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ORIGINAL ARTICLE

Preliminary studies on Antimicrobial, Antioxidant, Anti-inflammatory and wound healing potential of benzene crude extracts of *Quisqualis indica*

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ABSTRACT
The objective of this study was to investigate the possible antimicrobial, antioxidant anti-inflammatory and wound healing potential of Quisqualis indica. The effect of flowers, leaf and bark extract of Quisqualis indica with benzenewas evaluated in experimental models. Higher concentration (400 µg/ml) of flowers, leaf and bark extract of Quisqualis indica with benzene showed maximum zone of inhibition against selected gram positive and gram negative pathogens such as Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Bacillus subtilis, Staphylococcus epidermidis, Streptococcus pyogenes, Salmonella typhi. The benzene flowers, leaves and bark extracts of the Quisqualis indica at a concentration 400 µg/3 ml exhibited potential inhibiting activity in DPPH and nitric oxide scavenging antioxidant in vitro test. Oral Administration of 400 mg/kg of benzene flowers, leaves and bark extracts of the Quisqualis indica showed significant ($p<0.001$) inhibition in carrageenan induced paw edema. Higher dose of flowers, leaf and bark of Quisqualis indica with benzene extracts showed significant ($p<0.0001$) improvement in area of wound in mm² (% wound contractions) on day 8 and 12, and complete healing was achieved on day 22. Result was confirmed using histological assessment. Phytochemical screening has shown the presence of Alkaloids, Saponins, Sterols and Flavonoids in benzene extract. All the above results support the traditional uses of the plant in the treatment of wound healing.
Keywords*: Quisqualis indica, Antimicrobial, Antioxidant, Anti-inflammatory, Wound healing activity*

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INTRODUCTION

Traditional medicine transform into modern industry contributing a crucial role in delivery of healthcare by evaluation of herbal remedies and/or their phytoconstituents. This concept in plant derived herbal drugs is mainly due to the current belief that 'Green revolution' is safe, effective, less expensive and easily tolerated by patients [1]. Wounds are physical injuries that cause breaking of skin. Wound healing is not a linear process that consists of three phases like inflammation, proliferation and remodeling[2]. The inflammation stage begins immediately after injury leads to vasoconstriction that cause homeostasis and releasing of inflammatory mediators. The proliferative phase is characterized by granulation tissue proliferation formed mainly by fibroblast and the angiogenesis process. The remodeling stage is characterized by reformulations and improvement in the components of the collagen fibre that increases the tensile strength [3].A wound provides an ideal environment for the growth of microorganisms[4]. Wound infection is less likely to heal, therefore removal and prevention of infection is a key to rapid and effective healing [5]. Some plants with antibacterial, antioxidants and anti-inflammatory properties have shown to be effective for wound healing [6-7].Reactive Oxygen Species (ROS) is produced in high amounts at the site of wound as a defence mechanism against invading bacteria. [8]. Many oxidants produce toxicity during its catalytic cycle by different mechanisms like protein oxidation, enzyme inactivation and damage to cell membrane. Antioxidants are believed to protect against certain diseases by preventing the deleterious effects of free radical-mediated processes in cell membranes and by reducing the susceptibility of tissues to oxidative stress. Several studies have shown that medicinal plants

consist of a rich source of antioxidants, antimicrobial and anti-inflammatory rich plant extracts [9-10].Administration of antioxidant having antimicrobial property could reduce inflammations.

The plant *Quisqualis indica,* belongs to the family Combretaceae, commonly known as 'Madhumalti' in India, is a well-known traditional herb used for variety of ailments. It is also known asVilayatichambeli (Marathi), Madhumalti (Hindi) and Modhumalati (Bengali**)**. It is widely distributed all over the world especially on China, Philippines, Bangladesh, Myanmar and Malaysia and now also broadly grow in India as ornamental plant in most of the garden. The *Quisqualis indica* (QI) have been used traditionally as antihelmintic to expel parasitic worms [11]. The traditional use of this plant suggests Anti-inflammatory activity [12], Antidiabetic activity [13], Antipyretic activity[14], Immunomodulatory activity [15], Anti-staphylococcal activity [16], Anthelmintic activity [17] and Cytotoxic effects [18].Based on literature, the present study have been undertaken to find out the Antimicrobial, antioxidant, anti-inflammatory and wound healing activity of benzene crude extract of *Quisqualis indica* on different screening models.

MATERIAL AND METHODS

COLLECTION OF PLANT MATERIAL

Fresh flowers, leaves and bark of *Quisqualis indica* were collected from local area of Aravalli district, Gujarat, India in the months of July-October. This plant was identified and authenticated to Botanical Survey of India, Pune.

ANIMALS

Adult male Wistar albino rats, weighing between 180 - 220 g and albino mice (25-30 g) were used and acclimatized to laboratory condition for one week. All animals were housed in well ventilated polypropylene cages at 12 h light/dark schedule with 25±2ºC and 55-65% relative humidity. The rats had fed with commercial pelleted rats chow and water *ad libitum* as a standard diet. Institutional Animal Ethics Committee approved the experimental protocol in accordance with CPCSEA.

PREPARATION OF FLOWERS, LEAF AND BARK EXTRACT

The flowers, leaf and bark of *Quisqualis indica*were collected and dried in shade and ground. Coarsely powdered plant material was used for the study. Coarsely powdered plant material (1000 g) was subjected to successive extraction with benzene in a soxhlet extractor at a temperature of 45-50^{0}C to 40 cycles per batch for 2 batches. The extraction was continued until the solvent in the thimble becomes clear indicating the completion of the extraction. After each extraction the solvent was distilled off and concentrated extract was transferred to previously weighed petri dish and evaporated to dryness at room temperature (45-50^{0}C) to obtain dried extracts. After completion of drying the petri dish was weighed again. The yield of extract was calculated by subtracting original weight of empty petri dish [19-20]. The yield for flower, leaf and bark extracts were 4.8 g/100 g, 5.6 g/100 g and 5.5 g/100 g respectively.

PRELIMINARY PHYTOCHEMICAL STUDIES

Preliminary qualitative phytochemical screening for the identification of the phytoconstituents of the benzene flowers, leaf and bark extract of *Quisqualis indica*(L.) has been carried out [21-22].

ACUTE ORAL TOXICITY OF THE EXTRACT

Adult Albino mice (25-30 g) were divided into different groups containing ten mice each. The mice were fasted for 6 h and access only water *ad libitum* before experimrntal study. Group I received only vehicle (distilled water). Group II to XIII animals received with different doses of benzene flowers extract of *Quisqualis indica* (BFQI), benzene leaf extract of *Quisqualis indica* (BLQI)and benzene bark extract of *Quisqualis indica* (BBQI) i.e. 1000, 2000, 3000 and 4000 mg/kg respectively. All the doses and vehicle were administered orally. The animals were observed for 72 h for mortality[23-24].

ANTIMICROBIAL ACTIVITY [25]

Antimicrobial activity was tested against gram positive and gram negative Bacteria named *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Bacillus subtilis, Staphylococcus epidermidis, Streptococcus pyogenes, Salmonella typhi*using agar well diffusion method. The test bacteria was grown in sterile Nutrient broth and Sabouraud / Potato dextrose broth tubes respectively overnight. The broth cultures of bacteria was then aseptically swabbed on sterile Nutrient agar and Sabouraud / Potato dextrose agar respectively using sterile cotton swabs. Wells of 6 / 10 mm diameter was created in the inoculated plates using sterile cork borer. Different concentrations of plant extracts were filled in labeled wells. The plates were incubated and the zone of inhibition was recorded.

SCREENING OF ANTIOXIDANT ACTIVITY OF EXTRACTS [26]

Free Radical scavenging activity test (DPPH Method)

The DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical has a deep violet colour due to its unpaired electron, and radical scavenging activity can be followed by absorbance at 525 nm Sample stock solutions (1mg ml^{-1}) will be diluted to final concentrations of 100, 50, 10 and 5 µg ml^{-1} in 70% ethanol or DMSO. The DPPH

ethanol solution (0.2 mM, 0.5 ml) was added to 1 ml of sample solutions of different concentrations, shaken well by vortex, and allowed to react at room temperature. The absorbance values were measured after 10 min at 525 nm by UV/V is spectrophotometer.

$$\text{Percentage inhibition} = \frac{A_0 - A_t}{A_0} \times 100$$

where A_0 was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the samples of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

Nitric oxide scavenging activity

The nitric oxide radical scavenging activity was measured using Griess' reagent. 5 mL each of extract solutions of different concentrations (100–1000 µg/3mL) in standard phosphate buffer solution (pH 7.4) will be incubated with 5mL of sodium nitroprusside solution (10 mM) in standard phosphate buffer (pH 7.4) at 25°C for 2.5 hours. In an identical manner 5 mL of ascorbic acid solution (200 µg/mL) in standard phosphate buffer solution (pH 7.4) was also incubated with 5 mL of sodium nitroprusside solution (10 mM) in standard phosphate buffer (pH 7.4). Control experiments without the test compounds but with equivalent amount of buffer was also conducted. After incubation, 0.5 mL of the incubation mixture was mixed with 0.5 mL of Griess' reagent (Sulphanilamide 1%, *O*-phosphoric acid 2% and naphthyl ethylene diaminedihydrochloride 0.1%) and the absorbance was measured at 546 nm. From the absorbance, the percent scavenging activity was calculated.

$$\text{Percentage inhibition} = \frac{A_0 - A_t}{A_0} \times 100$$

where A_0 was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the samples of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

ANTI INFLAMMATORY AND WOUND HEALING ACTIVITY

Animal selection and treatment

The Wistar rats were starved overnight and divided into eleven groups of six animals each as follows Group I: Vehicle control rats received distilled water (10 ml/kg, p.o.), Group II: Diclofenac sodium (10 mg/kg, p.o.), Group III: BFQI (100 mg/kg, p.o.), Group IV: BFQI (200 mg/kg, p.o.), Group V: BFQI (400 mg/kg, p.o.), Group VI: BLQI (100 mg/kg, p.o.), Group VII: BLQI (200 mg/kg, p.o.), Group VIII: BLQI (400 mg/kg, p.o.), Group IX: BBQI (100 mg/kg, p.o.), Group X: BBQI (200 mg/kg, p.o.), Group XI: BBQI (400 mg/kg, p.o.).

Carrageenan induced rat paw Oedema

After selection of animals, 0.1 ml of 1% carrageenan solution was injected into the left hind paw. The pretreatment time was 1 h before carrageenan injection. The paw volume was recorded immediately and at 1 h, 2 h, 3 h, 4 h and 6 h by using plethysmometer. Mean increase in the volume of oedema was measured and percentage inhibition was calculated[27-29].

Excision wound method

An impression were made on the shaved dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear using a round seal of 2.5 cm diameter on the ether anaesthetized rat. A full circular piece (approximately 500 mm^2) of skin of full thickness (0.2 cm) will be excised from the predetermined area on the back of the rat. Haemostasis will be achieved by blotting the wound with cotton swab soaked in normal saline. The progressive changes in wound area were monitored by a camera on predetermined days, i.e., 2, 4, 8, 12, 16, 18 and 22. Later on wounds were traced on 1 mm^2 graph paper on the day of wounding and subsequently on alternate days until healing was completed [30]. Wound contraction was calculated as percentage of the reduction in original wound area size. It was calculated by using the following formula:

$$\text{Percentage wound contraction} = \frac{\text{Initial area of wound} - \text{Nth day area of wound}}{\text{initial area of wound}} \times 100$$

Number of days required for falling of scar without any residual raw wound gave the period of epithelisation. The actual value was converted into percentage value taking the size of the wound at the time of wounding as 100%. On day 12, one animal of each group was sacrificed by decapitation for skin collection for histological assessment. A section of the skin was fixed in 10 % neutral buffered formalin. The tissue was then processed and sectioned (5 µm thick) using a rotary microtome. The sections were stained with haematoxyline and eosin (H and E stain) dye for histopathological observations. The slides were the mounted with DPX (mixture of 10 g of sistyrene, 80.5 ml of dibutyl phthalate and 35 ml of xylene) and observed under the light microscope for morphological changes.

STATISTICAL ANALYSIS

The observations were expressed in mean ±S.E.M. The difference in response to test drug was determined by two way ANOVA followed by Bonferroni test. ***P < 0.001 was considered as significant.

RESULTS AND DISCUSSION

The different parts of the plant such as stem, flowers, leaf and bark of *Quisqualis indica* has been used since ancient times for its traditional uses. It consists of vide source of secondary metabolites or phytoconstituents such as glycosides, alkaloids, phytosterols proteins, saponins and phytosterols. While screening of the benzene flowers, leaf and bark extracts of *Quisqualis indica*for its phytoconstituents, it showed the presence of Alkaloids, Saponins, Sterols and Flavonoids as shown in Table 1.

Acute oral toxicity of the extract

Acute oral toxicity has been performed to find the safe dose of extracts. The BFQI, BLQI and BBQI were found to be safe at all doses used and oral administration of BFQI, BLQI and BBQI at a dose 4000 mg/kg were found no mortality. Therefore, we have taken 400 mg/kg as the therapeutic dose and made variations by taking 100 mg/kg as lower dose and 400 mg/kg as higher dose.

Antimicrobial activity

For the investigation of newer drugs, phytomedicines is a natural blue print, used for the management of diseases. The antimicrobial activity of benzene flowers, leaves and bark extracts of *Quisqualis indica*were investigated at different concentrations viz. 100, 200 and 400 µg/ml using agar well diffusion method against gram positive and gram negative pathogens such as *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia*, Bacillus subtilis, *Staphylococcus epidermidis, Streptococcus pyogenes, Salmonella typhi*.

Higher concentrations (400 µg/ml) of benzene flowers, leaves and bark extracts of *Quisqualis indica* showed maximum zone of inhibition against Gram positive as well as Gram negative bacteria. It indicates that *Quisqualis indica* could inhibit the growth of bacteria which cause infectious diseases. However, lower concentrations of *Quisqualis indica* benzene extract did not express any activity or exhibited low activity (Table 2).

Antioxidant activity

DPPH scavenging activity

DPPH is a stable free radical. It becomes a stable diamagnetic molecule on accepting an electron or hydrogen atom. Test substance has an ability to donate a hydrogen atom which gives purple color changes to yellow (diphenylpicrylhydrazine) by loosing of free radical. The bleaching of DPPH radical is one of the most widely used method to screen the antioxidant activity of herbal extracts. This method is very simple, fast and measures the capacity of herbal extract to bleach the DPPH radical. Also, this method is sensitive and requires small amount of samples. In the present study, free radical scavenging activity of flowers, leaves and bark of *Quisqualis indica* with benzene extracts monitored at 525 nm at different concentrations. The antioxidant activities of benzene flowers, leaves and bark extracts of *Quisqualis indica* exert in a dose dependent manner. Flowers, leaf and bark of *Quisqualis indica* with benzene extracts at a concentration 400 µg/3 ml exhibited 79% %, 75 % and 62 % DPPH scavenging activity respectively. Whereas, 200 and 400 µg/3 ml of Ascorbic acid exhibited 67 % and 96 % DPPH scavenging activity respectively. However, lower concentrations of extracts did not exhibit significant scavenging activity.It was evident that the active extracts showed hydrogen donating ability and therefore the extracts could serve as free radical scavengers, acting possibly as primary antioxidants [31]. At higher concentrationsof flowers, leaves and bark of *Quisqualis indica*with benzene extracts showed effective free radical scavenging in the DPPH assay. (Figure1)

Nitric oxide scavenging activity

In addition to reactive oxygen species, nitric oxide is also concerned in inflammatory disorders. The percentage inhibition of nitric oxide generation from sodium nitroprusside in buffered saline by flowers, leaves and bark of *Quisqualis indica* with benzene extracts at different concentrations were compared with standard. Nitric oxide is a potent pleiotropic mediator of physiological processes. At higher concentrations of flowers, leaves and bark of *Quisqualis indica* with benzene extracts showed potential inhibiting activity against NO generation. Flowers, leaf and bark of *Quisqualis indica* with benzene extracts at a concentration 400 µg/3 ml exhibited 71 %, 71 % and 75% nitric oxide scavenging activity respectively. Whereas, 200 and 400 µg/3 ml of Ascorbic acid exhibited 84 % and 92 % scavenging activity respectively. However, lower concentrations of extracts did not exhibit significant activity (Figure 2).

Antiinflammatory activity

Carrageenan induced rat paw Oedema

Carrageenan induced rat paw oedema is a multimediated phenomenon that liberates diversity of mediators. It is believed to be biphasic the first phase (60 min) involves the release of serotonin and histamine while the second phase (over 60 min) is mediated by prostaglandins, the cyclooxygenase products, and the continuing between the two phase is provided by kinins [32-33]. Development of oedema induced by carrageenan is commonly correlated with early exudative stage of inflammation [34].Inoculation of carrageenan induced significant raise in paw volume in all animals. Pretreatment with oral administration of diclofenac sodium (10 mg/kg) and 400 mg/kg of flowers, leaf and bark of *Quisqualis indica* with benzene extracts significantly ($p<0.001$)suppressed the level of carrageenan induced paw edema at 1, 2, 3, 4 and 6 h as compared to vehicle treated animals. However, lower doses (100 and 200 mg/kg, p.o.) did not show significant anti-inflammatory activity (Table 3).Since carrageenan induced inflammation model is a significant test for anti-inflammatory agent acting by the mediators of acute inflammation [35].

Excision wound method

Wound healing is a process in which a tissue (damaged) is regenerated to their normal state and contractions of wound is a process of shrinkage of wound area. In wound, collagen play a important role in contributing wound strength. Phytoconstituents like flavonoids, tannins, alkaloids promote the wound healing through several mechanisms like chelation of free radicals and reactive oxygen species, and promotes wound contractions and neovascularisation. The collagen is a major protein of the extracellular matrix composed of amino acid which gives strengthening and support to tissues. The wound healing activity of flowers, leaf and bark of *Quisqualis indica* with benzene extracts was screened on rats to confirm the traditional use of plant. The area of the wound was measured on the day 2, 4, 8, 12, 16, 18 and 22 in all groups. Higher dose of flowers, leaf and bark of *Quisqualis indica* with benzene extracts showed significant ($p<0.0001$) improvement in area of wound in mm^2 (% wound contractions) on day 8 and 12, and complete healing was achieved on day 22. Lower doses i.e. 100 and 200 mg/kg of flowers, leaf and bark of *Quisqualis indica* with benzene extracts exhibited mild to moderate healing potential respectively (Table 4 and Figure 3).Inflammation, proliferation, and remodeling are the various phases in wound healing processes that were observed during the experimental period. Delayed wound healing processes were recorded in control groups. Aggregation of tissues with poor collagenation was observed in control group of animals. Treatment with flowers, leaf and bark of *Quisqualis indica* with benzene extracts at 400 mg/kg resulted in decreased inflammation, increasing the rate of tissue perfusion and proliferation as well as remodeling, along with reepithelization (Figure 4).

The present investigation suggests a scientific support to the traditional antimicrobial, antioxidant, anti-inflammatory and wound healing activity in use of the plant *Quisqualis indica.* Further investigations are needed for identification of active principles responsible for multimechanistic role involved in various diseases. The present study suggests that the antimicrobial, antioxidant, anti-inflammatory and wound healing activity can be enhanced by the use of crude benzene extracts of *Quisqualis indica*.

Table 1: Phytochemical screening of the benzene flowers, leaf and bark extracts of *Quisqualis indica*

Sr. No.	TEST	BFQI	BLQI	BBQI
1	Alkaloids	+ve	+ve	-ve
2	Flavonoids	-ve	-ve	+ve
3	Saponins	+ve	+ve	-ve
4	Tannins	-ve	-ve	-ve
5	Sterols	-ve	-ve	+ve
6	Carbohydrates	-ve	-ve	-ve
7	Glycosides	-ve	-ve	-ve

Table 2: Antimicrobial effect of benzene flowers, leaf and bark extracts of *Quisqualis indica* against human pathogenic bacteria using Agar well diffusion method.

Treatment	Zone of Inhibition (mm)							
	B. cereus	***S. aureus***	***E. coli***	***P. Aeruginosa***	***K. pneumonia***	***S. epidermidis***	***S. pyogenes***	***S. typhi***
VEHICLE	4.5±0.25	3.9±0.16	3.7±0.16	3.2±0.35	4.7±0.23	3.4±0.37	4.1±0.16	5.4±0.35
STREPTOMYCIN (10 µg/ml)	21.9±0.29 ***	19.2±0.25 ***	17.9±0.32 ***	21.7±0.32 ***	26.7±0.25 ***	24.3±0.38 ***	21.9±0.32 ***	20.4±0.34 ***
BFQI (100 µg/ml)	6.2±0.25	5.9±0.35	7.6±0.34	5.1±0.64	6.8±0.43	5.6±0.34	4.5±0.62	5.5±0.48
BFQI (200 µg/ml)	9.8±0.65	8.2±0.35	9.8±0.34	8.5±0.54	7.8±0.34	7.6±0.34	6.6±0.66	7.3±0.47
BFQI (400 µg/ml)	13.2±0.23 ***	14.6±0.53 ***	13.2±0.54 ***	16.2±0.52 ***	15.7±0.52 ***	13.9±0.65 ***	16.3±0.23 ***	15.7±0.55 ***
BLQI (100 µg/ml)	6.4±0.56	5.7±0.54	7.2±0.52	5.5±0.33	6.6±0.65	5.4±0.24	4.6±0.23	5.4±0.24
BLQI (200 µg/ml)	9.4±0.84	8.7±0.54	9.8±0.45	8.7±0.64	7.6±0.89	7.7±0.64	6.2±0.55	7.4±0.84
BLQI (400 µg/ml)	13.5±0.58 ***	14.1±0.16 ***	13.4±0.28 ***	16.4±0.23 ***	15.5±0.68 ***	13.9±0.24 ***	16.6±0.45 ***	15.8±0.24 ***
BBQI (100 µg/ml)	6.2±0.68	5.5±0.26	7.3±0.92	5.6±0.45	6.6±0.42	5.8±0.25	4.6±0.45	5.7±0.23
BBQI (200 µg/ml)	9.5±0.26	8.6±0.63	9.5±0.34	8.8±0.23	7.3±0.82	7.5±0.25	6.9±0.53	7.4±0.23
BBQI (400 µg/ml)	13.5±0.59 ***	14.6±0.65 ***	13.2±0.58 ***	16.9±0.45 ***	15.9±0.22 ***	13.8±0.55 ***	16.5±0.75 ***	15.6±0.52 ***

Values are mean±S.E.M. of 3 replications. and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. ***P < 0.001 compared to vehicle treated group.

Table 3: Effect of benzene flowers, leaf and bark extracts of *Quisqualis indica* on change in paw volume in Carrageenan induced rat paw edema.

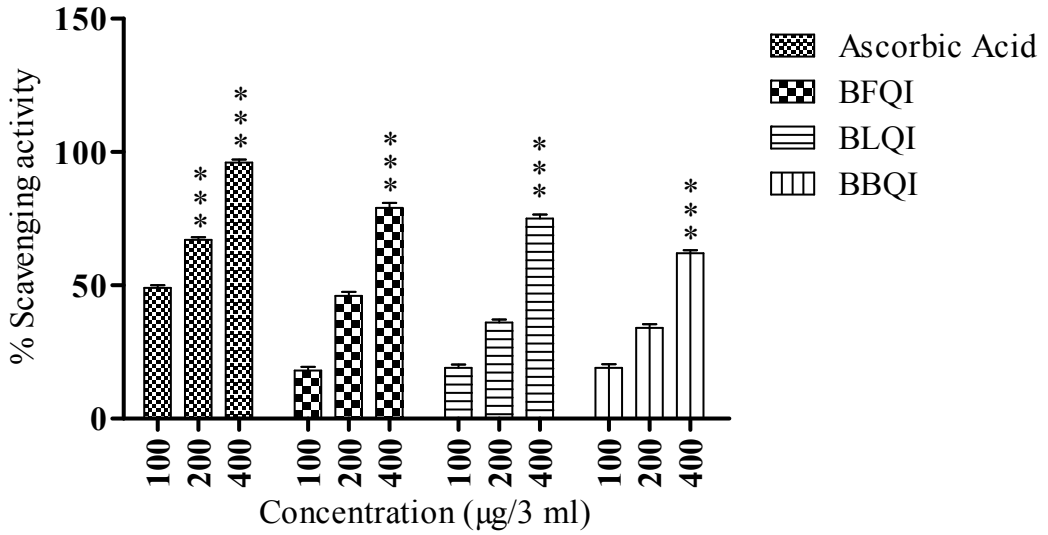
Treatment	Change in paw volume (ml)					
	0	**1**	**2**	**3**	**4**	**6**
VEHICLE	1.16±0.02	1.37±0.05	1.45±0.05	1.52±0.05	1.6±0.03	1.67±0.06
DICLOFENAC SODIUM (10 mg/ml)	1.15±0.05	1.24±0.04 ***	1.26±0.06 ***	1.27±0.08 ***	1.29±0.03 ***	1.28±0.05 ***
BFQI (100 mg/ml)	1.19±0.05	1.35±0.03	1.43±0.03	1.46±0.02	1.52±0.02	1.52±0.06
BFQI (200 mg/ml)	1.13±0.08	1.31±0.06	1.35±0.05	1.42±0.08	1.42±0.05	1.48±0.03
BFQI (400 mg/ml)	1.18±0.05	1.32±0.06 ***	1.33±0.08 ***	1.35±0.08 ***	1.37±0.08 ***	1.39±0.07 ***
BLQI (100 mg/ml)	1.18±0.09	1.35±0.04	1.42±0.04	1.49±0.07	1.54±0.05	1.56±0.05
BLQI (200 mg/ml)	1.15±0.05	1.34±0.08	1.34±0.04	1.42±0.04	1.46±0.05	1.42±0.02
BLQI (400 mg/ml)	1.18±0.05	1.31±0.06 ***	1.32±0.08 ***	1.34±0.09 ***	1.37±0.02 ***	1.39±0.03 ***
BBQI (100 mg/ml)	1.19±0.04	1.34±0.05	1.46±0.04	1.48±0.09	1.51±0.08	1.53±0.02
BBQI (200 mg/ml)	1.19±0.05	1.3±0.08	1.31±0.08	1.38±0.08	1.46±0.08	1.46±0.05
BBQI (400 mg/ml)	1.19±0.05	1.31±0.08 ***	1.34±0.03 ***	1.35±0.09 ***	1.38±0.03 ***	1.4±0.03 ***

Values are Mean±S.E.M. from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. ***P < 0.001 compared to vehicle treated animals.

Table 4 : Effect of benzene flowers, leaf and bark extracts of *Quisqualis indica* on area of wound and contractions of wound on excision wound method.

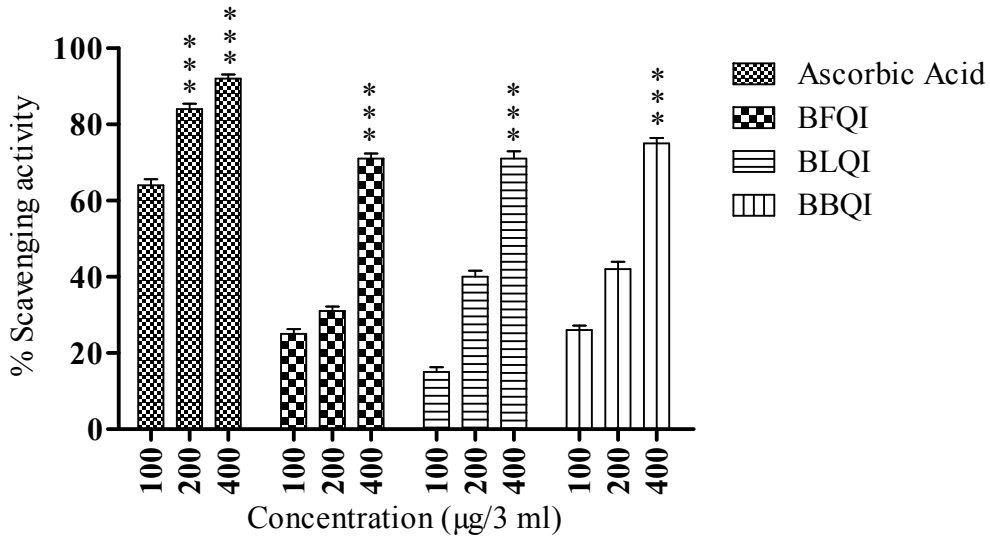
Treatment	Area of wound (mm²) &% wound contraction					
	1	4	8	12	16	22
VEHICLE	519.5±1.52	490.6±1.98 (5.56)	394.3±1.48 (24.10)	207±1.52 (60.15)	137±1.7 (73.63)	66±1.59 (87.30)
DICLOFENAC SODIUM (10 mg/ml)	508.6±1.66	320.8±1.65 (36.92)	45.2±1.54 (91.11) ***	93±1.63 (81.71) ***	0±1.45 (100.00) ***	0±1.63 (100.00) ***
BFQI (100 mg/ml)	523.4±1.89	428.8±1.64 (18.07)	331.2±1.52 (36.72)	197.9±1.49 (62.19)	126.5±1.54 (75.83)	24.3±1.86 (95.36)
BFQI (200 mg/ml)	528.5±1.82	394.8±1.61 (25.30)	307.6±1.94 (41.80)	184.4±1.54 (65.11)	94.2±1.57 (82.18)	0±1.86 (100.00)
BFQI (400 mg/ml)	516.3±1.78	378.1±1.44 (26.77)	95.5±1.55 (81.50) ***	42.1±1.42 (91.85) ***	0±1.52 (100.00) ***	0±1.83 (100.00) ***
BLQI (100 mg/ml)	524.9±1.93	418.7±1.59 (20.23)	334.9±1.62 (36.20)	197.2±1.68 (62.43)	128.5±1.66 (75.52)	24.4±1.61 (95.35)
BLQI (200 mg/ml)	533.2±1.82	385.2±1.58 (27.76)	312.3±1.47 (41.43)	182.6±1.52 (65.75)	94.9±1.58 (82.20)	0±1.84 (100.00)
BLQI (400 mg/ml)	536.5±1.85	376.4±1.99 (29.84)	96.2±1.84 (82.07) ***	35.2±1.58 (93.44) ***	0±1.52 (100.00) ***	0±1.83 (100.00) ***
BBQI (100 mg/ml)	527.1±1.89	428.2±1.83 (18.76)	334.2±1.49 (36.60)	185.5±1.44 (64.81)	128.9±1.88 (75.55)	28.5±1.48 (94.59)
BBQI (200 mg/ml)	524.2±1.68	396.8±1.52 (24.30)	306.3±1.88 (41.57)	180.3±1.47 (65.60)	92.5±1.64 (82.35)	0±1.83 (100.00)
BBQI (400 mg/ml)	523.5±1.69	377.3±1.69 (27.93)	94.8±1.84 (81.89) ***	45.3±1.84 (91.35) ***	0±1.43 (100.00) ***	0±1.77 (100.00) ***

Figure1: Effect of benzene flowers, leaf and bark extracts of *Quisqualis indica* on DPPH scavenging activity.



Values are Mean±S.E.M (n=3). and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. ***P < 0.001 compared to vehicle treated animals.

Figure 2: Effect of benzene flowers, leaf and bark extracts of *Quisqualis indica* on Nitric oxide scavenging activity.



Values are Mean±S.E.M (n=3). and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. ***P < 0.001 compared to vehicle treated animals.

Figure 3: Effect of benzene flowers, leaf and bark extracts of *Quisqualis indica*on various phases of wound contractions in excision wound method.

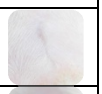
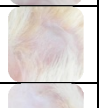
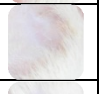
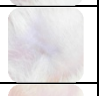
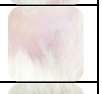
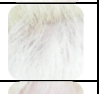
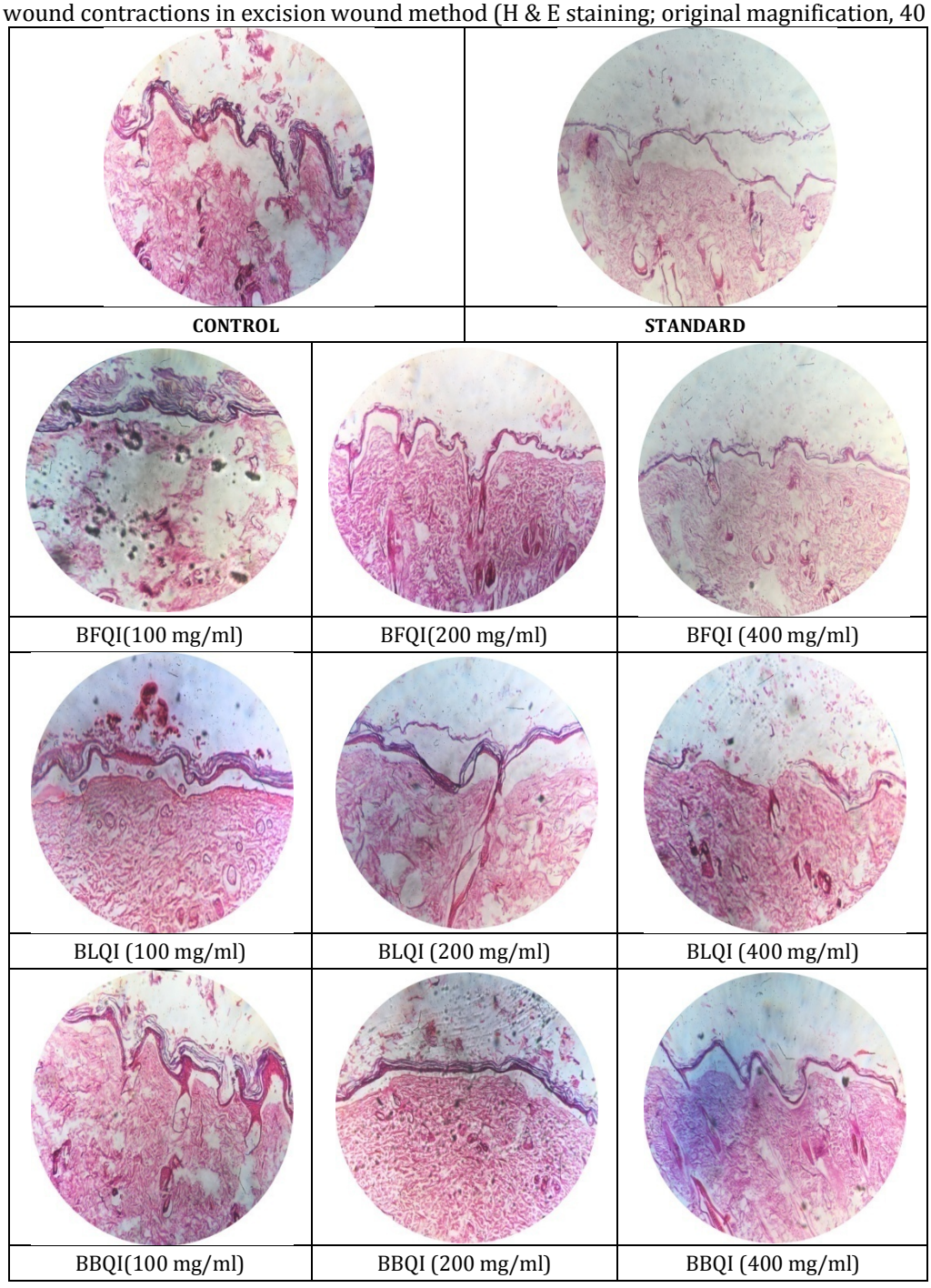
Treatment	Days				
	1	4	8	12	22
Control					
Std					
BFQI (100 mg/ml)					
BFQI (200 mg/ml)					
BFQI (400 mg/ml)					
BLQI (100 mg/ml)					
BLQI (200 mg/ml)					
BLQI (400 mg/ml)					
BBQI (100 mg/ml)					
BBQI (200 mg/ml)					
BBQI (400 mg/ml)					

Figure 4: Effect of benzene flowers, leaf and bark extracts of *Quisqualis indica* on histopathological studies of wound contractions in excision wound method (H & E staining; original magnification, 40 X).



CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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