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Wound healing and antiinflammatory properties of *Allophylus abyssinicus* (Hochst.) Radlk

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Abstract

The leaves of *Allophylus abyssinicus* (Hochst.) Radlk. (Sapindaceae) are used for the treatment of wounds, burns, skin diseases and to arrest bleeding in the Ethiopian folk medicine. In this study, the hydroalcoholic extract and the different solvent fractions obtained from the leaves of *A. abyssinicus* were evaluated for their wound healing and antiinflammatory activities. Wound healing activity was studied using excision, incision and dead space wound models whilst carrageenan-induced mouse paw oedema model was used to evaluate antiinflammatory activity. The methanolic fraction levigated in simple ointment at concentrations of 5% and 10% was found to be the most active in the excision wound model. Also, the same fraction exhibited good healing effect in incision and dead space models in a dose dependant manner. At a dose of 200 mg/kg, all the test substances except the chloroform fraction exerted significant antiinflammatory effects when compared to the control, the methanolic fraction being the most active. The present study supports the folkloric use of the plant for the treatment of wounds and inflammatory conditions.

Keywords: *Allophylus abyssinicus*; hydroalcoholic extract; solvent fractions; wound healing; antiinflammatory

Introduction

Since time immemorial man has used various parts of plants in the treatment and prevention of many ailments (Chah *et al*., 2006). About 60% of the world population and 60-90% of the population of developing countries rely on traditional medicine for their primary health care (Kunwar and Bussmann, 2008). It has been reported that one-third of all traditional medicines in use are for the treatment of wounds and skin disorders, compared to only 1-3% of synthetic modern drugs (Mantle *et al*., 2001). Phytomedicines are not only cheap and affordable but also purportedly safe as hypersensitive reactions are rarely encountered. These natural agents induce healing by multiple mechanisms. However, there is a need for

scientific validation, standardization and safety evaluation of natural medicines before they could be recommended for use.

Several medicinal plants are used in the Ethiopian folkloric medicine for wound management. One such plant is *Allophylus abyssinicus* (Hochst.) Radlk. (Sapindaceae), a medium to large sized tree with grayish-green bark (Vollsen, 1989). Traditionally, the dried and powdered leaves of *A. abyssinicus* are either topically applied to treat wounds or taken orally to counteract different inflammatory conditions. The leaves are also used for the treatment of boils and sexually transmitted diseases. The ethnomedicinal uses of this plant have stimulated our interest to study the extracts of the leaves for potential application in wound care and inflammatory conditions.

Materials and Methods

Collection of plant material

The leaves of *Allophylus abyssinicus* were collected in December 2009 from the Science Faculty campus, Addis Ababa University, Addis Ababa. The authenticity of the plant material was confirmed by Ato Melaku Wondaferash, the National Herbarium, Department of Biology, Addis Ababa University, where voucher specimen was deposited (collection number AY0001).

Animals

Rabbits, Wistar rats weighing 125-175 g and Swiss Albino mice weighing 25-30 g were used for the experiments. The animals were procured from the Ethiopian Health and Nutrition Research Institute (EHNRI), Addis Ababa, Ethiopia. Animal quarters were maintained at a temperature of 22±2 °C with 12-h light/12-h dark cycle. All animal experiments were conducted in accordance with the internationally accepted laboratory animal use, care and guideline (ILAR, 1996) and approved by the Institutional Review Board of the School of Pharmacy, Addis Ababa University.

Extraction

Air-dried leaves of *A. abyssinicus* (100 g) were extracted using 80% (v/v) methanol by maceration. Maceration was carried out for 72 h with intermittent agitation. The extract was filtered (Whatman No. 3, Whatman Ltd. England) and the marc remacerated 3x for another 72 h and filtered. The combined filtrates were concentrated using Rota Vapor (Büchi Rota Vapor R-205, Switzerland) and dried in a vacuum oven and kept in a desiccator until used.

Preparation of solvent fractions

The dried hydroalcoholic extract was placed in a thimble and extracted exhaustively with chloroform, acetone and methanol sequentially in a Soxhlet apparatus. The final residue was dissolved in distilled water to prepare the aqueous fraction. The organic solvents were evaporated to dryness at a reduced pressure while the water fraction was lyophilized.

Formulation of test substances

The hydroalcoholic and the different solvent fractions were formulated using simple ointment base and hydroxylpropyl methylcellulose (HPMC) gel at 5% and 10% concentrations. Fusion method was employed in the preparation of medicated ointments. Levigation on the surface of ointment slab was carried out to make ointments and jells of uniform consistency and smooth texture. The smooth and uniform gels were packed in wide-mouthed ointment jars and stored in a refrigerator until used.

Acute toxicity test

A. Acute dermal toxicity test

Skin irritation test for the different extracts was conducted on rabbits by using occluded dermal irritation test (Robinson and Perkins, 2002). The skin of each rabbit was shaved at two different positions on the dorsal side, each with an area of about 1200 mm^2. The first position was kept as a control on which non-medicated ointment was applied, and test substances were applied on the second site (Gfeller *et al*., 1985). On day one of the test period the preparations were evenly applied on the shaven area of the animals' skin. Immediately after application it was covered by dressing gauze over which a plastic sheet (occlusive material) was placed, and the covering was loosely held in contact with the skin by means of a non-irritating adhesive tape (Teshome *et al*., 2008). After 24 h of exposure period, the elastic bandage, the adhesive plaster, the plastic sheet and the gauze were carefully removed so as not to damage the skin and the test site was rinsed with distilled water. The animals were examined for the presence of erythema and oedema according to Draize dermal irritation scoring system at grading intervals of 1, 24, 48 and 72 h (Draize, 1959). The degree of erythema and oedema was determined based on the scores given in Table 1.

Primary irritation index (PII) which is a parameter that indicates the potential of a given substance for skin irritation was also calculated for the different test substances by summing up all the erythema and oedema scores of all the 4 time intervals of grading (1, 24, 48, and 72 h) and dividing by the number of test sites (2) multiplied by the grading interval (4) (Teshome *et al*., 2008). According to Draize classification, substances scoring PII of < 2 are mildly irritant, 2-5 moderately irritant, and > 5 severely irritant.

B. Acute oral toxicity test

Swiss albino mice of either sex weighing 30-40 g were used for acute oral toxicity study. The study was conducted as per the protocol drawn under Organization for Economic

Table 1: Draize dermal irritation scoring system.

Erythema and eschar formation	Value	Oedema formation	Value
No erythema	0	No oedema	0
Very slight erythema	1	Very slight oedema	1
Well defined erythema	2	Slight oedema	2
Moderate to severe erythema	3	Moderate oedema	3

Cooperation and Development (OECD) guidelines 420 in Swiss albino mice starting at a dose 2000 mg/kg up to 5000 mg/kg of the extract (Karodi *et al.*, 2009). First, the animals were dosed with the extract and observed periodically for signs of acute toxicity within 48 h. Then, they were further observed for 14 days for signs of acute toxicity like diarrhoea, seizure, weight reduction etc.

Wound healing activity tests

A. Excision model

Each group containing six rats was anaesthetized by open mask method with anesthetic ether. Each animal was depilated at the back and one excision was inflicted by cutting out 500 mm^2 full thickness of skin of a predetermined area. The rats were left undressed to the open environment. Then, the positive control (0.2% w/v nitrofurazone ointment) or the negative control (simple ointment BP/HPMC gel) or the test samples were administered till the wound gets completely healed. This was followed by monitoring wound contraction and epithelization time. Epithelization time was noted as the number of days after wounding required for the scar to fall off leaving no raw wound behind. Wound contraction was calculated as percent reduction in wound area. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on a graph paper every other day. To determine the changes in healing of wound, measurements of wound area on graph paper were expressed in mm^2 (Saha *et al*., 1997).

B. Incision model

Six mice in each group were anaesthetized and a paravertebral-long incision was made through the skin and cutaneous muscles at a distance of about 1.5 cm from the midline of the depilated back of the mice. Then, the negative control [1% carboxymethyl cellulose (CMC)] or different concentrations of the test substances suspended in 1% CMC were orally administered once daily for 9 days. No ligature was used for stitching and full aseptic measures were not taken and no local or systemic antimicrobials were used throughout the experiment (Udupa *et al*., 1995). After the incision was made, the parted skin was kept together and stitched with black silk at 0.5 cm intervals; surgical thread (No. 000) and a curved needle (No. 11) were used for stitching. The wound was left undressed and the sutures removed on the 7^{th} day. On the tenth day the mice were again anaesthetized and each mouse was placed on the middle of a board towel. The clamps were then carefully attached to the skin on the opposite sides of the wound at a distance 0.5 cm away from the wound. The longer pieces of the fishing line were placed on a pulley and then on to a polyethylene bottle. The position of the board was adjusted so that the bottle received a rapid and constant rate of water from a large reservoir until the wound began to open. The amount of water in the polyethylene bag was weighed and considered as an indirect measure of the tensile strength of the wound. The tensile strength of the extract treated wounds was compared with controls. The tensile strength increment indicates better wound healing stimulation by the applied test substance. Tensile strength was calculated using the following formula given by Reddy *et al*. (2008):

$$\text{Tensile strength} = \frac{\text{Breaking strength}}{\text{Cross-sectional area of skin } (mm^2)}$$

C. Dead space model

Dead space wounds were inflicted by implanting sterile cotton pellets (5 mg) on one side of the groin on the ventral surface of each mouse as described by Neuman and Logan (1950). The animals were randomly divided into groups of six mice. The control group animals were provided with 1% CMC and the test group mice were given different concentrations of the test substances dispersed in 1% CMC orally. On the 10^{th} post wounding day, the granulation tissue formed on the implanted cotton pellets was carefully removed under anaesthesia. These tissue samples were dried at 60 ^{o}C for 12 h. The dried tissue was mixed with 5 ml 6N HCl and kept at 110 ^{o}C for 24 h. The neutralized acid hydrolysate of the dry tissue was used for the determination of hydroxyproline.

i. Estimation of hydroxyproline

Dry granulation tissue from both control and treated groups was used for estimation of hydroxyproline. Hydroxyproline present in the neutralized acid hydrolysate was oxidized by hydrogen peroxide in the presence of copper sulfate, and subsequently complexed with *p*-dimethylaminobenzaldehyde to develop a pink colour that was measured spectrophotometrically at 572 nm.

ii. Preparation of calibration curve

Standard L-hydroxyproline (0.05 g) was dissolved in water and diluted to about 400 ml. Concentrated HCl (20 ml) were added and the solution made up to 500 ml with water. Then, 100 µg/ml solution was diluted to give concentrations of 5, 10, 15, 25 and 50 µg/ml of hydroxyproline.

Nineteen test tubes were arranged sequentially. In each of test tubes 1-3, l ml of 5 µg/ml, test tubes 4-6, 1 ml of 10 µg/ml, test tubes 7-9, 1 ml of 15 µg/ml, test tubes 10-12, 1 ml of 25 µg/ml and test tubes 13-15, 1 ml of 50 µg/ml of hydroxyproline solution was added. To each of test tubes 16-18, 1 ml of the test solution and to test tube 19, 1 ml of water was added. After gentle mixing of the contents of each test tube with 1 ml of 0.05 M $CuSO_4$ and 1 ml of 2.5 N NaOH, the tubes were placed on a water bath at 40 °C for 3-5 min. This was followed by the addition of 1 ml of 6% hydrogen peroxide to each test tube; thorough mixing of the contents by swirling was carried out before addition is made to the next tube. The tubes were left on the water bath for 10 min with occasional swirling and then cooled with tap water. Finally, 4 ml of 3N H_2SO_4 and 2 ml of 5% *p*-dimethylaminobenzaldehyde soluteons were added with mixing and swirling after each addition. Caps were placed on the tubes, which are kept on a water bath at 70 °C for 16 min. The solutions were then cooled, mixed and their extinctions measured against the blank solution at a wavelength of 572 nm in 1 cm cell. The average reading for each set of tubes was used in the calculation. Standard absorbance versus concentration curve was drawn and based on the curve, hydroxyproline concentration of the unknown was determined.

In vivo antiinflammatory activity test

In vivo antiinflammatory activity was evaluated on the basis of inhibition of carrageenan-induced mice hind paw oedema as described by Dongmo *et al*. (2003). The extracts/

fractions, indomethacin and vehicle were administered orally to the experiment, reference and control groups, respectively. The oedema inducing agent, i.e. 0.1 ml of 1% carrageenan in normal saline was then injected into the plantar surface of the left hind paw 30 min after oral administration of the test substances. The volumes of injected paws were measured before, and 60, 120, 180 and 240 min after injection of carrageenan using Ugo Basile plethysmometer (Italy, model 7140). Each group was composed of six mice (three male and three female). The increase in paw volume, i.e. inflammation (%I) was calculated according to the equation given by Delporte *et al*. (1998):

$$\%I = \frac{Vf - Vi}{Vi} X\,100$$

Where Vf and Vi are the final and initial paw volumes of each animal, respectively. The mean %I was then calculated and a curve of mean %I versus time was plotted. In addition, the antiinflammatory effect (%A) was calculated according to the formula given below (Delporte *et al.*, 1998) and data were presented as mean ± standard error of the mean (SEM).

$$\%A = \frac{\%I_c - \%I_e}{\%I_c} X\,100$$

Where I_c and I_e are the mean inflammation values attained in control and experimental groups, respectively.

Statistical analysis

Results obtained have been expressed as mean ± SEM and were compared with the corresponding control group by applying analysis of variance (ANOVA) test followed by dunnett test (comparing all values vs. control). $P < 0.05$ was the probability level taken to determine statistical significance. Statistical analysis was done using Graph Pad Instat®.

Results and Discussion

Extraction

The dried leaves of *A. abyssinicus* were extracted by maceration using 80% methanol to obtain the total extract. Solvent fractions were prepared by successive Soxhlet extraction using chloroform, acetone, methanol and water.

Oral acute toxicity

No death or perception of adverse reactions was observed within the fourteen days follow up period. This is an indication that the extracts and the fractions may not be toxic at the doses employed in this study.

Skin irritation test

No irritation symptoms were developed over the test period. Neither erythema nor skin swelling were developed during a 72 h time period for all test substances. Hence, the PII

Table 2: Effect of topical application of ointments containing 80% methanolic extract of the leaves of *Allophylus abyssinicus* on contraction of excision wound.

Group	Day 0	Day 4	Day 8	Day 12	Day 16	Period of epithelization (days)
0.2% NF	510.67 ± 3.73	313.46 ±3.14 (38.62)	163.20 ±3.42 (68.04)***	63.94 ±1.30 (87.48)***	10.32 ±1.98 (97.98)***	15.33 ±0.61***
5% ME in SO	514.22 ±3.45	348.67 ±3.21 (32.19)	218.67 ±4.32 (57.48)	120.76 ± 2.71 (76.52)***	47.11 ±0.32 (90.84)***	20.97 ±0.76**
10% ME in SO	520.40 ±4.23	340.43 ±1.94 (34.58)	187.23 ±3.12 (64.02)**	72.11 ±1.69 (86.14)***	14.22 ±0.32 (97.27)***	16.68 ±0.43***
5% ME in HPMC	522.12 ±5.43	415.54 ±5.64 (20.41)	307.65 ±4.87 (41.08)	245.56 ±3.65 (52.97)	194.23 ±1.80 (62.80)*	22.76 ±0.62
10% ME in HPMC	505.34 ±4.54	385.09 ±4.29 (23.80)	267.43 ±3.07 (47.08)	162.98 ±3.07 (67.75)***	97.76 ±1.32 (80.65)***	21.76 ±0.71***
SO	516.50 ±2.34	374.76 ±2.08 (27.44)	304.72 ±2.40 (41.00)	260.47 ±1.75 (49.57)	208.00 ±2.45 (59.73)	24.78±0.49
HPMC	498.21 ±6.75	412.76 ±5.01 (17.15)	345.98 ±4.60 (30.56)	280.65 ±3.54 (43.67)	224.87 ±1.71 (54.86)	25.55±0.83

Values are expressed as mean ± S.E.M (n = 6), percentage of contraction are in parenthesis, *P < 0.05 vs. control, **P < 0.01 vs. control, ***P < 0.001 vs. control; NF = nitrofurazone, ME in SO = 80% methanolic extract in simple ointment, ME in HPMC = 80% methanolic extract in hydroxypropyl methyl cellulose, SO = simple ointment, HPMC = hydroxypropyl methyl cellulose.

was found to be zero. This indicates that all the test substances from the leaves of *A. abyssinicus* do not have irritant property.

Wound healing activity

Excision model

In excision wound healing model, the 80% methanolic extract was formulated using simple ointment and HPMC gel as a base. As presented in Table 2, the hydroalcoholic extract showed significant increase in percentage closure of excision wounds and enhanced epithelization. This effect was observed in a dose dependant manner. At 10% concentration, the 80% methanolic extract levigated in simple ointment showed comparable activity with that of the positive control whilst its activity was lower when formulated in HPMC gel. This could be due to the better release of the ingredients from the simple ointment than from HPMC gel, an indication that the components responsible for activity are polar.

The methanolic and aqueous fractions embedded in simple ointment base show significant wound healing activity in a dose dependant manner on excision wound healing model that was comparable with that of nitrofurazone (Table 3). The percentage contraction on day

Table 3: Effect of topical application of ointments containing different solvent fractions of the leaves of *Allophylus abyssinicus* on contraction of excision wound.

Group	Day 0	4th day	8th day	12th day	16th day	Period of epithelization (days)
5% CHL	523.75±5.34	426.72±4.05 (18.53)	336.41±3.85 (35.77)	201.54±1.91 (61.52)**	124.43±0.78 (76.24)***	22.78±0.63
10% CHL	521.43±5.43	411.76±4.82 (21.03)	288.42±3.76 (44.69)	164.96±2.75 (68.36)***	69.71±1.65 (86.63)***	21.34±0.73*
5% ACN	505.33±5.32	407.32±4.71 (19.40)	300.12±3.02 (40.61)	189.28±2.19 (62.54)**	106.37±1.36 (78.95)***	22.01±0.46
10% ACN	518.34±5.54	399.84±4.71 (22.86)	267.65±3.23 (48.36)	145.32±1.87 (71.96)***	53.82±0.76 (89.62)***	20.96±0.62*
5% ME	516.76±6.43	392.65±4.54 (24.02)	243.76±3.70 (52.83)*	139.91±4.34 (72.93)***	56.91±3.03 (88.99)***	21.02±0.42*
10% ME	490.21±5.74	352.37±4.45 (28.12)	198.34±4.78 (59.54)***	89.09±1.34 (81.83)***	27.22±0.32 (94.44)***	17.54±0.33***
5% WA	498.56±6.43	404.98±5.44 (18.77)	288.43±3.08 (42.15)	176.34±3.28 (64.63)*	93.32±1.98 (81.28)***	21.44±0.65
10% WA	524.55±5.22	367.91±4.04 (29.86)	239.23±3.21 (54.39)**	103.72±2.93 (80.23)***	39.87±1.01 (92.40)***	19.34±0.76***
0.2% NF	510.67 ±3.73	313.46±3.14 (38.62)*	163.20±3.27 (68.04)***	63.94±1.30 (87.48)***	10.32±1.98 (97.98)***	16.33±0.61***
SO	526.32±3.43	435.18±2.91 (17.32)	345.23±2.34 (34.41)	250.75±1.38 (52.36)	176.32±0.98 (66.50)	23.83±0.48
HPMC	519.76±4.34	427.34±4.21 (17.78)	347.38±3.01 (33.17)	276.62±1.98 (46.78)	197.12±1.01 (62.07)	24.11±0.46

Values are expressed as mean ± S.E.M (n = 6), percentage of contraction are in parenthesis. *P < 0.05 vs. control, **P < 0.01 vs. control, ***P < 0.001 vs. control, CHL = chloroform fraction, ACN = acetone faction, ME = methanolic fraction, WA = aqueous fraction, NF = nitrofurazone, SO = simple ointment, HPMC = hydroxypropyl methyl cellulose.

eight post treatment was significant for the methanolic fraction and nitrofurazone as compared with the negative control. The remaining fractions possessed better wound contraction than the respective negative controls but statistically insignificant.

On day twelve and day sixteen all the fractions displayed significant wound contraction rate as compared with the control. This indicates that the components are active mainly in the late phase of the wound healing cascade. The period of epithelization for the 10% methanol and aqueous fractions was found to be 17.54 ± 0.33 and 19.34 ± 0.76, respectively. These were comparable with 0.2% nitrofurazone (16.33 ± 0.61), and highly significant when compared with the negative control group. Ointments prepared from the 10% chloroform, 10% acetone and 5% methanol fractions also showed faster period of epithelization compared with the respective negative control groups.

Incision model

In the wound healing process, deposition of newly synthesized collagens at the wound site increases collagen concentration per unit area and hence the tissue tensile strength (Deshmukh *et al*., 2009). Table 4 compares the tensile strength of the healing skin treated with different formulations for 10 days.

Table 4: Effect of oral administration of the different solvent fractions obtained from the leaves of *Allophylus abyssinicus* on tensile strength of skin with an incision wound.

Extract/fraction (mg/kg)	Average tensile strength
Negative control (1% CMC)	267.64±6.28
80% methanol extract (200)	386.91±5.67***
80% methanol extract (400)	451.32±4.76***
Chloroform fraction (200)	301.43±5.40**
Chloroform fraction (100)	286.09±4.32
Acetone fraction (200)	360.43±6.83***
Acetone fraction (100)	322.87±5.91***
Methanol fraction (200)	412.87±6.43***
Methanol fraction (100)	384.32±5.57***
Water fraction (200)	367.54±5.98***
Water fraction (100)	332.87±6.21***

Values are expressed as mean ± S.E.M (n = 6), Dose levels are in parenthesis, **P < 0.01 vs. control, ***P < 0.001 vs. control.

Wound treated with non medicated ointment (negative control) had the minimum strength (267.64 g). Tensile strength of the tissue treated with other formulations was significantly higher in treated than untreated wounds. Tensile strength of the wound treated with 200 mg/kg of the methanolic fraction was the highest. Those which received 200 mg/kg of the methanolic and acetone fractions showed higher and statistically significant tensile strength when compared with the remaining groups. At a dose of 100 mg/kg the chloroform fraction did not show significant increase in tensile strength. Increased tensile strength indicates increase in collagen synthesis and strength by the formation of inter- and intra-molecular cross links which facilitate wound healing (Reddy *et al*., 2002).

Dead space model

Wound healing mainly depends on the repairing ability of the tissue, type and extent of damage and general state of the health of the tissue. The granulation tissue of a wound is primarily composed of fibroblast, collagen and small new blood vessels. The undifferentiated mesenchymal cells of the wound margin modulate themselves into fibroblast, which start migrating into the wound gap along with the fibrin strands. The collagen composed of the amino acid hydroxyproline is the major component of extra cellular tissue, which gives strength and support. Breakdown of collagen liberates free hydroxyproline and its peptides (Nayak and Pereira, 2006). Hence, estimation of hydroxyproline in the granulation tissue may throw light on the maturation and healing process (Azeez *et al*., 2007). The data depicted in Table 5 indicate that hydroxyproline content of the granulation tissue of animals treated with 400 mg/kg of the hydroalcoholic extract (P < 0.001) and 200 mg/kg of the methanolic fraction (P < 0.01) is higher than those in the other groups.

Antiinflammatory activity

Chronic ulcers will not heal until the chronic inflammation is reduced (Diegelmann and Evans, 2004). The inflammation in a chronic wound serves only to cause further injury and promote inflammation (Menke *et al.*, 2006). In the present study, carrageenan-induced oedema was used as a prototype to induce inflammation. The development of oedema has

Table 5: Effect of oral administration of the 80% methanolic extract and the different solvent fractions (in mg/kg) obtained from the leaves of *Allophylus abyssinicus* on dead space wound model

Extract/fraction (mg/kg)	Hydroxyproline (mg/g tissue)
Negative control (1% CMC)	21.53 ± 4.23
80% methanol extract (200)	46.76± 10.65
80% methanol extract (400)	63.25 ± 4.12***
Chloroform fraction (200)	28.09±3.43
Chloroform fraction (100)	23.66±5.92
Acetone fraction (200)	42.37±4.27
Acetone fraction (100)	34.87±3.65
Methanol fraction (200)	56.66±4.67**
Methanol fraction (100)	38.69±5.99
Aqueous fraction (200)	44.65±4.09
Aqueous fraction (100)	33.93±6.32

Values are expressed as mean ± S.E.M (n = 6). Dose levels are in parenthesis, **P < 0.01 vs. control, ***P < 0.001 vs. control.

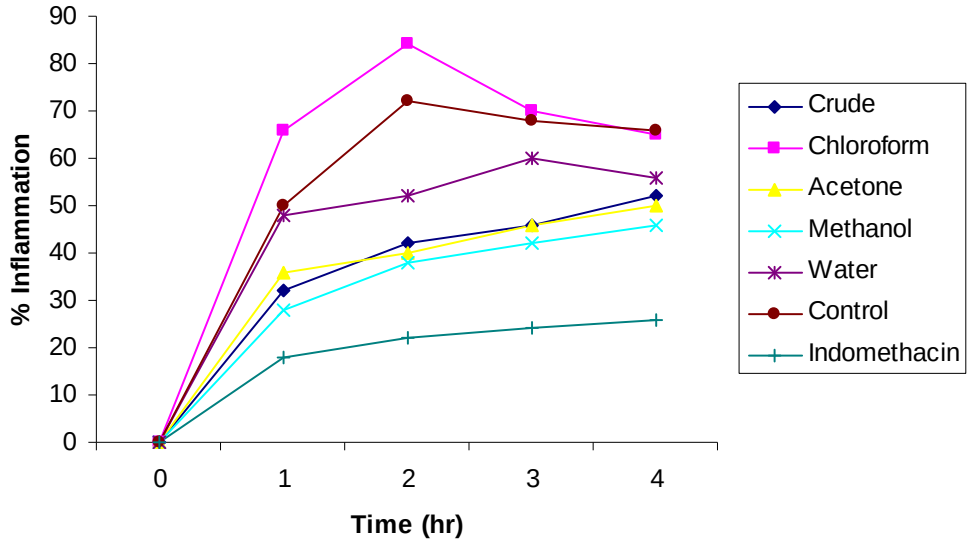

Figure 1. Effect of oral administration of various extracts of *Allophylus abyssinicus* (200 mg/kg) and indomethacin (10 mg/kg) on carrageenan induced paw oedema.

been described as biphasic. The initial phase is due to release of histamine, serotonin and kinins in the first h after injection of carrageenan. The more pronounced second phase is related to the release of a prostaglandin-like substance in 2–3 h. Indomethacin is known to show significant antiinflammatory effect, which may be due to inhibition of the mediators of inflammation induced by the phlogogenic stimuli (Lalitha and Sethuraman, 2010).

In the present study, all the test substances except the chloroform fraction exerted antiinflammatory effects, with respect to the control at a dose level of 200 mg/kg (Figure 1). Indomethacin was found to be the most effective both in the first and second phases of inflammation. In the first h of inflammation, the methanolic fraction and indomethacin showed significant antiinflammatory activity as compared with the control group ($P < 0.05$ and $P < 0.001$, respectively). In the subsequent h of inflammation, indomethacin showed statistically significant activity as compared with the rest of test substances. The 80% methanolic extract, the acetone, aqueous and methanolic fractions showed significant activity when compared

Table 6: Inhibition of carageenan-induced mice paw oedema by the 80% methanolic extract and the different solvent fractions of the leaves of *Allophylus abyssinicus.*

Treatment	Dose (mg/kg)	%A ± SEM (n = 6)			
		1 h	2 h	3 h	4 h
80% methanol extract	200	36.00 ± 4.40	41.67 ± 8.12 ***	32.35 ± 5.10***	21.21 ± 6.11*
Chloroform fraction	200	2.00 ± 5.08	5.56 ± 4.63	-2.94 ± 9.02	-9.09 ± 1.81
Acetone fraction	200	28.00 ± 0.84	44.44± 8.50***	32.35± 0.93**	24.24 ± 5.98**
Methanol fraction	200	44.00 ± 3.17*	47.22± 2.74***	38.24± 2.56***	30.30 ±2.71***
Aqueous fraction	200	4.00 ± 0.82	27.78± 4.34**	11.76 ± 4.83	15.15 ± 7.54
Indomethacin	10	64.00 ± 5.4***	69.44 ± 6.22***	64.71± 10.40***	60.61± 2.81***

Values are expressed as mean ± S.E.M, *P < 0.05, **P < 0.01, ***P < 0.001

with that of the control group in the second h. In the third and fourth h, all the test substances except the aqueous and chloroform fractions displayed statistically significant antiinflammatory activity (Table 6). This could be by blocking the release of prostaglandins.

The methanolic fraction and indomethacin seem to block both phases, which means blocking histamine and serotonin release in the first phase and preventing the release of some of the inflammatory mediators by blocking prostaglandin's action in the second phase. Although the methanolic fraction was found to be effective in both phases, its effect was not as pronounced as indomethacin. So, it is very likely that the antiinflammatory components of the plant reside in the methanolic fraction.

Conclusion

From the present study, it can be concluded that leaf extracts of *A. abyssinicus* possess genuine wound healing and antiinflammatory activities. The active ingredient(s) appear to reside mainly in the methanolic fraction suggesting that they are polar. The findings also provide scientific evidence for the traditional uses of the leaves *A. abyssinicus* in the treatment of wound and different inflammatory conditions

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Conflict of interest

There is no conflict of interest declared by the authors.

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