieces of 3 to 5 mm, thickness were collected from tissues showing gross morbid changes along with normal tissue. Kept the tissue in fixative for 24-48 hours at room temperature The fixation was useful in the following ways: Serves to harden the tissues by coagulating the cell protein, Prevents autolysis, Preserves the structure of the tissue, and Prevents shrinkage.

Common Fixatives: 10% Formalin.

STATISTICAL ANALYSIS

Data's of all the parameters were analyzed using the Graph pad 5.0 software. Analysis of Variance (ANOVA); one way ANOVA followed by Tukey's multiple comparison test was performed. The values were expressed as Mean ± SEM. P value<0.05was considered as significant.

3. RESULTS

3.1 *Invitro* Antioxidant Study

Dpph Radical Scavenging Assay

Table. 2: Percentage inhibition and IC_{50} values of DPPH radical by Quercetin and AQZR.

Sample	Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
Standard	5	41.2	1.55
	10	55.7	
	15	69.4	
	20	86.4	
	25	96.5	
	30	99.38	
AQZR	10	26.69	17.77
	15	43.89	
	20	50.12	
	25	59.67	
	50	69.77	
	100	91.36	

Abts Radical Scavenging Assay

Table. 3: Percentage inhibition and IC_{50} values of ABTS radical by Quercetin and AQZR.

Sample	Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
Standard	0.25	70.08	0.1142
	0.5	75.22	
	0.75	79.62	
	1.0	85.88	
	1.25	91.9	
	1.75	98.85	
AQZR	1	53.61	1.160
	2	57.41	
	3	61.63	
	4	71.18	
	5	76.92	
	10	97.37	

Nitric Oxide Radical Scavenging Assay

Table. 3: Percentage inhibition and IC_{50} values of NO radical by Quercetin and AQZR.

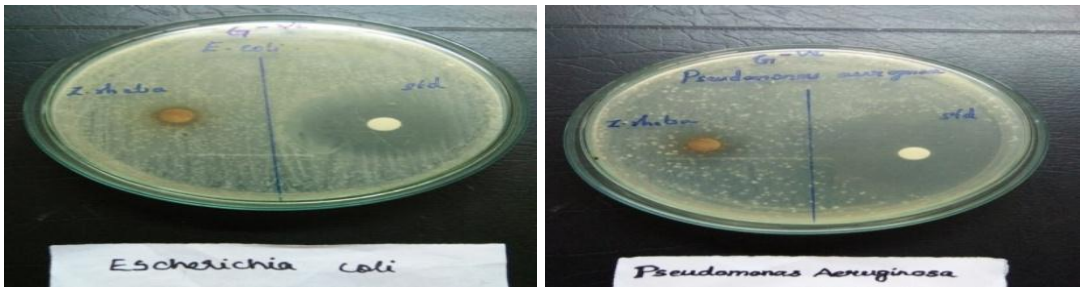
	Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
Sample	1	41.2	1.55
	2	55.7	
	3	69.4	
	4	86.4	
	5	96.5	
AQZR	10	24	30.5
	20	35.9	
	30	49,7	
	40	66.4	
	50	74.8	

3.2 Anti-Microbial Activity of Aqzr[52]

Anti Bacterial Activity

Table. 4: Zone of inhibition for Gram +ve and Gram -ve organisms.

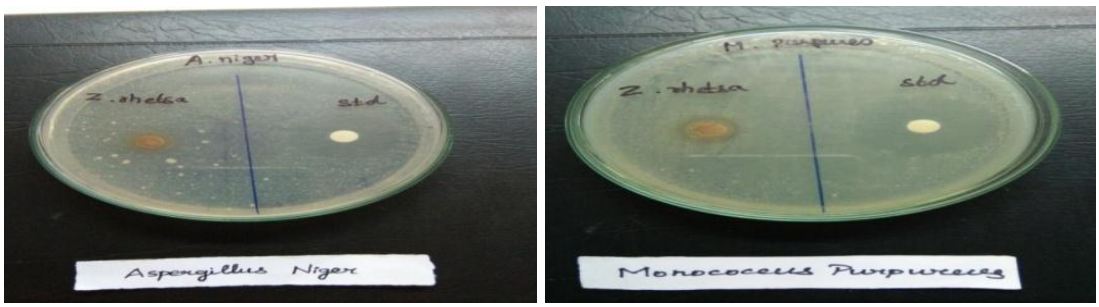
Sl No	Organisms	Standard(mm) (Ciprofloxacin)	AQZR(mm)
1	*Bacillus subtilis*	25	10
2	*Staphylococcus aureus*	26	14
3	*E.coli*	26	09
4	*P Pseudomonas auregenosa*	27	10



Antifungal Activity

Table. 5: Antifungal activity of AQZR.

Sl No	Organisms	Standard(mm) (Fluconazole)	AQZR(mm)
1	*Aspergillus niger*	21	12
2	*Monascus purpureus*	24	15



3.3 Determination of Minimum Inhibitory Concentration of Aqzr[53,54]

Bacterial Organisms

Table. 6: MIC values of AQZR.

SI NO	GRAM +VE & GRAM –VE ORGANISMS	MIC VALUE OF AQZR
1	*Bacillus subtilis* (µg/mL)	250
2	*Staphylococcus aureus* (µg/mL)	125
3	*E.coli* (µg/mL)	125
4	*Pseudomonas auregenosa* (µg/mL)	125

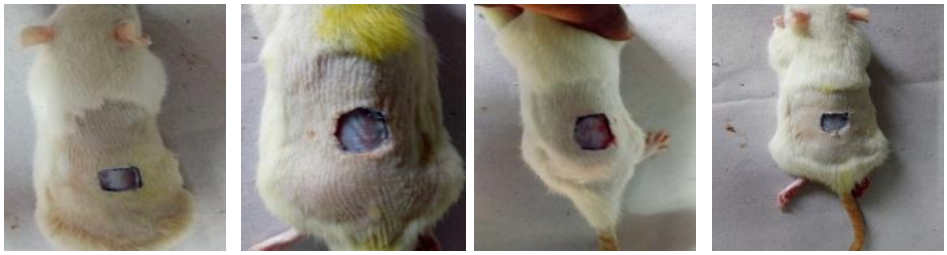
Fungal Organisms

Table 7: MIC values of AQZR

SI NO	Fungal Organisms	Mic Value of Aqzr
1	*Aspergillus niger* (µg/mL)	250
2	*Monascus purpureus* (µg/mL)	250

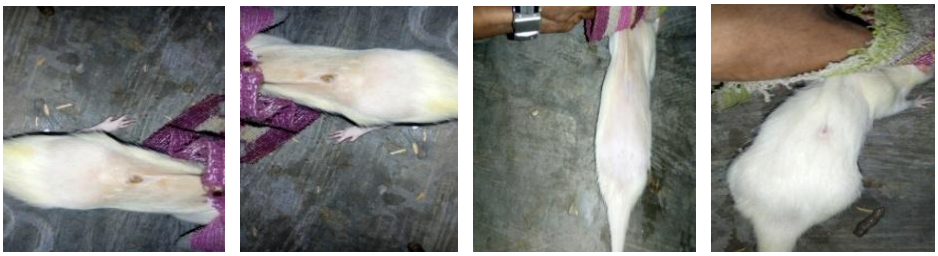
3.4. Evaluation of Wound Healing Effect of Aqzr In Excision Wound Model

On1st day



Control **Simple base** **ointment** **Standard AQZR**

On 21st day



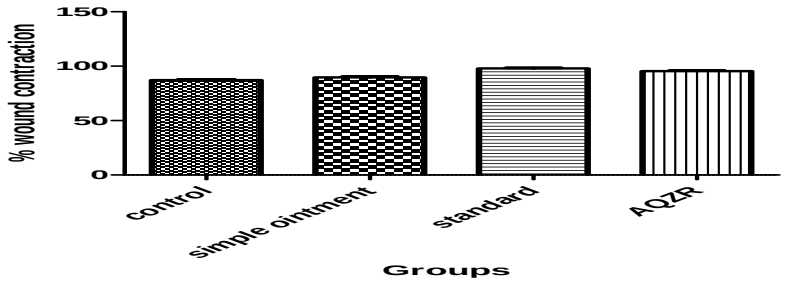
Control **Simple base ointment** **Standard** **AQZR**

3.5 Estimation of Parameters

Percentage Wound Contraction

Table. 8: Percentage wound contraction in excision wound model.

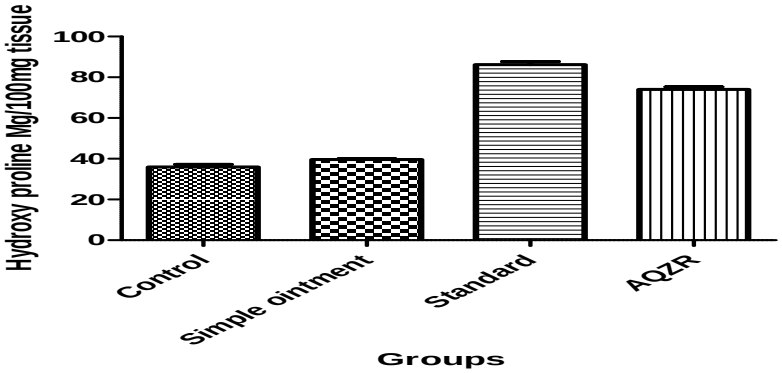
Compounds	0thDay	3rdDay	7thDay	13thDay	17thDay	19thDay
	% WC	% WC	% WC	% WC	% WC	% WC
Control	0	27.5±0.58	23.6±2.03	74.2±0.99	72±0.99	85.1±0.99
Ointment Base	0	13.5±0.49	27±0.83	52.5±1.24	66.8±1.24	88.2±1.88
Standard	0	15.7±0.29	39.8±0.77	81.8±1.09	88.5±1.25	99.5±1.10
Aqzr Ointment	0	10±0.42	13.8±1.48	55.4±1.08	84.1±1.08	96.9±1.08



Estimation of Hydroxyproline

Table 9: Hydroxy proline levels in Excision wound model.

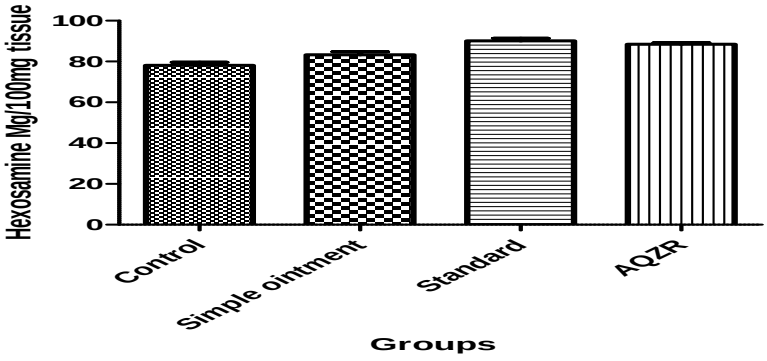
Group	Hydroxy Proline (µG/GM)
Control	36.64±2.107
Simple base ointment	39.00±3.123^{ns}
Standard	81.12±3.142***
AQZR	70.11±2.010**



Estimation of Hexosamine

Table. 10: Hexosamine levels in Excision wound model.

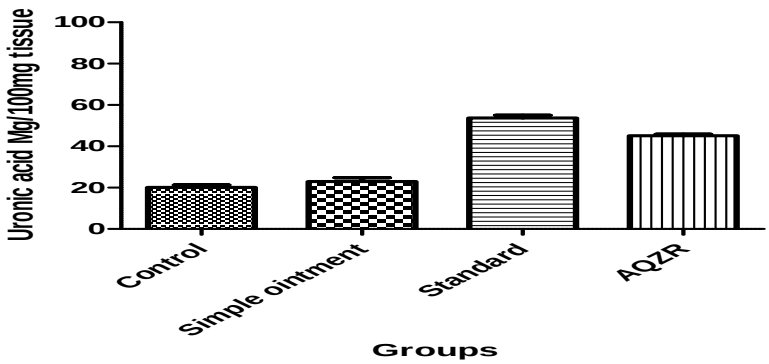
GROUP	Hexosamine (µg/gm)
Control	73.81±1.271
Simple base ointment	79.81±1.701 ^{ns}
Standard	88.12±1.412***
AQZR	85.10±1.812**



Estimation of Uronic Acid

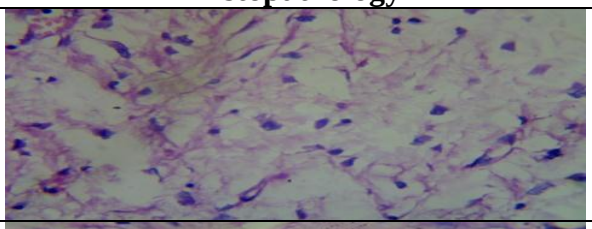
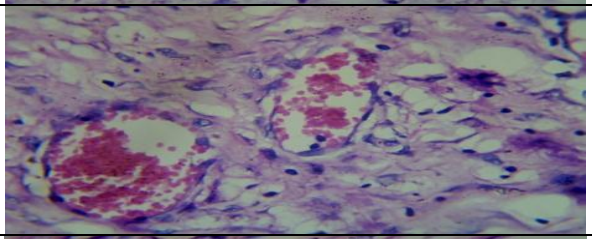
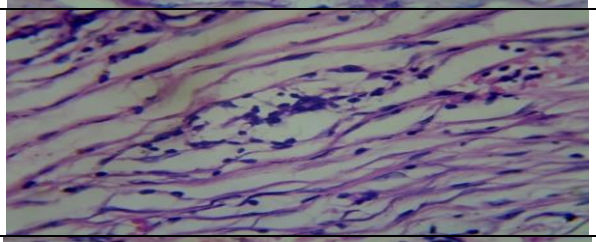
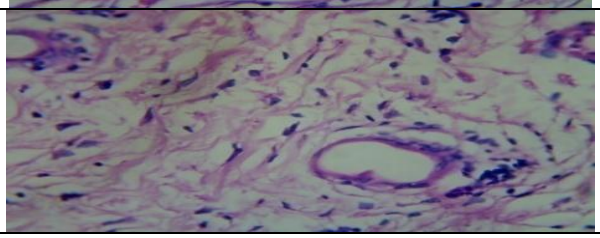
Table. 11: Uronic acid levels in Excision wound model.

GROUP	Uronic acid (µg/gm)
Control	17.12±0.412
Simple base ointment	19.41±0.481 ^{ns}
Standard	50.17±0.644***
AQZR	42.49±0.556**



Histopathological Study

Table. 12: Histopathological study.

Group	Histopathology
Group 1- Control	
Group 2- Simple base ointment	
Group 3- Standard	
Group 4 - AQZR sample treated	

4. DISCUSSION

Wound is a clinical entity and is as old as mankind, often considered as major problem in clinical practice. Each year, millions of people experience burns, suffer from chronic wounds, or have acute wounds that become complicated by infection. The bark, seed, and stem extract of *Zanthoxylum rhetsa* is well known for its different types of pharmacological properties such as Digestive problem, Inflammatory dermatosis, Tooth ache, Cytotoxic properties, Anti

nociceptive, Antioxidant, Antibacterial, Antifungal. Externally it is used for wound healing. The major active constituents are budrungaine, budrungainine, rhetine, rhetsine, evodiamine, chelerythrine, lupeol. The present study was aimed to evaluate the wound healing potential of aqueous stem extract of *Zanthoxylum rhetsa* (AQZR) in excision wound model of rats. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. The free radical scavenging activity of the extract was estimated by comparing the % inhibition of AQZR with standard Quercetin. IC_{50} was also calculated to determine the amount of extract needed to quench 50% of radicals. Aqueous Stem extract of *Zanthoxylum rhetsa* exhibited a dose dependent scavenging activity with IC_{50} values of, 17.77 µg/mL respectively for AQZR. Where the IC_{50} for standard Quercetin was found to be 1.55 µg/mL. The ABTS.+ scavenging assay, which employs a specific absorbance (734nm) at a wavelength remote from the visible region and requires a short reaction time, can be used as an index that reflects the antioxidant activity of the test sample. The presence of specific chemical compounds in the aqueous stem extract of *Zanthoxylum rhetsa* may inhibit the potassium persulfate activity and hence reduced the production of ABTS•+. *Zanthoxylum rhetsa* extracts were found to be effective in scavenging radicals and the increase was concentration-dependent. IC_{50} value of Quercetin was 0.1142 µg/mL whereas 1.160 µg/mL for AQZR respectively. This shows that *Zanthoxylum rhetsa* extract presents a good ability to scavenge the ABTS radical. The Nitric Oxide scavenging assay, which employs a specific absorbance (546nm) at a wavelength remote from the visible region and requires a short reaction time, can be used as an index that reflects the antioxidant activity of the test samples. *Zanthoxylum rhetsa* extracts were found to be effective in scavenging radicals and the increase was concentration-dependent. IC_{50} value of Quercetin was 1.55µg/mL whereas 30.5 µg/mL for AQZR respectively. This shows that *Zanthoxylum rhetsa* extract presents a good ability to scavenge the NO radical. The anti microbial study was carried out for aqueous stem extract of *Zanthoxylum rhetsa* against different strains of bacteria (2 Gram + ve and 2 Gram – ve) and fungi (2 strains), that are known to cause infection in human and plants, by disc diffusion method at 200µg/disc. The ciprofloxacin 10µg/disc and fluconazole 10µg/disc were used as standard for bacteria and fungi respectively. The standard ciprofloxacin and fluconazole was found to have significant antimicrobial activity against bacteria and fungi respectively. The various zone of inhibition were observed from the extract against various strains. Among the extract of AQZR was observed to have significant antimicrobial activity. The zone of inhibition was observed from both Gram +ve and Gram –ve bacteria and fungal strains. The maximum zone of inhibition (14mm) was found in AQZR against *Staphylococcus aureus* for the Gram +ve organism and

for Gram –ve organisms. The maximum zone of inhibition (10mm) was found in AQZR against *Pseudomonas auregenosa.* The anti fungal activity was observed maximum in AQZR. Among fungi species, the zone of inhibition was found to be maximum in *Monoscus purpureus* (15mm). The aqueous stem extract of *Zanthoxylum rhetsa* demonstrated wound healing properties comparable with that of antibiotic standard. Animals in the untreated group showed some degree of healing. As earlier suggested, healing in this untreated group may be due to self-immunity. It is important to note that throughout the period of wound treatment, the extract did not cause any irritation or pain to the animals as the rats neither show any signs of restlessness nor scratching/biting of wound site when the extract were applied. Extract treated excision wounds showed an increased rate of wound contraction, leading to faster healing as confirmed by the increased healed area when compared to the control untreated group. The AQZR was recorded similar effectiveness when compared to the group treated with a commercial brand of Povidone iodine ointment, but the magnitude was lesser than standard. From histopathological studies, it was observed that the phases of wound healing occurred in a timely manner. The untreated (control) wound healed slowly when compared to the wounds treated with AQZR. Extract treated group shows large number of collagen tissue.

5. CONCLUSION

Wound healing is a complex and continuous process that begins immediately after injury, followed by homeostasis, blood clotting, inflammation, proliferation and remodeling phases. All these phases can promote or prolong healing by influencing external or internal factors including infection, sex hormones and nutrition. Delay in healing process increases the possibility of getting infected, improper recovery, and formation of unpleasant scar. The study thus demonstrated the wound healing activity of aqueous stem extract of *Zanthoxylum rhetsa* (Roxb) DC and found to be effective in the functional recovery of the wound. The extract promotes wound contraction; increases the hydroxyproline, hexosamine and uronic acid of excision wounds as compared to control group. The result may be attributed to the phytoconstituents such as flavonoids and phenolics present in it which may be due to their individual or cumulative effect that enhanced wound healing and provided scientific evidence to the ethnomedicinal futures of *Zanthoxylum rhetsa* (Roxb) DC. These findings could justify the inclusion of this plant in the management of wound healing.

REFERENCES

1. Herbalism, http://enwikipedia.org/wiki, Herbalism.
2. Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardins SE; Nielsen; Andersen; Girardin. "Chronic Inflammation: Importance of NOD2 And NALP3 In Interleukin-1betaGeneration". Clin.Exp.Immunol, February 2007; 147(2): 061127015327006 DOI:10.1111/J.1365-2249.2006.03261.X. PMC 1810472.
3. S.S. HandaandV.K. Kapoor: Pharmacognosy; 44-67.
4. Raghavendra H Lakshmanashetty, Vijayananda B Nagaraj, Madumathi G, Hiremuth and Vadalapudi Kumar. Invitro antioxidant activity of Vitex negundo L. Leaf extract. Chiang. Mai. J. Sci., 2010; 37(3): 489-497.
5. Shekhar TC, Anju G. Antioxidant activity by DPPH radical scavenging method of Ageratum conyzoides Linn. leaves. American Journal of Ethnomedicine. 2014 Aug 31; 1(4): 244-9.
6. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of agricultural and food chemistry. 2005 May 18; 53(10): 4290-302.
7. Paulsamy S, Karthika K. Screening of in vitro antioxidant activity of methanolic leaf and root extracts of Hypochaeris radicata L.(Asteraceae). Journal of Applied Pharmaceutical Science. 2012 Jul 1; 2(7): 149.
8. Fadzai boora, Elaine chrisa, Evaluation of Nitrite radical scavenging properties of selected Zimbabwean plant extract and their phyto constituents, Journal of food processing volume, ID 918018, (2014).
9. Chavan chetan, Mulik suraj, Chevan, Maheswari, Screening of anti oxidant activity and phenolic content of whole plant of bacteria prisonities linn, IJRAP, 2011; 1313-1319.
10. Lakshmi KS, Sangeetha D, Sivamani S, Tamilarasan M, Rajesh TP, Anandraj B. In vitro antibacterial, antioxidant, haemolytic, thrombolytic activities and phytochemical analysis of Simarouba glauca leaves extracts. International Journal of Pharmaceutical Sciences and Research, Feb 1, 2014; 5(2): 432.
11. Ullah N, Parveen A, Bano R, Zulfiqar I, Maryam M, Jabeen S, Liaqat A, Ahmad S. In vitro and in vivo protocols of antimicrobial bioassay of medicinal herbal extracts: A review. Asian Pacific Journal of Tropical Disease. Aug 31, 2016; 6(8): 660-7.
12. Sen A, Batra A. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: Melia azedarach L. Int J Curr Pharm Res., Mar, 2012; 4(2): 67-73.

13. Dr. Shankara Sharma, N.Sriram. Anti-ulcer activity of Simarouba glauca against ethanol and indomethacin induced ulcer in rats. Int. J. of Res.in pharmacology and Pharmacotherapeutics, 2014; 3(2): 85-89.
14. Azubuike chukwuemeka evaluation anti microbial properties of herbal ointments formulated with ethanolic extract of acalypha wilkesiana. Journal of biological and scientific opinion, 2013; 1(2).
15. Das K. Wound healing potential of aqueous crude extract of Stevia rebaudiana in mice. Revista Brasileira de Farmacognosia, 2013 Apr; 23(2): 351-7.
16. Rashed AN, Afifi FU, Disi AM. Simple evaluation of the wound healing activity of a crude extract of Portulaca oleracea L.(growing in Jordan) in Mus musculus JVI-1. Journal of Ethnopharmacology, Oct 31; 2003; 88(2): 131-6.
17. Prashanthi R, Mohan N, Siva GV. Wound healing property of aqueous extract of seed and outer layer of Momordica charantia L. on albino rats. Indian Journal of Science and Technology, Jan 1; 2012; 5(1): 1936-40.
18. Edwards CA, O'Brien WD. Modified assay for determination of hydroxyproline in a tissue hydrolyzate. Clinica chimica acta. Jun., 10, 1980; 104(2): 161-7.
19. Rondle CJ, Morgan WT. The determination of glucosamine and galactosamine. Biochemical Journal, Dec., 1955 61(4): 586.