Anti-inflammatory activities of ethanol leaves extract and solvent fractions of *Zehneria scabra* (Cucurbitaceae) in rodents

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Abstract. Belay R, Makonnen E. 2020. Anti-inflammatory activities of ethanol leaves extract and solvent fractions of *Zehneria scabra* (Cucurbitaceae) in rodents. *Biofarmasi J Nat Prod Biochem* 18: 42-56. *Zehneria scarba* (L.f.) Sond is one of the medicinal plants used in folkloric medicine of Ethiopia for years to treat various inflammatory disorders. The present study was aimed to validate the anti-inflammatory activity of crude 70% ethanol leaves extract (70EE) against a sub-acute model and further evaluate the solvent fractions (AF, BF, and CF) in an acute (carrageenan-induced paw edema), sub-acute (formaldehyde induced arthritis) and chronic (cotton pellet induced granuloma) inflammatory models. The 70EE was first prepared by maceration, and the fractions were obtained by sequential partitioning with chloroform and n-butanol from the aqueous suspension of crude 70EE. The test groups then received 100, 200, and 400 mg/kg of the crude 70EE or the fractions (AF, BF, and CF) at the same dose levels. In contrast, positive controls received aspirin (200 mg/kg) or dexamethasone (0.5mg/kg) and negative controls received vehicle (2% tween 80 or distilled water, 10 mL/kg). All tested doses of the crude 70EE showed significant inhibition of formaldehyde-induced arthritis on the 10th day of treatment. The 400 mg/kg dose showed the maximum anti-arthritic effect (%A = 60.5; $p < 0.001$). In the carrageenan-induced paw edema, all three fractions showed a statistically significant effect, in fact, with different onset and magnitude. In this model, the AF was found to be the most active fraction, and the 400mg/kg dose demonstrated the maximum effect (%A = 76.25; $p < 0.001$) at 5h post-induction, which is much better than the effect of aspirin at the dose employed. The overall order of efficacy in inhibiting the exudative component of carrageenan-induced paw edema was found to be AF > BF > CF. The AF was also the most active fraction in inhibiting the exudative component of chronic inflammation in the cotton pellet-induced granuloma model, where the maximum effect (%A = 43.10, $p < 0.001$) was exhibited by a dose of 400mg/kg. The AF was also the most active fraction in inhibiting formaldehyde-induced arthritis, in which the BF and CF relatively showed a comparable effect throughout days 4-10. On the contrary, in the cotton pellet-induced granuloma model, the CF was the most active fraction in inhibiting the proliferative and granulomatous component of chronic inflammation. The overall order of effectiveness was found to be CF > AF > BF. Besides, 400mg/kg of CF demonstrated the maximum inhibition of granuloma formation (%A = 55.52; $P < 0.001$). The phytochemical analysis revealed the differential distribution of secondary metabolites into the three fractions, which either singly or in concert appeared to be responsible for the observed effects. The data obtained from the present study collectively indicate that the extract and fractions of leaves of *Z.scabra* possessed a significant anti-inflammatory activity, upholding the folkloric use of the plant.

Keywords: Anti-inflammatory, arthritis, granuloma, phytochemical, *Zehneria scarba*

INTRODUCTION

Inflammation is central to many diseases that affect both developed and emerging nations. Virtually all acute and chronic diseases are either driven or modulated by inflammation (Vodovotz et al., 2010). Despite this fact, the complex interplay between beneficial and harmful arms of the inflammatory response underlies the lack of safe and fully effective therapies for many pathologies (Vodovotz et al., 2009). On top of this, most existing drugs suffer from diverse adverse events, especially at higher doses and longer duration of therapy (Spies et al., 2011).

Non-steroidal anti-inflammatory drugs (NSAIDs), for example, are associated with the development of gastric or duodenal ulceration, nephrotoxicity, bronchospasm, exacerbation of symptoms of asthma, an increase in blood pressure, and increased incidence of myocardial infarction and stroke (Ong et al. 2007; Stanos 2013). On the other hand, corticosteroids are associated with numerous side effects, such as diabetes mellitus/glucose intolerance,

hypertension, obesity, osteoporosis, immune suppression, glaucoma, and growth retardation in children (Spies et al. 2011; Rhen and Cidlowski 2005). Because of this, WHO advocates the inclusion of herbal medicines with proven safety and efficacy in healthcare programs because of the high potential they hold in combating various diseases (WHO 2002).

Z.scabra is one of these plants whose 80% methanol leaf extract has been proven anti-inflammatory and analgesic activity. Such a plant exhibiting anti-inflammatory, analgesic (Akele 2012), antifungal (Arulappan et al. 2015), and antibacterial activity (Anand et al. 2012) would improve patient compliance and has economic importance. However, there is a shortage of scientific evidence to substantiate further the plant's therapeutic potential in different inflammatory models.

Hence, this study focuses on an *in-vivo* anti-inflammatory activity of 70EE, and solvent fractions of *Z. scabra* leave using acute (carrageenan-induced paw edema), sub-acute (formaldehyde induced arthritis), and

chronic (cotton pellet induced granuloma) models of inflammation to validate its acclaimed use by the traditional practitioners.

Moreover, it also tries to identify the most active fraction to give a clue for further investigation in search of the specific agent(s) responsible for the anti-inflammatory effect of the plant. Besides, the finding of this study might provide a clue about the possible mechanisms of anti-inflammatory action of the plant. It might serve as baseline information for the scientific community to further investigate the plant by initiating advanced studies on molecular mechanisms to identify the specific agent(s) responsible for the anti-inflammatory effect of the most active fraction.

The aims of this research were (i) To assess the acute toxicity of 70EE and solvent fractions of *Zehneria scabra* leaves in mice, (ii) To evaluate the effect of crude 70EE of *Zehneria scabra* leaves on formaldehyde-induced arthritis in mice, (iii) To assess the effect of chloroform, n-butanol and aqueous fractions of *Zehneria scabra* leaves on carrageenan-induced paw edema in mice, (iv) To evaluate the effect of chloroform, n-butanol and aqueous fractions of *Zehneria scabra* leaves on formaldehyde-induced arthritis in mice, (v) To assess the impact of chloroform, n-butanol and aqueous fractions of *Zehneria scabra* leaves using cotton pellet induced granuloma in rats, (vi) To determine the phytochemical constituents of the 70EE and solvent fractions of *Zehneria scabra* leaves.

MATERIALS AND METHODS

Drugs and chemicals

Aspirin active (EPHARM, Ethiopia), Dexamethasone (Medico Labs, Lot E6A00, Syria), Carrageenan (Sigma Aldrich, Germany), thiopental sodium (NEON Labs, India); ethanol (Lot 30320601EX) and formaldehyde (Research-Lab Fine Chem Industries- India); n-butanol (Lot 10061), chloroform (Lot 10077), and glacial acetic acid (Lot MR0478) (BDH chemical LTD Poole, England); acetic anhydride (Lot A13/45/67/A) and Mayer's reagent (May & Baker LTD Dagenham, England); Dragendroff's reagent and sulfuric acid (Lot 30326) (Fisher Scientific, UK); ammonia, hydrochloric acid, and ferric chloride (Lot 10111) (BDH Laboratory Supplies Poole, England); normal saline (Addis Pharmaceutical Factory, Ethiopia), distilled water (Biochemistry Laboratory of AAU, Ethiopia), tween 80 (UNI-CHEM Chemical Reagents, India), were used in the study and all were of analytical grade.

Materials and instruments

Rotary evaporator (Heidolph, Germany), lyophilizer (OPERON, OPR-FDU-5012, Korea), digital plethysmometer (Ugo Basile-Cat no 7140, Italy), electronic balance (KERN-ALJ 220-4, Germany), mini orbital shaker (SSM1-STUART), Tissue Drying Oven (Medite - Medizin Technik, Germany), separatory funnel, flasks, Cotton pellets, syringes with needles, feeding tube, blunt forceps, scissors, suturing set.

Plant material collection and authentication

The fresh leaves of *Zehneria scabra* were collected in the Lideta sub-city, Addis Ababa, Ethiopia, in December 2014. Identification and authentication of the plant specimen were made at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University, and a voucher specimen was deposited with voucher number RB 001/2014 for future reference. The leaves were washed gently by rinsing with running water to remove dust particles, air-dried under the shade, and then reduced into a coarse powder with mortar and pestle.

Experimental animals

Healthy male (for the anti-inflammatory test) and female (for acute toxicity test) Swiss albino mice weighing 20-30 g and male albino Wistar rats weighing 180-220 g were obtained from the animal house of the Ethiopian Public Health Institute and Department of Pharmacology, School of medicine, Addis Ababa University were used. They were kept in plastic cages at room temperature on a 12 h light-dark cycle with free access to pellet food and water *ad libitum*. The animals were acclimatized to laboratory conditions for one week before the commencement of the experiments. All experiments were carried out during the light period of the day (9:00 a.m. - 5:00 p.m.) and followed the guideline for the care and use of laboratory animals (Institute for Laboratory Animal Research 1996; OECD 2008). The study was conducted after approval by Addis Ababa University, College of Health Sciences Institution Review Board (IRB).

Preparation of crude extracts and solvent fractions of *Z. scabra* leaves

The air-dried and powdered leaves of *Z. scabra* were first defatted by macerating with petroleum ether for 24 hours at room temperature and occasionally shaking, followed by filtration. The solvent (petroleum ether) was removed from the residue by exposing it to open air. Then the defatted coarse powder was divided into two halves of 665gm each for the extraction process. The first half (665gm of the leave) was macerated in a flask containing 70% ethanol (1:5 w/v) for 72 hrs, and the other half was macerated with distilled water with the same ration and for the same period. The maceration was undertaken with occasional shaking using a mini orbital shaker tuned to 120 rpm for 72 hrs at room temperature. Then, the extract was filtered first using a muslin cloth and then using Whatman filter paper (No 1), and the marc was re-macerated for a second and third time by adding another fresh solvent.

The three batches of the 70EE filtrates were combined and concentrated in a rotary evaporator with a temperature of 40 °C. The concentrate was then placed in a deep freezer operating at negative 20°C until it formed a block of ice, and then the remaining solvent (water) was removed using a lyophilizer. After water removal, a black powder residue weighing 88 gm was obtained, giving rise to a percentage yield of 13.23%. The filtrates from the three batches of the aqueous extract were also combined, placed in a deep freezer at -20°C to form ice, and lyophilized. The water extract yields 39.34 gm of black powder (5.92% w/w). The

powders were kept in tightly stoppered bottles and stored in a deep freezer at -20°C until the commencement of the pilot study.

The hydroalcoholic extract (70EE) (which had a better activity) was selected for further fractionation based on the pilot experiment performed. A total of eighty grams of the powdered residue of the 70EE was divided into four equal parts (20gm each) and then employed for the fractionation. At a time, the 70EE (20 g) aliquot was suspended in distilled water (100 mL) and then sequentially partitioned with chloroform and *n*-butanol at room temperature using a separatory funnel. Partitioned layers of each solvent were pooled together and concentrated on a rotary evaporator at 40°C , followed by an oven at room temperature for 48 h, yielding 13.4 gm of black gummy residue from the chloroform fraction (16.75% w/w), and 16.73 gm of light brown slightly hygroscopic powder from the *n*-butanol fraction (20.91% w/w). The aqueous residue was lyophilized to give 38.85gm of black dried powder (48.56% w/w) fraction. The fractions were kept in tightly stoppered bottles and stored in a deep freezer at -20°C until the commencement of the actual experiment. Finally, the portions were reconstituted in distilled water/2% tween 80 at appropriate concentrations for the various trials.

Acute oral toxicity test

An acute oral toxicity test for 70EE and solvent fractions of the leaves of *Z. scabra* was performed according to the Organization for Economic Cooperation and Development (OECD) guideline 425; "Limit Test at 2000 mg/kg" (2008). Five female albino mice of 6-8 weeks were used for each test. All mice fasted for four h before and two h after administering the extract/fractions. First, a sighting study was performed to determine the starting dose, in which a single female mouse was given 2000 mg/kg of the extract/fractions as a single dose by oral gavage. Since no death was observed within 24 h, four additional mice were used for each extract and fraction and administered the same dose of extract/fractions. The animals were observed continuously for 4 h with 30 min intervals and then for 14 consecutive days with a range of 24 h for the general signs and symptoms of toxicities such as changes in skin and fur, eyes and mucous membranes, somatomotor activity, and behavioral pattern, salivation and diarrhea, weight loss, tremor and convulsions, lethargy and paralysis, food and water intake and mortality.

Pilot study

The pilot study was done using the Carrageenan-induced paw edema acute model of inflammation on 70% ethanol leave extract (70EE), aqueous leave extracts (AE), and petroleum ether extract (PE) of *Z. scabra*. All three extracts were administered at doses of 100, 200, and 400mg/kg, and three animals per group were used at all the dose levels. The result indicated that both the AE and 70EE have anti-inflammatory activity; even though the 70EE had shown a better anti-inflammatory effect (%A) at all employed doses (maximum %A at the 5th h = 37% for 100mg/kg, 45% for 200mg/kg, and 52% for 400mg/kg) than the AE (maximum %A at the 5th h = 19% for

100mg/kg, 28% for 200mg/kg, and 41% for 400mg/kg). On the other hand, the PE extract failed to demonstrate anti-inflammatory activity at all employed doses. Hence, the 70EE opted for further study and fractionation.

Animal grouping and dosing

The animals were randomly assigned into twelve groups of six to perform the anti-inflammatory activity test in three models. The first two groups served as negative controls, and the vehicles for the fractions (distilled water and 2% tween 80 at a dose of 10 mL/kg) were administered. The third group served as a positive control. The standard drugs aspirin (200mg/kg p.o. in the acute and sub-acute models) or dexamethasone (0.5mg/kg p.o. in the chronic model) was administered to this group. The first three test groups (4-6) received three doses (100, 200, and 400mg/kg) of the aqueous fraction. The next three test groups (7-9) received *n*-butanol fraction at doses of 100, 200, and 400mg/kg, while the final three test groups (10-12) received the chloroform fraction at the same three dose levels. The same dose levels were also applied during an anti-inflammatory test of the crude extract using formaldehyde-induced arthritis in mice, during which a total of five groups, i.e., two control groups (positive and negative) and three test groups of 70EE (100, 200, and 400mg/kg) were used. The dose levels (100, 200, and 400mg/kg) were selected based on the acute oral toxicity test results and pilot study.

Determination of anti-inflammatory activity

Carrageenan-induced paw edema

The method described by Mequanint et al. (2011) was followed in this model to study the effect of solvent fractions of *Z. scabra* leaves on acute inflammation. Mice fasted for 12 h with free access to water until the experiment started and grouped and treated by oral gavage as described under section 3.8. Aspirin 200mg/kg p.o. was administered as a standard drug. The right hind paw was marked with ink at the level of lateral malleolus so that it could always be immersed to the same extent in the measurement chamber of the plethysmometer.

An hour later, edema was induced by injecting 0.05 mL of 1% w/v carrageenan in normal saline into the right hind paw of each mouse. The increased volume of the right hind paws was taken to signify paw edema. Paw volume was determined by volume displacement technique using the Ugo-Basile plethysmometer just before carrageenan injection (initial amount (V_0)), and 1, 2, 3, 4, and 5 h after carrageenan injection (final volumes (V_f)). The degree of swelling, i.e., edema, was evaluated by the delta volume ($V_f - V_0$) (Sanusi et al., 2013).

$$\text{Oedema (I)} = V_f - V_0$$

Where: V_0 is the paw volume before the carrageenan injection, and V_f is the paw volume after the carrageenan injection at a given time.

Also, the anti-inflammatory effect of the fractions expressed in percentage (%A) was calculated according to the formula given by Sanusi et al. (2013):

$$\% \text{ inhibition (\%A)} = \left(1 - \frac{I_t}{I_c}\right) \times 100$$

Where: I_t and I_c are the mean inflammation (Oedema) values reached at a given time in treatment and control groups, respectively.

Formaldehyde induced arthritis

The method described by Mehta et al. (2012) and Cui et al. (2014) was used as a subacute inflammation model. Mice (20-30 g) fasted for 12 h with free access to water until the commencement of the experiment. The right hind paw was marked with ink at the level of lateral malleolus so that it could always be immersed to the same extent in the measurement chamber of the plethysmometer. The control, standard, and test groups of mice received distilled water (2% tween 80 in case of chloroform fraction), Aspirin (200mg/kg p.o.), and extract/fractions, respectively, as described in section 3.8. On the first day, the basal paw volume (V_0) of the right hind paw of each mouse was measured using a plethysmometer. On day one and day 3, mice were injected into the sub-plantar region of the right hind paw with 0.05 mL of 2 % v/v formaldehyde in normal saline. Dosing with vehicles, standard drug (Aspirin), and extract/fractions was started on the same day an hour before induction of arthritis and continued for ten consecutive days by oral gavage. The mice paw volume was recorded daily using a plethysmometer after one h of drug administration, but on day one, the measurement was taken three h after formaldehyde injection. Finally, the percentage of inhibition of edema was calculated, as described in section 3.9.1.

Cotton pellet induced granuloma method

The method previously described by Afsar et al. (2013) was used to assess chronic inflammation's transudative and proliferative (granulomatous) components. Male albino Wistar rats (180-220 g) fasted for 12 h with free access to water until the commencement of the experiment. The control, standard, and test groups of rats received distilled water (2% tween 80 in case of chloroform fraction), dexamethasone (0.5 mg/kg p.o.), and fractions, respectively, as described in section 3.8.

Cotton pellets weighing 10 ± 1 mg were sterilized in an autoclave for 30 min at 120°C under 15lb pressure. Twenty minutes after treatment with the standard drug and fractions, the rats were anesthetized with thiopental sodium (25 mg/kg, i.p.). The subcutaneous tunnel was made aseptically using blunted forceps on both sides of each rat's previously shaved groin region. Two sterilized cotton pellets weighing 10 ± 1 mg each were implanted bilaterally in the subcutaneous tunnel and sutured with chromic catgut (0/4metric-1/2 Circle). Treatment with the standard drug (dexamethasone) and fractions continued for seven consecutive days (p.o., once a day). On the 8th day, the rats were sacrificed with ether anesthesia; then, the pellets surrounded by granuloma tissue were dissected carefully and freed from extraneous tissue. The wet weight of the cotton was taken immediately after removal and then dried

up to constant weight at 60°C for 24hrs, and the net dry weight, that is, after subtracting the weight of the cotton pellets.

The exudate amount (mg), granulation tissue formation (mg), and percent inhibition of exudate and granuloma tissue formation were calculated according to the formula given by Aziz et al. (2014):

$$\text{Exudate inhibition (\%)} = \left(1 - \frac{\text{Exudate in treated group}}{\text{Exudate in controls}}\right) \times 100$$

$$\text{Granuloma inhibition (\%)} = \left(1 - \frac{\text{Granuloma in treated group}}{\text{Granuloma in controls}}\right) \times 100$$

Where:

The measure of exudate formation = immediate wet weight of pellet - Constant dry weight of the pellet

The measure of granuloma tissue formation = Constant dry weight - Initial weight of the cotton pellet.

Preliminary phytochemical screening

The initial phytochemical screening of secondary metabolites of 70EE, and chloroform, n-butanol, and aqueous fractions of leaves of *Z. scabra* were carried out using standard tests (Debella 2002; Sasidharan et al. 2011).

Test for saponins

To 0.25 g of 70EE and each fraction (AF, BF, and CF), 5 mL of distilled water was added to a test tube. Then, the solution was shaken vigorously and observed for a stable, persistent froth. The formation of a stable froth that persisted for about half an hour indicated the presence of saponins.

Test for terpenoids

To 0.25 g of 70EE and each fraction, 2 mL of chloroform was added. Then, 3mL concentrated sulfuric acid was carefully added to form a layer. A reddish-brown coloration of the interface indicated the presence of terpenoids.

Test for tannins

About 0.25 g of 70EE, each fraction was boiled in 10 mL of water in a test tube and filtered with filter paper (Whatman No. 1). A few drops of 0.1% ferric chloride were added to the filtrate. A brownish-green or a blue-black precipitate indicated the presence of tannins.

Test for flavonoids

About 10 mL of ethyl acetate was added to 0.2 g of 70EE, and each fraction and heated in a water bath for 3 min. The mixture was cooled and filtered. Then, about 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. The layers were allowed to separate, and the yellow color in the ammonia layer indicated the presence of flavonoids.

Test for cardiac glycosides

To 0.25 g of 70EE and each fraction diluted with 5 mL of water, 2 mL of glacial acetic acid containing one drop of

ferric chloride solution was added. This was underlaid with 1 mL of concentrated sulfuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides.

Test for steroids

Two mL of acetic anhydride was added to 0.25 g of 70EE and each fraction with 2 mL sulfuric acid. The color change from violet to blue or green in some samples indicated the presence of steroids.

Test for alkaloids

0.5 g of 70EE, and each fraction was diluted to 10 mL with acid alcohol, boiled, and filtered. To 5 mL of the filtrate, 2 mL of dilute ammonia and 5 mL of chloroform were added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 mL of acetic acid. This was divided into two portions. Mayer's reagent was added to one part, and Dragendorff's reagent to the other. The formation of cream (with Mayer's reagent) or reddish-brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids.

Statistical analysis

The data were analyzed using SPSS version 16.0 for Windows. The experimental results are expressed as mean $\pm$ standard error of the mean (SEM), and statistical significance was carried out by employing a one-way analysis of variance (ANOVA) followed by the Tukey post hoc test for multiple comparisons to compare results among groups, where p values < 0.05 were considered statistically significant. The analyzed data were then presented using tables. Linear regression was also used where appropriate.

RESULTS AND DISCUSSION

Acute oral toxicity test

The acute oral toxicity test of 70EE and fractions (AF, BF, and CF) of leaves of *Z. scabra* showed that neither the 70EE nor the solvent fractions caused gross behavioral changes, toxic effects, or mortality within 24 h and in the next 14 days. According to the "Limit Test at 2000 mg/kg" of OECD guideline 425 (2008), it can be concluded that the oral LD₅₀ of both the crude 70EE and solvent fractions are more significant than 2000 mg/kg in mice.

Carrageenan induced paw edema

Subplantar injection of 0.05 mL of 1% carrageenan to the mice's hind paw produced a progressive increment of paw thickness that reached its maximum value after 3h of induction in both distilled water and 2% tween vehicle controls (Table 1). All tested doses of the aqueous fraction (100, 200, and 400mg/kg of AF) showed significant inhibition of paw edema that started from 1 h ($p < 0.01$), and the effect lasted till 5 h post-induction ($p < 0.001$ from 2nd - 5th h) as compared to the distilled water vehicle control.

Maximum anti-inflammatory effect (%A) by the 100, 200 and 400 mg/kg of AF was observed at 5 h post-

induction, with respective values of 50.97%, 72.02 %, and 76.25 %, and the effect at this hour was found to increase in dose-dependent manner ($R^2 = 0.721$). Intergroup comparison among doses of the AF also showed a statistically significant different effect in both 200 versus 100mg/kg ($p < 0.05$ at 4h and 5h), and 400 versus 100mg/kg ($p < 0.05$ at 4 h, and $p < 0.01$ at 5 h).

Unlike the AF, only the higher doses of butanol fraction (200mg/kg and 400mg/kg of BF) showed statistically significant inhibition of paw edema at 1h post-induction as compared to distilled water vehicle control, with $p < 0.05$ and $p < 0.01$, respectively. The effect in both dose levels then persisted from the 2nd - 5th h post-induction with $p < 0.001$, except at the 3rd h where $p < 0.01$ for 200mg/kg compared to the negative control. However, the 100mg/kg of BF failed to demonstrate statistically significant inhibition of paw edema compared to the negative control except at two h post-induction, where $p < 0.05$.

Like the AF, maximum percent inhibition by the 100, 200, and 400 mg/kg of BF was observed at five h post-induction, with respective values of 19.93%, 43.29%, and 51.33%, and the anti-inflammatory effect was found to increase in a dose-dependent manner ($R^2 = 0.799$). Intergroup comparison among doses of the BF also showed a statistically significant different effect in both 200 versus 100 mg/kg (5h, $p < 0.05$) and 400 versus 100 mg/kg (2 h, $p < 0.05$ and 3 - 5 h, $p < 0.01$).

The higher doses of chloroform fraction (CF), on the other hand, significantly inhibited paw edema as compared to the 2% tween vehicle control only late at the 4th h ($p < 0.05$ for 200mg/kg; $p < 0.01$ for 400mg/kg), and 5th h ($p < 0.01$ for 200mg/kg; $p < 0.001$ for 400mg/kg) post induction. The 100mg/kg of CF, however, did not show significant inhibition of paw edema as compare to the negative control throughout the observation period. Interestingly, no significant difference was noted among the doses of CF, except at 5h post induction where 400mg/kg showed a statistically significant inhibition ($p < 0.05$; %A = 39.31) as compared to 100mg/kg (%A = 12.63), and the effect at this hour was found to increase dose dependently ($R^2 = 0.931$).

Significant inhibition of paw edema occurred with 200mg/kg of aspirin from the 1st h ($p < 0.01$) till the 5th h after carrageenan injection ($p < 0.001$ from 2nd - 5th h) as compared to the negative control. Moreover, no difference in onset and duration of action was observed among all tested doses of the AF, 200 and 400mg/kg of the BF, and 200mg/kg of aspirin, as all showed significant inhibition of paw edema from the 1st h till the 5th h post-induction. Nevertheless, 200 and 400mg/kg of the AF showed a higher anti-inflammatory effect (%A) than that of 200mg/kg of aspirin throughout the observation period, whereas 100mg/kg of AF had shown a comparable anti-inflammatory impact with 200mg/kg of aspirin (Table 1).

Therefore, it can be concluded that the AF was the most active fraction in terms of anti-inflammatory effects on carrageenan-induced mice paw edema. This is evidenced by the higher percent inhibition (%A) values of all tested doses of AF compared to the equivalent doses of the BF and CF.

The reference drug, 200mg/kg of aspirin, showed significant inhibition of paw edema beginning from day 2 of treatment. The effect lasted till day 10 with $p < 0.001$, but on day 4 $p < 0.01$ compared to the negative control. Maximum percentage inhibition (57.44%) by 200mg/kg of aspirin was noted on day 3 of treatment. Moreover, no significant difference in onset of action was observed among aspirin; all three doses of AF, 200 and 400 mg/kg of BF, and the highest dose (400mg/kg) of CF all showed a statistically significant inhibition starting from the 2nd day of treatment. Furthermore, 100mg/kg of AF and the BF at 200 and 400mg/kg showed a comparable anti-inflammatory effect (%A) with 200mg/kg of aspirin. The 200 and 400mg/kg of AF, on the other hand, exhibited a significantly higher anti-inflammatory effect than 200mg/kg aspirin throughout days 3 - 10, as shown in Table 3.

Even though all three fractions, of course, showed significant inhibition of paw edema at different doses compared to their respective negative controls, the AF was found to be the most active fraction in terms of the anti-inflammatory effect on formaldehyde-induced arthritis in mice. This is evidenced by the higher percent inhibition value of the AF throughout the observation period compared to the equivalent doses of BF and CF.

Cotton pellet induced granuloma

Subcutaneous implantation of two pellets of cotton, each weighing 10 ± 1 mg in the groin region of rats, induced granulomatous inflammation with a maximum granuloma weight and exudates observed in distilled water and 2% tween 80 received negative controls as shown in Table 4. The aqueous fraction (AF) at all tested doses significantly inhibited the formation of inflammatory exudates ($p < 0.001$) and granuloma mass ($P < 0.01$ for 100mg/kg; $p < 0.001$ for 200 and 400mg/kg) as compared to the negative control.

Intergroup comparisons among doses of the AF revealed a statistically significant different effect in 400 versus 200mg/kg ($p < 0.05$ in exudates inhibition; $p < 0.001$ in granuloma inhibition), 400 versus 100mg/kg ($P < 0.001$ in both exudates and granuloma inhibition), and 200 versus 100mg/kg ($p < 0.001$ in exudates inhibition). Furthermore, the anti-inflammatory effect of the AF was found to increase in dose dependent manner ($R^2 = 0.829$ for exudates inhibition; $R^2 = 1$ for granuloma inhibition).

All tested doses of the chloroform fraction (CF) significantly ($p < 0.001$) inhibited the formation of both inflammatory exudates and granuloma mass as compared to the 2% tween negative controls. Comparison among doses of the CF revealed a statistically significant different effect in 200 versus 100mg/kg ($p < 0.01$ in exudates inhibition), 400 versus 200mg/kg ($P < 0.01$ in granuloma inhibition), and 400 versus 100mg/kg ($p < 0.001$ in both exudates and granuloma inhibition). Besides, the anti-inflammatory effect of the CF was ascertained to increase in a dose-dependent manner ($R^2 = 0.928$ for exudates inhibition; $R^2 = 0.998$ for granuloma inhibition). Furthermore, the maximum anti-proliferative effect (peak

percentage inhibition of granuloma formation, 55.52%) was shown at 400mg/kg of CF compared to all other CF, BF, and AF doses.

The butanol fraction (BF), on the other hand, showed significant inhibition of both inflammatory exudates ($p < 0.001$) and granuloma mass ($p < 0.01$) only at the highest tested dose (400mg/kg) as compared to the distilled water vehicle control. The 200mg/kg of BF showed a significant ($p < 0.05$) inhibition of exudate formation, but no considerable protection against granuloma formation was noted compared to the negative control.

The 100mg/kg of BF, on the other hand, failed to demonstrate significant inhibition of both exudates and granuloma formation as compared to the negative control. Intergroup comparisons among doses of the BF revealed a statistically significant different protection against exudates formation in 400 versus 200mg/kg ($p < 0.05$) and 400 versus 100mg/kg ($p < 0.01$). Still, no significant difference was observed among all three doses of BF regarding inhibition of granuloma mass formation.

The reference drug, 0.5mg/kg of dexamethasone, significantly ($p < 0.001$) inhibited the formation of both exudates (%A = 45.07) and granuloma mass (%A = 65.99) as compared to the negative control. The highest tested dose (400mg/kg) of both the AF and CF showed a comparable inhibition of exudate formation with the reference drug. But a significant ($P < 0.01$ and $P < 0.001$) difference was noted when all doses of the three fractions were compared with dexamethasone in terms of granuloma inhibition (Table 4).

As the results of this model revealed, the AF and CF were comparably effective at all tested doses in inhibiting cotton pellet induced exudates formation, whereas the CF was the most active fraction in inhibiting the formation of granuloma mass, as evidenced by the higher percentage of granuloma inhibition as compared to the respective doses of the AF and BF.

Phytochemical screening

Preliminary phytochemical screening for secondary metabolites was carried out on the crude 70EE, and solvent fractions of *Z. scabra*. The result revealed a differential distribution of secondary metabolites in the solvent fractions, as shown in Table 5.

Discussion

Zehneria scabra has been used in the folk medicine of Ethiopia for the management of different inflammatory pathologies. Its use in various inflammatory conditions is recorded in ethnobotanical studies of Ethiopia with a high fidelity rate; e.g., FL= 100% for febrile conditions by the ethnic groups of Gondar Zuria district (Birhanu 2013); 86% for 'mich' by people in Zegie Peninsula (Teklehaymanot and Giday 2007); 95% for febrile conditions by people of Ankober District, North Shewa Zone (Lulekal et al. 2013) and people of Bahirdar Zuria district (Ragunathan and Abay 2009).

Table 1. Effects of the solvent fractions of *Zehneria scabra* on carrageenan-induced mouse paw edema

Treatment group	Mean increase in paw Volume $\pm$ S.E.M and [% Inhibition (%A)]				
	1hr	2hr	3hr	4hr	5hr
Distilled H ₂ O	0.490 $\pm$ 0.037	0.560 $\pm$ 0.020	0.598 $\pm$ 0.028	0.5167 $\pm$ 0.030	0.4350 $\pm$ 0.024
2% Tween 80	0.537 $\pm$ 0.025	0.563 $\pm$ 0.027	0.610 $\pm$ 0.025	0.543 $\pm$ 0.023	0.475 $\pm$ 0.026
ASA200mg/kg	0.343 $\pm$ 0.025 ^{a2} [29.94]	0.355 $\pm$ 0.026 ^{a3} [36.61]	0.388 $\pm$ 0.033 ^{a3} [35.10]	0.282 $\pm$ 0.023 ^{a3} [45.48]	0.180 $\pm$ 0.023 ^{a3} [58.62]
AF 100mg/kg	0.345 $\pm$ 0.023 ^{a2} [29.59]	0.338 $\pm$ 0.022 ^{a3} [39.59]	0.378 $\pm$ 0.022 ^{a3} [36.77]	0.303 $\pm$ 0.024 ^{a3d1e1} [41.3]	0.213 $\pm$ 0.020 ^{a3d1e2} [50.97]
AF 200mg/kg	0.318 $\pm$ 0.021 ^{a2} [35.04]	0.277 $\pm$ 0.027 ^{a3} [50.59]	0.288 $\pm$ 0.028 ^{a3} [51.81]	0.185 $\pm$ 0.024 ^{a3} [64.2]	0.122 $\pm$ 0.012 ^{a3} [72.02]
AF 400mg/kg	0.317 $\pm$ 0.024 ^{a2} [35.37]	0.272 $\pm$ 0.015 ^{a3} [51.48]	0.267 $\pm$ 0.034 ^{a3b1} [55.42]	0.176 $\pm$ 0.026 ^{a3b1} [65.8]	0.103 $\pm$ 0.014 ^{a3} [76.25]
BF 100mg/kg	0.428 $\pm$ 0.023 [12.59]	0.465 $\pm$ 0.018 ^{a1b1e1} [16.96]	0.493 $\pm$ 0.022 ^{b1e2} [17.55]	0.433 $\pm$ 0.023 ^{b2e2} [16.14]	0.348 $\pm$ 0.026 ^{b3d1e2} [19.93]
BF 200mg/kg	0.377 $\pm$ 0.022 ^{a1} [23.12]	0.403 $\pm$ 0.030 ^{a3} [27.98]	0.425 $\pm$ 0.028 ^{a2} [28.97]	0.333 $\pm$ 0.035 ^{a3} [35.49]	0.247 $\pm$ 0.030 ^{a3} [43.29]
BF 400mg/kg	0.350 $\pm$ 0.018 ^{a2} [28.57]	0.357 $\pm$ 0.015 ^{a3} [36.30]	0.363 $\pm$ 0.011 ^{a3} [39.28]	0.272 $\pm$ 0.016 ^{a3} [47.42]	0.212 $\pm$ 0.013 ^{a3} [51.33]
CF 100mg/kg	0.495 $\pm$ 0.023 ^{b2} [7.77]	0.518 $\pm$ 0.026 ^{b2} [7.99]	0.567 $\pm$ 0.029 ^{b2} [7.10]	0.490 $\pm$ 0.028 ^{b3} [9.81]	0.415 $\pm$ 0.024 ^{b3e1} [12.63]
CF 200mg/kg	0.480 $\pm$ 0.031 ^{b1} [10.60]	0.512 $\pm$ 0.038 ^{b2} [9.16]	0.537 $\pm$ 0.044 ^{b1} [12.02]	0.427 $\pm$ 0.025 ^{f1b2} [21.50]	0.343 $\pm$ 0.025 ^{f2b2} [27.73]
CF 400mg/kg	0.482 $\pm$ 0.031 ^{b1} [10.20]	0.500 $\pm$ 0.022 ^{b1} [11.20]	0.488 $\pm$ 0.025 [19.95]	0.403 $\pm$ 0.036 ^{f2b1} [25.80]	0.288 $\pm$ 0.030 ^{f3b1} [39.31]

Note: Values are expressed as Mean $\pm$ S.E.M.; n = 6; Values in parenthesis shows % inhibition of paw edema; ^a compared with distilled H₂O, ^b compared with ASA 200mg/kg, ^d compared with 200 mg/kg of respective fraction, ^e compared with 400 mg/kg of respective fraction, ^f compared with 2% tween 80; ¹*p*<0.05, ²*p*<0.01, ³*p*<0.001; AF: aqueous fraction; BF: butanol fraction; CF: chloroform fraction.

Table 2. Effects of crude 70EE of *Zehneria scabra* on formaldehyde-induced arthritis in mice

Treatment group	Mean increase in paw volume (mL) $\pm$ S.E.M and [% Inhibition (%A)]				
	Day 1	Day 2	Day 3	Day 4	Day 5
Distilled H ₂ O	0.450 $\pm$ 0.029	0.533 $\pm$ 0.023	0.645 $\pm$ 0.024	0.878 $\pm$ 0.025	0.887 $\pm$ 0.028
ASA 200mg/kg	0.347 $\pm$ 0.029 ^{a1} [22.89]	0.295 $\pm$ 0.031 ^{a3} [44.65]	0.238 $\pm$ 0.025 ^{a3} [63.10]	0.597 $\pm$ 0.037 ^{a3} [32.00]	0.550 $\pm$ 0.028 ^{a3} [37.99]
70EE 100mg/kg	0.367 $\pm$ 0.024 [18.44]	0.436 $\pm$ 0.027 ^{b2} [18.20]	0.480 $\pm$ 0.024 ^{a2b3d1e2} [25.58]	0.767 $\pm$ 0.029 ^{b2e2} [12.64]	0.748 $\pm$ 0.020 ^{a1b3e2} [15.67]
70EE 200mg/kg	0.353 $\pm$ 0.010 [21.56]	0.398 $\pm$ 0.016 ^{a1} [25.33]	0.368 $\pm$ 0.025 ^{a3b1} [42.95]	0.666 $\pm$ 0.031 ^{a3} [24.15]	0.656 $\pm$ 0.030 ^{a3} [26.04]
70EE 400mg/kg	0.332 $\pm$ 0.026 ^{a1} [26.22]	0.363 $\pm$ 0.033 ^{a2} [31.89]	0.307 $\pm$ 0.031 ^{a3} [52.40]	0.607 $\pm$ 0.023 ^{a3} [30.87]	0.578 $\pm$ 0.032 ^{a3} [34.84]
	Day 6	Day 7	Day 8	Day 9	Day 10
Distilled H ₂ O	0.808 $\pm$ 0.031	0.693 $\pm$ 0.031	0.642 $\pm$ 0.033	0.566 $\pm$ 0.035	0.477 $\pm$ 0.034
ASA 200mg/kg	0.512 $\pm$ 0.025 ^{a3} [36.63]	0.452 $\pm$ 0.026 ^{a3} [34.85]	0.400 $\pm$ 0.025 ^{a3} [37.69]	0.310 $\pm$ 0.029 ^{a3} [45.23]	0.232 $\pm$ 0.028 ^{a3} [51.40]
70EE 100mg/kg	0.713 $\pm$ 0.030 ^{b3e3} [11.76]	0.610 $\pm$ 0.031 ^{b2e3} [12.02]	0.502 $\pm$ 0.030 ^{a2e3} [21.81]	0.455 $\pm$ 0.026 ^{a1b2e3} [19.61]	0.382 $\pm$ 0.015 ^{a1b2e3} [19.92]
70EE 200mg/kg	0.607 $\pm$ 0.018 ^{a3e1} [24.88]	0.518 $\pm$ 0.014 ^{a2e1} [25.24]	0.460 $\pm$ 0.016 ^{a3e2} [28.35]	0.383 $\pm$ 0.009 ^{a3e1} [32.33]	0.308 $\pm$ 0.016 ^{a3e1} [35.33]
70EE 400mg/kg	0.487 $\pm$ 0.033 ^{a3} [39.73]	0.407 $\pm$ 0.027 ^{a3} [41.34]	0.322 $\pm$ 0.018 ^{a3} [49.84]	0.257 $\pm$ 0.017 ^{a3} [54.59]	0.188 $\pm$ 0.019 ^{a3} [60.50]

Note: Values are expressed as Mean $\pm$ SEM; n = 6; Values in parenthesis shows % inhibition of paw edema (%A); ^a compared with distilled H₂O, ^b compared with 200mg/kg ASA, ^d compared

Table 3. Effects of the solvent fractions of *Zehneria scabra* on formaldehyde-induced arthritis in mice

Treatment group	Mean increase in paw volume (mL) $\pm$ S.E.M and [% Inhibition (%A)]				
	Day 1	Day 2	Day 3	Day 4	Day 5
Distilled H ₂ O	0.482 $\pm$ 0.026	0.558 $\pm$ 0.023	0.627 $\pm$ 0.025	0.832 $\pm$ 0.017	0.855 $\pm$ 0.015
2% Tween	0.515 $\pm$ 0.031	0.615 $\pm$ 0.030	0.640 $\pm$ 0.019	0.812 $\pm$ 0.024	0.827 $\pm$ 0.021
ASA200mg/kg	0.363 $\pm$ 0.027 [24.58]	0.308 $\pm$ 0.032 ^{a3} [44.78]	0.267 $\pm$ 0.023 ^{a3} [57.44]	0.643 $\pm$ 0.025 ^{a2} [22.65]	0.563 $\pm$ 0.026 ^{a3} [34.12]
AF100mg/kg	0.383 $\pm$ 0.029 [20.43]	0.262 $\pm$ 0.028 ^{a3} [53.13]	0.192 $\pm$ 0.031 ^{a3} [69.41]	0.600 $\pm$ 0.045 ^{a3e2} [27.86]	0.580 $\pm$ 0.047 ^{a3d2e3} [32.16]
AF200mg/kg	0.408 $\pm$ 0.045 [15.24]	0.258 $\pm$ 0.026 ^{a3} [53.73]	0.153 $\pm$ 0.025 ^{a3b1} [75.54]	0.492 $\pm$ 0.026 ^{a3b1} [40.88]	0.402 $\pm$ 0.046 ^{a3b1} [53.02]
AF400mg/kg	0.393 $\pm$ 0.025 [18.35]	0.235 $\pm$ 0.031 ^{a3} [57.91]	0.138 $\pm$ 0.022 ^{a3b1} [77.93]	0.447 $\pm$ 0.027 ^{a3b2} [46.29]	0.333 $\pm$ 0.026 ^{a3b2} [61.02]
BF100mg/kg	0.425 $\pm$ 0.031 [11.77]	0.372 $\pm$ 0.034 ^{a2} [33.42]	0.323 $\pm$ 0.037 ^{a3} [48.41]	0.715 $\pm$ 0.038 ^{e1} [14.03]	0.768 $\pm$ 0.043 ^{b2d1e3} [10.14]
BF200mg/kg	0.400 $\pm$ 0.030 [16.96]	0.345 $\pm$ 0.029 ^{a3} [38.21]	0.280 $\pm$ 0.025 ^{a3} [55.32]	0.662 $\pm$ 0.026 ^{a2} [20.44]	0.617 $\pm$ 0.026 ^{a3e1} [27.87]
BF400mg/kg	0.387 $\pm$ 0.030 [19.72]	0.288 $\pm$ 0.028 ^{a3} [48.36]	0.222 $\pm$ 0.026 ^{a3} [64.62]	0.578 $\pm$ 0.034 ^{a3} [30.47]	0.492 $\pm$ 0.033 ^{a3} [42.49]
CF100mg/kg	0.455 $\pm$ 0.016 [11.65]	0.525 $\pm$ 0.023 ^{b3} [14.63]	0.497 $\pm$ 0.026 ^{f2b3} [22.39]	0.737 $\pm$ 0.022 [9.24]	0.720 $\pm$ 0.030 ^{b2e2} [12.91]
CF200mg/kg	0.445 $\pm$ 0.032 [13.59]	0.522 $\pm$ 0.030 ^{b3} [15.17]	0.472 $\pm$ 0.031 ^{f2b3} [26.3]	0.695 $\pm$ 0.029 ^{f1} [14.38]	0.672 $\pm$ 0.029 ^{f2} [18.75]
CF400mg/kg	0.423 $\pm$ 0.023 [17.81]	0.468 $\pm$ 0.029 ^{f1b2} [23.85]	0.388 $\pm$ 0.034 ^{f3b1} [39.33]	0.627 $\pm$ 0.037 ^{f2} [22.79]	0.567 $\pm$ 0.033 ^{f3} [31.45]
	Day 6	Day 7	Day 8	Day 9	Day 10
Distilled H ₂ O	0.797 $\pm$ 0.022	0.715 $\pm$ 0.027	0.610 $\pm$ 0.039	0.522 $\pm$ 0.034	0.450 $\pm$ 0.035
2% Tween	0.758 $\pm$ 0.020	0.698 $\pm$ 0.016	0.600 $\pm$ 0.017	0.553 $\pm$ 0.023	0.492 $\pm$ 0.031
ASA200mg/kg	0.498 $\pm$ 0.026 ^{a3} [37.45]	0.442 $\pm$ 0.022 ^{a3} [38.22]	0.370 $\pm$ 0.019 ^{a3} [39.34]	0.327 $\pm$ 0.027 ^{a3} [37.38]	0.262 $\pm$ 0.024 ^{a3} [41.84]
AF100mg/kg	0.505 $\pm$ 0.048 ^{a3d2e3} [36.61]	0.423 $\pm$ 0.049 ^{a3d2e3} [40.80]	0.353 $\pm$ 0.039 ^{a3d2e3} [42.08]	0.273 $\pm$ 0.036 ^{a3d2e2} [47.61]	0.201 $\pm$ 0.036 ^{a3d1e2} [55.18]
AF200mg/kg	0.315 $\pm$ 0.037 ^{a3b2} [60.46]	0.227 $\pm$ 0.032 ^{a3b2} [68.29]	0.172 $\pm$ 0.027 ^{a3b2} [71.85]	0.122 $\pm$ 0.020 ^{a3b3} [76.67]	0.078 $\pm$ 0.015 ^{a3b3} [82.60]
AF400mg/kg	0.243 $\pm$ 0.027 ^{a3b3} [69.46]	0.185 $\pm$ 0.017 ^{a3b3} [74.13]	0.132 $\pm$ 0.013 ^{a3b3} [78.41]	0.097 $\pm$ 0.011 ^{a3b3} [81.46]	0.052 $\pm$ 0.010 ^{a3b3} [88.51]
BF100mg/kg	0.675 $\pm$ 0.047 ^{b2e3} [15.28]	0.590 $\pm$ 0.041 ^{b1d1e3} [17.48]	0.518 $\pm$ 0.041 ^{b1d1e3} [15.03]	0.427 $\pm$ 0.037 ^{e2} [18.21]	0.347 $\pm$ 0.037 ^{e2} [22.96]
BF200mg/kg	0.540 $\pm$ 0.028 ^{a3} [32.22]	0.457 $\pm$ 0.029 ^{a3e1} [36.13]	0.373 $\pm$ 0.033 ^{a3} [38.8]	0.316 $\pm$ 0.038 ^{a2} [39.29]	0.262 $\pm$ 0.030 ^{a2} [41.84]
BF400mg/kg	0.408 $\pm$ 0.036 ^{a3} [48.75]	0.330 $\pm$ 0.028 ^{a3} [53.85]	0.265 $\pm$ 0.025 ^{a3} [56.56]	0.220 $\pm$ 0.022 ^{a3} [57.83]	0.168 $\pm$ 0.017 ^{a3} [62.6]
CF100mg/kg	0.657 $\pm$ 0.031 ^{b2e2} [13.40]	0.592 $\pm$ 0.038 ^{b2e2} [15.27]	0.483 $\pm$ 0.037 ^{f1b1e3} [19.45]	0.422 $\pm$ 0.034 ^{f1e3} [23.78]	0.346 $\pm$ 0.031 ^{f2e3} [29.49]
CF200mg/kg	0.597 $\pm$ 0.035 ^{f2e1} [21.31]	0.503 $\pm$ 0.034 ^{f3} [27.92]	0.420 $\pm$ 0.034 ^{f2e1} [30.00]	0.368 $\pm$ 0.035 ^{f2e1} [33.44]	0.302 $\pm$ 0.027 ^{f3e2} [38.64]
CF400mg/kg	0.473 $\pm$ 0.028 ^{f3} [37.58]	0.406 $\pm$ 0.026 ^{f3} [41.76]	0.286 $\pm$ 0.020 ^{f3} [52.22]	0.227 $\pm$ 0.021 ^{f3} [59.03]	0.150 $\pm$ 0.015 ^{f3b1} [69.49]

Note: Values are expressed as Mean $\pm$ SEM; n = 6; Values in parenthesis shows % inhibition of paw edema; ^a compared with distilled H₂O, ^b compared with ASA 200mg/kg, ^d compared with 200 mg/kg of respective fraction, ^e compared with 400 mg/kg of respective fraction, ^f compared with 2% Tween 80; ¹p<0.05, ²p<0.01, ³p<0.001; AF: aqueous fraction; BF: butanol fraction; CF: chloroform fraction

Table 4. Effects of solvent fractions of *Zehneria scabra* on cotton pellet induced granuloma in rats

Treatment group	Mean weight of exudates (mg) $\pm$ S.E.M and [% inhibition]	Mean weight of granuloma (mg) $\pm$ S.E.M and [% inhibition]
Distilled H ₂ O	159.28 $\pm$ 1.93	32.61 $\pm$ 0.76
2% Tween	152.72 $\pm$ 2.08	34.2833 $\pm$ 0.44
Dexa 0.5mg/kg	87.50 $\pm$ 2.05 ^{a3} [45.07]	11.0917 $\pm$ 0.44 ^{a3} [65.99]
AF 100mg/kg	122.02 $\pm$ 2.37 ^{a3b3d3e3} [23.39]	28.9333 $\pm$ 0.83 ^{a2b3e3} [11.27]
AF 200mg/kg	99.81 $\pm$ 1.91 ^{a3b2e1} [37.34]	26.3833 $\pm$ 0.66 ^{a3b3e3} [19.09]
AF 400mg/kg	90.63 $\pm$ 2.34 ^{a3} [43.10]	21.3250 $\pm$ 0.50 ^{a3b3} [34.61]
BF 100mg/kg	152.23 $\pm$ 1.77 ^{b3e2} [4.43]	30.6750 $\pm$ 1.09 ^{b3} [5.93]
BF 200mg/kg	150.82 $\pm$ 1.58 ^{a1b3e1} [5.31]	29.6917 $\pm$ 0.86 ^{b3} [8.95]
BF 400mg/kg	142.21 $\pm$ 1.29 ^{a3b3} [10.72]	27.7483 $\pm$ 0.65 ^{a2b3} [14.91]
CF 100mg/kg	109.07 $\pm$ 3.09 ^{f3b3d2e3} [28.58]	21.01 $\pm$ 0.84 ^{f3b3e3} [38.72]
CF 200mg/kg	97.40 $\pm$ 2.38 ^{f3b1} [36.22]	18.85 $\pm$ 0.59 ^{f3b3e2} [45.02]
CF 400mg/kg	88.61 $\pm$ 0.95 ^{f3} [41.98]	15.25 $\pm$ 0.66 ^{f3b2} [55.52]

Note: Values are expressed as Mean $\pm$ S.E.M; n = 6; Values in parenthesis shows % inhibition (%A); ^a compared with distilled H₂O, ^b compared with dexa 0.5 mg/kg, ^d compared with 200 mg/kg of respective fraction, ^e compared with 400 mg/kg of respective fraction, ^f compared with 2% Tween 80; ¹p<0.05, ²p<0.01, ³p<0.001; AF: aqueous fraction; BF: butanol fraction; CF: chloroform fraction; Dexa: dexamethasone.

Table 5. Preliminary phytochemical screening of 70EE and solvent fractions of *Zehneria scabra*

Secondary metabolites	Crude 70EE	AF	BF	CF	Distilled water	2% Tween 80
Alkaloids	+	++	+	-	-	-
Tannins	+	+	++	-	-	-
Saponins	+	++	-	-	-	-
Terpenoids	+	++	+	-	-	-
Steroids	+	-	-	++	-	-
Flavonoids	+	-	-	++	-	-
Anthraquinones	-	-	-	-	-	-
Cardiac glycosides	++	+	+	-	-	-

Note: (+, Present); (-, Absent); (++, relatively concentrated as compared to the crude extract)

In line with this notion, Akele (2012) has elucidated the anti-nociceptive and acute anti-inflammatory activity of the hydro-alcoholic crude leave extract of *Z. scabra* using the acetic acid-induced writhing test and carrageenin-induced edema model, respectively. Despite this, the literature recorded no reports about the anti-inflammatory activity of the crude extract and further fractions in experimental models of sub-acute and chronic inflammation. Thus, the present study is the first one to demonstrate the anti-inflammatory activity of 70% ethanol leave extract and solvent fractions of *Z. scabra* using a battery of pharmacological inflammatory models ranging from acute to chronic ones.

The inflammatory response is a polyphasic tissue reaction, ranging from a temporary increase in vascular permeability to a prolonged cellular infiltration and proliferation. So, it is essential to evaluate the performance of agents claimed for anti-inflammatory effects via a battery of tests valid for various phases of inflammation (Kumar et al. 2012).

The carrageenan-induced paw edema model is a prototype of the exudative phase of acute inflammation

(Divakar et al., 2010; Sarkar, 2015). As a phlogistic agent, Carrageenan is not antigenic and is devoid of apparent systemic effect (Igbe and Inarumen 2013). Hence, carrageenan injection induces localized inflammation in two different phases through the subsequent release of several mediators. The initial period, which occurs between 0 and 2.5 h after carrageenan injection, has been attributed to the action of mediators such as histamine, serotonin, and bradykinin on vascular permeability. Histamine and serotonin are mainly released during the first 1.5 h, while bradykinin is released from 1.5 to 2.5 h after carrageenan injection (Masresha et al. 2012).

The second phase (2.5 - 6 h post-carrageenan injection) is a result of the overproduction of COX-2 and its pro-inflammatory PG products, with infiltration of polymorphonuclear leukocytes (neutrophils) (Dawson et al. 1991; Coura et al. 2015). The peak inflammation is usually seen approximately 2 - 3 h post carrageenan injection and is attributed to PG release (Kumar et al., 2012; Silva-Neto et al., 2014). Hence, this second phase appears to be the most exciting in terms of inflammatory processes. The carrageenan-induced paw edema is known to be sensitive to COX inhibitors but not to 5- LOX inhibitors and hence has been used to evaluate the effect of NSAIDs, which primarily inhibit the COX pathway, i.e., single-action NSAIDs such as aspirin. It has been demonstrated that the suppression of carrageenin-induced hind paw edema after the 3rd h correlates reasonably with therapeutic doses of most clinically useful anti-inflammatory agents (Panthong et al., 2007; Mathew et al., 2014). Thence, in this study, carrageenan-induced hind paw edema was used as an appropriate acute inflammation model, and aspirin has opted as a reference drug.

The formaldehyde-induced arthritis model, on the other hand, represents a sub-acute phase characterized by increased migration of leukocytes and phagocytes to the area of inflammation (Divakar et al., 2010). Hence, the inhibition of formaldehyde-induced edema is one of the

most suitable methods for evaluating the antiproliferative activity and screening anti-arthritic agents. Injection of formaldehyde into mice's hind paws produces localized inflammation and pain, which is biphasic. During the first neurogenic phase (0-5 min), pain is initiated due to the direct chemical stimulation of nociceptors. It is thought to be mediated by substance-P and bradykinin (Kaithwas et al., 2012; Sanusi et al., 2013). The second phase (15 min post-induction) appears to be an inflammatory phase during which histamine, serotonin, prostaglandin, and bradykinin become key mediators and thus could be inhibited by peripherally acting anti-inflammatory drugs such as aspirin. Leukocyte migration into the inflamed site will commence late in this phase (2.5 - 6 h) and is considered the most crucial inflammatory response process (Silva-Neto et al., 2014; Cui et al., 2014). In line with this notion, on the 1st day of induction, paw edema was determined three h following formaldehyde injection.

The cotton pellet-induced granuloma model is one of the most commonly employed models in animal research to screen for the chronic anti-inflammatory activity of drugs and novel natural products (Roome et al., 2014). In this model, the transudative phase causes an increase in the wet weight of the cotton pellet while hosting an inflammatory response to the implanted cotton between days 3 – 6, causing granuloma formation due to the proliferation of fibroblasts and infiltration of modified macrophages and the lymphocytes. Hence, the increase in dry weight is considered a measure of chronic inflammation's proliferative component (Bagad et al., 2013). Therefore, this model was used to verify further the anti-inflammatory activity of solvent fractions of *Z. scabra* on the transudative and proliferative components of chronic inflammation. Steroidal anti-inflammatory drugs demonstrated higher activity in this model (Andrade et al. 2007), and hence dexamethasone was opted as a reference drug.

The anti-inflammatory activity of hydroalcoholic crude leaf extracts of *Z. scabra*, in carrageenan-induced paw edema was reported by Akele (2012). In the present study, the anti-inflammatory potential of 70% ethanol crude leaves extract of *Z. scabra* was first tested against formaldehyde-induced arthritis, which is one of the most suitable test procedures to screen for the anti-arthritic and sub-acute anti-inflammatory activity of natural products as it closely resembles human arthritis (Deshpande et al. 2011). In this model, the crude 70EE at 200 and 400mg/kg doses demonstrated statistically significant inhibition of paw edema from day two to day 10 of treatment compared to the negative control (Table 2). In addition, 400mg/kg of 70EE showed a comparable anti-inflammatory effect (%A) with 200mg/kg of aspirin. This could be due to certain alterations in the inflammatory response with possible anti-arthritic potential comparable with the standard drug aspirin.

Furthermore, the result was also in agreement with the anti-inflammatory effects of the hydroalcoholic crude extract on carrageenan-induced acute inflammation, as reported by Akele (2012), and expands the evidence of its anti-arthritic and anti-proliferative efficacy on the sub-acute model of inflammation. The result obtained for the

70EE in formaldehyde induced arthritis model was also in line with those reported elsewhere (Umukoro and Ashorobi 2006; Deshpande et al. 2011; Reddy et al. 2015), where extracts of *Momordica charantia*, *Coccinia grandis*, and *Momordica cymbalaria* showed statistically significant inhibition of formaldehyde induced paw edema, signifying the potential anti-arthritis and anti-proliferative activity of plants in the Cucurbitaceae family.

Besides, for further evaluation of the nature of the active constituents and to state the possible mechanism(s) of the anti-inflammatory activity of the plant, the 70EE was successively fractionated by partitioning into solvents of differing polarity, and the anti-inflammatory activity of the fractions was evaluated by using acute, sub-acute and chronic models of inflammation.

The aqueous fraction (AF), at all tested doses, showed statistically significant inhibition of inflammatory parameters in all three employed models of inflammation. In the acute model (carrageenan-induced paw edema), all employed doses of the AF showed a significant ($p < 0.01$ and $p < 0.001$) inhibition of paw edema starting from 1h post-induction, and the effect was maintained till the 5th h (Table 1). The significant anti-inflammatory activity of this fraction during the first 2 hours of the initial phase of inflammation could be due to the inhibitory effect on mediators like histamine and 5-HT. This is further evidenced by the higher inhibitory effect of all doses of the AF compared to the particular time the inhibitory effect of aspirin, which like most other NSAIDs, has less effect on the first phase of carrageenan-induced inflammation. No significant increment in percent inhibition values of all tested doses of the AF was noted from the 2nd to the 3rd h. As noted above, this is the period where the release of the mediator bradykinin reaches its peak. Hence, it can be generalized that the AF may not have a significant inhibitory effect on the release or activity of bradykinin.

Furthermore, all tested doses of the AF showed more pronounced edema inhibition in the second phase of inflammation on the 4th and 5th h, compared to their respective inhibitory values in the first phase (0 - 2.5 h), achieving the maximum anti-inflammatory effect on the 5th h. This indicates that the primary mechanism of the anti-inflammatory effect of the AF could be via inhibition of COX and its pro-inflammatory metabolites, such as PGs. This is substantiated by the fact that even the lower employed dose of the AF (100mg/kg) showed a comparable inhibitory effect with that of aspirin, which like most other NSAIDs, exert a more pronounced effect on the second phase (Panthong et al. 2007; Mathew et al. 2014). Moreover, the higher employed doses, 200 and 400mg/kg, of the AF even showed a better inhibitory effect than aspirin in this late phase of inflammation, which is primarily mediated through products of the inducible COX.

This finding is also in line with Akele's (2012) previous report. The crude hydroalcoholic extract of *Z. scabra* showed significant inhibition of carrageenan-induced paw edema in the second phase of inflammation, with the maximum effect observed at the 3rd h. In comparison with Akele's (2012) report on the crude extract, it could be concluded that the phytochemicals responsible for the

pronounced anti-inflammatory effect on the second phase of acute inflammation could be highly concentrated in the AF. This could be explained since the equivalent doses of the crude extract showed a better percent inhibition value in the first phase (~ up to the 3rd h) than treatments of the AF at a given point of time; whereas from the 3rd to 5th h of the second phase, all tested doses of the AF showed a remarkably higher percent inhibition than the equivalent doses of the crude extract at the respective point of time. This could be possibly due to preferential partitioning of the secondary metabolites responsible for a better anti-inflammatory effect on the second phase, perhaps through COX inhibition, into the AF.

The results from the sub-acute and chronic models also revealed the anti-inflammatory effects of this fraction. For example, in the cotton pellet-induced granuloma model, all tested doses of the AF showed statistically significant inhibition of both exudates and granuloma formation. In this model, the vital ($P < 0.001$) inhibitory effect of the AF on the formation of exudates (Table 5) substantiates the finding of the carrageenan-induced acute model, i.e., both findings solidify the effectiveness of the AF in inhibiting the exudative and transudative component of inflammation. On the other hand, the statistically significant ($P < 0.01$ and $P < 0.001$) inhibition of granuloma formation justifies the effectiveness of this fraction in inhibiting the proliferative phase of inflammation. This could also be ascertained from the findings of the formaldehyde-induced arthritic model, in which all tested doses of the AF significantly ($p < 0.001$) inhibited the development of arthritis from day 2 to 10 of treatment (Table 3). Moreover, the AF at all tested doses demonstrated a considerably better inhibitory effect on formaldehyde-induced arthritis as compared to equivalent doses of the crude 70EE (Table 2) from day two throughout to day 10 of treatment. This could be possibly due to preferential partitioning of the secondary metabolites responsible for a better anti-arthritic activity in the AF.

Phytochemical screening for secondary metabolites revealed the presence of alkaloids, saponins, tannins, and terpenoids in the aqueous residue. The anti-inflammatory activity of this fraction could emanate from the presence of these secondary metabolites whose protection against inflammation is also reported elsewhere; alkaloids (Küpeli et al. 2002; Souto et al. 2011), saponins (Navarro et al. 2001; Ahn et al. 2005; Chen et al. 2014), and terpenoids (Heras and Hortelano 2009; Bellik et al. 2012; Ku and Lin 2013).

The mechanism of the anti-inflammatory effect of these different secondary metabolites is also documented in the literature. Various terpenoids were reported to exert their anti-inflammatory influence by inhibiting PLA2 activity, inhibition of TNF- α production, inhibition of iNOS expression, inhibition of COX-2 expression, and inhibition of NF- κ B activation (Bellik et al. 2012). Saponins were also reported to exert an anti-inflammatory effect by inhibiting iNOS expression, COX-2 expression, and subsequent production of PGE2 (Ahn et al. 2005). The anti-inflammatory activity of different alkaloids was also reported to be mediated through inhibition of COX

expression and production of PGE2 (Fukuda et al. 1999; Kuo et al. 2004), inhibition of pro-inflammatory cytokines production (IL-1 β , IL-6, TNF- α) and inhibition of the expression of ICAM-1 and VCAM-1 adhesion molecules (Bellik et al. 2012).

From the results of the phytochemical screening, terpenoids were found to be highly concentrated in the AF, less in the BF, and none in the CF. Polar solvents such as ethanol and water led to the extraction of highly oxygenated polar triterpenes and triterpenoid glycosides. In contrast, non-polar solvents such as chloroform and petroleum ether extracted most other lipid-soluble terpenoids such as Sesquiterpene lactones, diterpenes, and sterols (Citoglu and Acikara 2012). From this fact and the findings of the phytochemical screening, highly oxygenated polar triterpenes or triterpenoid glycosides could be the significant terpenoids concentrated in the AF and responsible for their anti-inflammatory activity. This could further be substantiated by a lack of activity of the petroleum ether extract in the pilot study and the negative terpenoid test result of the CF for the lipid-soluble terpenoids.

Other plants in the Cucurbitaceae family such as *Cayaponia tayuya* (Escandell et al. 2007), *Cucurbita andreana* (Jayaprakasam et al. 2003), *Picrorhiza scrophulariaeflora* (Smit et al. 2000), *Wilbrandia ebracteata* (Peters et al. 1999), and *Citrullus lanatus* (Abdelwahab et al. 2011), were also reported to contain highly oxygenated triterpenoid molecules called cucurbitacins, which were proved to have potent anti-inflammatory and anti-proliferative activities (Wakimoto et al. 2008; Duangmano et al. 2012).

All in all, from the results of the acute (Table 1) and sub-acute (Table 3) models of inflammation and percentage inhibition values of exudate formation in the chronic model (Table 4), the AF was found to be the most active fraction in inhibiting the exudative and transudative component of inflammation. It was also found to exhibit significant anti-proliferative activity, as revealed by the results of the sub-acute model (Table 3) and percentage inhibition values of granuloma formation in the chronic granulomatous inflammatory model (Table 4).

Moreover, the probable mechanism of the anti-inflammatory effect of the phytochemicals concentrated in the AF could be explained by inhibiting mediators like histamine, serotonin, and products of COX, such as pro-inflammatory PGs. The latter mechanism, i.e., inhibition of COX, could be the primary mechanism of the anti-inflammatory effect of this fraction since it showed a more significant effect during the second phase of the acute model of inflammation. This is further substantiated by the type of secondary metabolites found in this fraction, most of which were proved to possess COX inhibitory activities.

The butanol fraction (BF), at doses of 200 and 400mg/kg, significantly inhibited mice paw edema in both acute (Table 1) and sub-acute (Table 3) models of inflammation. In the cotton pellet-induced granuloma model, on the other hand, only the highest tested dose (400mg/kg) showed statistically significant inhibition of both inflammatory exudates ($P < 0.001$) and granuloma

formation ($P < 0.01$). A phytochemical test (Table 5) of this fraction revealed the presence of tannins, alkaloids, and terpenoids, albeit the last two secondary metabolites with a relatively lower concentration than the AF.

The moderate anti-inflammatory effect of the BF in the acute and sub-acute model, compared to the AF, could be accounted for by the presence of alkaloids and terpenoids, which were the main constituents of the most active AF. The anti-inflammatory effect of the BF could also emanate from tannins, the primary concentrate. The anti-inflammatory effect of tannins was reported to be mediated through inhibition of leukocyte migration by their well-known astringent properties, which cause precipitation of cell membrane proteins and hence affect cellular movements, recruitment, and membrane permeability (Mota et al. 1985) and/or through inhibition of expression of pro-inflammatory cytokines and chemokines by blocking of transcription factors, NF- κ B and AP-1 (Erdélyi et al. 2005). These different mechanisms underlying tannins may at least partly be responsible for the moderate anti-inflammatory effect observed for higher doses of the BF in inhibition of edema (carrageenan and formaldehyde-induced edema models) and cellular proliferation in the chronic model.

Unlike the AF and BF, the higher doses of chloroform fraction (200 and 400mg/kg CF) showed a statistically significant anti-inflammatory effect only during the second phase (4th and 5th h) of carrageenan-induced paw edema (Table 1). Similarly, the anti-inflammatory effect of this fraction was delayed until the 3rd day of formaldehyde-induced arthritis. This could probably be due to the specific inhibitory effect of phytochemicals in the CF on the synthesis or outcome of pro-inflammatory PGs, which mainly mediate the late phase of carrageenan-induced paw edema.

In the cotton pellet-induced granuloma model, on the other hand, the CF at all employed doses showed a comparable inhibitory effect on exudate formation with the equivalent doses of the AF (Table 4). Moreover, in this model, the CF at all treatments was found to be the most active fraction of inhibition of granuloma formation, the maximum inhibitory effect (%A = 55.52, $p < 0.001$) being observed at a dose of 400mg/kg. From these findings, i.e., the delayed anti-inflammatory effect in the acute and sub-acute model and its profound inhibitory effect on granuloma formation in the chronic model, it can be concluded that the CF's phytochemicals may be most effective in inhibiting pro-inflammatory prostanoids and cytokines induced cellular proliferation.

The results of the phytochemical screening revealed the preferential partitioning of steroids and flavonoids into the CF. Different kinds of literature support the anti-proliferative efficacy of these phytochemicals; Loizou et al. (2009) and Bigoniya et al. (2013), for example, showed phytosterols to inhibit TNF- α induced endothelial activation and expression of ICAM-1 and VCAM-1 adhesion molecules which mediate cellular recruitment to sites of inflammation. Hernández-Valle et al. (2014), Han et al. (2015), and Wagle et al. (2016), on the other hand, showed phytosterols to inhibit the inflammatory cytokines

IL-6, IL-1 β , TNF- α , and on the contrary induce the production of anti-inflammatory cytokines IL-4 and IL-10.

On the other hand, Flavonoids were reported to exhibit anti-inflammatory and anti-proliferative effects through selective inhibition of 5-LOX (Schewe et al. 2002; Redrejo-Rodriguez et al. 2004), COX-2, and/or iNOS (Tunon et al. 2009). Aquila et al. (2009) further reported the anti-inflammatory effect of flavonoids from *Cayaponia tayuya* (Cucurbitaceae) to be mediated through inhibition of COX-2 and iNOS induction. The possibility of selective COX-2 inhibition by flavonoids in the CF is further evidenced by the delayed anti-inflammatory effect shown in the second phase of the acute model.

The major products of the 5-LOX pathway, LTB₄, and CysLTs, in concert with the adhesion molecules ICAM-1 and VCAM-1, are known to mediate chemokinesis, firm adhesion, and subsequent extravasation of leucocytes to sites of inflammation (Werz et al. 2002; Pelletier et al. 2003; Weber et al. 2007; Afonso et al. 2012). On the other hand, products of the COX pathway, especially PGE₂ and PGI₂, are well known for their potent vasodilatory actions and increasing vascular permeability and leukocyte infiltration (Pelletier et al., 2003; Smyth et al., 2009). Hence, the effectiveness of the CF in inhibiting the formation of inflammatory exudates and, more profoundly, granuloma in the chronic model could emanate from the possible inhibitory effect of the flavonoids and phytosterols on the COX and 5-LOX pathways and/or inhibition of inflammatory cytokines induced endothelial activation and expression of ICAM-1 and VCAM-1.

All in all, the results of this study revealed that the crude extract (70EE) and solvent fractions of *Z.scabra* leaves possess anti-inflammatory activities. The overall order of efficacy in inhibiting the exudative phase of acute inflammation, as evidenced by the percentage inhibition of paw edema in the critical model, was found to be AF > BF > CF. Hence the phytochemicals in the AF could be most effective in inhibiting the acute phase of inflammation. While on the contrary, the overall order of effectiveness in inhibiting the proliferative phase of chronic inflammation, as evidenced by the percentage of granuloma inhibition in the chronic model, was found to be CF > AF > BF. This indicates that the phytochemicals concentrated in the CF may be specifically useful in inhibiting the cellular response of the proliferative phase of inflammation.

Such disparity in order of effectiveness of the fractions in modulating the acute and chronic phases of inflammation could be due to the differential partitioning of phytochemicals into the three portions (Table 5) and the associated difference in the mechanism of action of the secondary metabolites. Furthermore, the greater efficacy of the AF in comparison with other fractions in inhibiting the acute phase of inflammation supports the traditional method of extraction of the leaves of *Z.scabra* in Ethiopia, where the leaves are boiled in water, and the vapor inhaled, or they leave juice is given orally for the treatment of inflammatory conditions (Teklehaymanot and Giday 2007; Ragunathan and Abay 2009; Birhanu 2013).

From the overall results of this study, it can be postulated that *Z. scabra* is rich in secondary anti-

inflammatory metabolites that act by different mechanisms to inhibit acute, sub-acute, and chronic inflammatory conditions. Moreover, the potent anti-inflammatory activity of the 70EE and solvent fractions of *Z. scabra* in this study as compared to the standard drug aspirin may be due to the cumulative effects of different active constituents in reducing the synthesis, release, and/or action of various inflammatory cytokines, chemokines and mediators such as histamine, serotonin, prostaglandins, and Leukotrienes. This notion is in line with the proven concept that medicinal plants possess a combination of phytoconstituents with different anti-inflammatory mechanisms, offering synergistic or additive effects (Liu 2003; Csaki et al. 2009).

The phytochemical screening in the present study revealed the presence of secondary metabolites such as alkaloids, tannins, saponins, terpenoids, steroids, flavonoids, and cardiac glycosides in the crude 70EE and their differential partitioning into employed solvents of differing polarity (Table 5). Tesfaye and Alamneh (2014) reported the absence of saponins in the crude 80% methanol extract of the leaves of *Z. scabra*. However, the test for other secondary metabolites was in line with the present study's findings. On the other hand, Tadesse et al. (2014) reported the presence of saponins, which is in agreement with the results of the present study, while Alkaloids and steroids were tested negative in 80% methanolic leaves extract of *Z. scabra*. Such discrepancy in the presence and/or absence of secondary metabolites could be accounted for by seasonal and geographical variations, which are known to influence the expression of phytochemical constituents in plants (Jayanthi et al., 2013).

Arulappan et al. (2015), conversely, showed the presence of phenolic compounds, steroids, and glycosides and the absence of tannins, flavonoids, alkaloids, and saponins in the ethanolic (absolute) and aqueous tuber extract of *Z. scabra*. Such disagreement with the present study results could be due to the differential distribution of the secondary metabolites into different parts of the plant, i.e., the tuber and leaves, on top of the geographic variation and the difference in the extraction solvents.

In this study, ethanol specifically was chosen as the solvent of extraction as it can extract a wide variety of polar and nonpolar phytochemical constituents in medicinal plants (Herman et al., 2013; Tatke and Rajan, 2014). Hydroalcoholic solvents (a mixture of alcohol and water in varying proportions) are generally considered to give high extraction yields, owing to their expanded polarity range (Gupta et al. 2012). It is also hypothesized that alcoholic solvents efficiently penetrate the cell membranes, permitting the extraction of high amounts of endocellular components, including phytochemicals produced in plants. Furthermore, ethanol is widely used to obtain crude extracts of phytochemicals in the herbal medicine industry for therapeutic applications due to its relative safety (Wendakoon et al., 2012). Hence, 70% v/v ethanol was the solvent of choice in the present study for extracting the leaves of *Z. scabra*.

Moreover, male mice and rats were preferably used in this study for all the anti-inflammatory models. The rationale for using the male sex in inflammation models is because estrogen, the primary female sex hormone, has been confirmed to possess anti-inflammatory activity by a line of evidence (Miyamoto et al. 1999; Cuzzocrea et al. 2000; Vegeto et al. 2002).

The results of phytochemical tests revealed that leaves of *Z. scabra* are chemically enriched with alkaloids, tannins, saponins, terpenoids, steroids, flavonoids, and cardiac glycosides. The results from the pharmacological tests further confirmed the aqueous fraction to be the most efficient fraction in inhibiting the exudative component of acute inflammation. In contrast, the chloroform fraction showed the highest activity in inhibiting the cellular response of the proliferative part of chronic inflammation. On the other hand, the butanol fraction was found to possess a moderate activity in both the acute (carrageenan-induced) and sub-acute (formalin-induced) paw edema models and was found to be the least active in the cotton pellet-induced chronic granuloma model.

In conclusion, the data obtained in this study demonstrated that the leaves of *Z. scabra* possess different secondary metabolites, which, by acting through an array of possibly different mechanisms, are useful in treating both acute and chronic inflammatory conditions. The current findings also demonstrated the scientific rationale for the traditional use of this plant in different inflammatory conditions. It also confirms the presence of biologically active components, which are worth further investigation and elucidation.

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