Investigation into the Wound Healing and Antimicrobial Potential of AzadirachtrailIndica and Neocarya macrophylla Seeds

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ABSTRACT

This work was designed to investigate the wound healing, antimicrobial and phytochemical constituents of *Azadirachta indica* and *Neocarya macrophylla* seeds. The hexane extracts of *A. indica* and *N. macrophylla* were prepared, labeled as AIHE and NMHE and studied for phytochemical constituents, antimicrobial activity on transient flora microorganisms of the skin using standard methods. Wound healing study was conducted on 42 Wistar rats distributed into six groups of seven rats each and incised with wound area of 550mm^2. Rats from Group A were treated with 100 % ointment (positive control), Groups B and C with 15 % of AIHE and NMHE and group D and E with 20 % AIHE and NMHE respectively, while Group F were left untreated (negative control). The wound healing activity of the seed extracts were assessed by the rate of wound contraction, wound area and epitheliazation time on every four days interval. The wound effect on the rats (feed intake, growth parameters, change in body weight, and specific growth rate) were also assessed. The phytochemical analysis of AIHE and NMHE revealed the presence of different bioactive compounds such as alkaloid, reducing sugar, cardiac glycosides, flavonoids, terpenoids and quinones in AIHE, while reducing sugar, alkaloids, cardiac glycosides, quinones and terpernoids were detected in NMHE. The two extracts exhibited significant antimicrobial activity on *S. aureus*, *B. subtilis* and *P. aeroginosa*. The results of the wound healing activity indicated that 20 % AIHE had the most potent wound healing capacity as evident from epitheliazation time (18.33±1.86 days), followed by its 15 % counterpart with the epitheliazation time of 18.67±2.16 days. The epitheliazation time of 15 % and 20 % NMHE groups was 19.29±1.25 and 20.17±2.64 respectively. The activity of the endogenous enzymes on the scar areas were also found to be regulated by the seed extracts. The increased wound contraction, the antioxidative and moderate antimicrobial activities of the extracts are indicative of the wound healing potential of *A. indica* and *N. macrophylla* seeds.

Keywords: Seed extract; phytochemical; antimicrobial; wound healing; epitheliazation period

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1. INTRODUCTION

Neem (*Azadirachta indica*) A. Juss (syn. *Melia azadirachta*) is an evergreen tree, cultivated in various parts of the Indian subcontinent. It is well known in India and its neighbouring countries for more than 2000 years as one of the most versatile medicinal plants having a wide spectrum of biological activity. *A. indica* A. Juss and *M. azedarach* are

two closely related species of Meliaceae. Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization. Every part of Neem tree has been used as traditional medicine for household remedy against various human ailments, from antiquity1–6; the tree has been known from time immemorial for its versatile applications. It has medicinal properties such as antimalaria activities, antiulcer effect, hypoglycaemic and immune stimulant activities (Biswas *et al*., 2002).

N. macrophylla commonly known as gingerbread can be used as a food-product that is sweet in nature and flavoured with ginger typically using honey or molasses (treacle) instead of sugar. *N. macrophylla* foods could be soft, moist loaf cake to something close to a ginger biscuits. Its seeds have purgative and anthelminthic properties (Dalziel, 2000) and are also a good source of ointment.

2. MATERIALS AND METHODS

Preparation of Seed Extracts

The seeds collected were sun dried and reduced to coarse powder using an electronic blender. Approximately 500 g of dried powdered samples were put in 2 aspirator bottles; 2 L of hexane was added to the two bottles and left at room temperature (30±2 °C) for 5 days with constant stirring, to allow proper mixing with the solvent. Filtration was carried out after the extraction. The filtrates were distilled to recover the solvent from the oil for further analysis. The extracts were stored in universal bottles and refrigerated at 4 °C prior to use.

Phytochemical Analysis and Screening

Phytochemical analyses were done on seed samples using the methods of Harborne (1998), Sofowora (1993) and Solomon *et al.* (2013). All determinations were done in triplicates.

Test Microorganisms

Multi Drug Resistant (multi) clinical isolates of tested organisms *Pseudomonas aerugonisa*, *Staphylococcus aureus* and *Bacillus subtilis,* were used as the bacterial tested organisms. The pure bacterial strains were obtained from the Department of Pharmacy, University of Ibadan, Ibadan, Nigeria. The bacterial strains were cultured overnight at a temperature of 37 °C in nutrient agar.

Antimicrobial Activity Assay

The antimicrobial activity of the seed extracts were evaluated against a few pathogenic bacteria such as *S. aureus, B. subtilis,* and *P. aeroginosa*.

Pour Plat Method (Bacteria)

A culture of each of the organisms was prepared by taking a loop, which is full of the organism from stock and each was inoculated into the sterile nutrient broth of 5 mls each. This was incubated for 18-24 h at 37 ^{o}C. From the culture made overnight, 0.1 ml of each organism was taken and placed into 9.9 mls of sterile distilled water in order to get 1:100 (10^{-2}) of the dilution of the organism. After this, 0.2 mls was taken from the diluted organism and placed into the prepared sterile nutrient agar which was at 45 ^{o}C, this was aseptically poured into sterile petri dishes and allowed to solidify for about 45-60 mins. A sterile cork borer of 8 mm diameter was used to make wells according to the number of graded concentration of the sample. In each well, different grades of concentrations of sample were produced, this was done in duplicates. The plates were allowed to stay on the bench for 2 h in order to allow pre-diffusion after which the plates were incubated uprightly in the incubator for 18-24 h at 37 ^{o}C.

Minimum Inhibitory Concentrations (MIC) of Seed Extract
Minimum inhibitory concentrations for both bacterial and fungal strains were measured as reported in literature by Sarker *et al.* (2007).

Experimental Animals
42 Wistar rats, all females of weight 60-80 g were used for the study. The rats were properly kept in polypropylene cages; food and water were provided for them with easy access. All The experimental work done was in compliance with University of Ibadan Ethics Committee on Research in Rats(14/0059/UIECRA) as well as international accepted principles for laboratory animal use and care.

Evaluation of wound healing activity
The excision wound model was used to evaluate the wound healing activity of AIHE and NMHE. The rats were divided into six groups, each containing seven rats and the 50 mg ointments formulated were applied topically once a day. The ratsof group1 received ointment base (control), group 2 and 3 rats were treated with 15 % (v/v) of AIHE and NMHE ointments, 4 and 5 were treated with 20 % (v/v) of AIHE and NMHE ointments. Group 6 was a negative control (no ointment applied). The rats were anaesthetized with ketamine hydrochloride (100 mg/kg, i.p.) prior to and during infliction of the wound (Nayak *et al.,* 2009). All rats were closely observed for any infection, so that the infected rats can be excluded from the study.

Oil Extract Formulation
A 15 % and 20 % (v/v) oil extracts of *A. indica* and *N. macrophylla* were prepared by mixing the extract (7.50 mls and 10.00 mls respectively) in yellow soft paraffin (50 mL) obtained from Chemistry Department, University of Ibadan store unit (Carter, 2016).

Excision Wound Model
The rats were anaesthetized before and during the creation of experimental wounds with ketamine hydrochloride (100 mg/kg, i.p.) (Nayak *et al.*, 2009). Rats were inflicted with excision wound according to the method described by Anusha (2002). The dorsal fur of the dorsolateral flank area was shaved using an electrical clipper. After the wound area has been prepared using 70 % alcohol, the skin from the predetermined shaved area was excised to its full thickness to ensure a wound area of about 500 $mm^2$was obtained using forceps, a surgical blade and scissor. Excision wounds were created on the dorsal thoracic region 2.4 cm from the vertebral column on either side. Haemostasis was achieved by blotting the wound with a cotton swab soaked in normal saline. The wound was left open and all the rats were treated using the ointments which was prepared and formulated, the healing of wound was monitored by tracing and measuring the wound contraction on the first, 4th, 8th, 12th, 16th and 20th, after the creation of wound. The wound closure was measured at regular intervals in order to calculate the percentage wound closure and the epitheliazation time which indicates the formation of new epithelial tissue to cover the wound (Sharath, 2010).

Wound healing activity study
To evaluate the wound healing ability of the prepared formulations, the following parameters were used:
1. Wound area (mm^2) of the excision wound,
2. Rate of wound contraction and epithelialization time (excision wound)
3. Histopathological studies of healed tissues were measured.

Rate of wound contraction

The rate of wound contraction was measured as percentage reduction of size at every 4 day interval. Progressive decrease in the wound size was measured periodically using transparency paper and a maker, and the wound area was measured graphically to monitor the percentage of wound closure which indicates new epithelial tissue to cover the wound. The percentage wound contraction was measured according to Srivastava and Durgaprasad (2008) formula:

$$\text{Percentage wound contraction} = \frac{A_0 - A_t}{A_o} \times 100\ \%$$

Where A_0 = Initial area of wound at day "0'' of experiment
A_t = Area of wound at day "t'' of experiment.
The number of days required for filling of the scar without any residual of the raw wound is the period of epithelization
.

Hispathological Study

On the 24th day of the experiment, the skin tissues were collected from all the five groups of the rats and processed for histological study to determine the pattern of lay-down for collagen. The skin specimens from the treated rats were collected in 10 % buffered formalin and subjected to sectioning and 6 m thickness sections were stained with hematoxylin and eosin. The stained slides were visualized for histological changes using a light microscope.

Assay of Superoxide Dismutate (SOD)

1 ml of the sample was diluted in 9 mls of distilled water in order to make 1:10. An aliquot of 0.2 ml of the diluted sample was the added to 2.5 mls of 0.05 M carbonate buffer (pH 10.2), this was done to equilibrate in spectrophotometer and the reaction began by the addition of 0.3 mls of freshly prepared 0.3 M sample adrenaline to the mixture. This was quickly mixed by inversion. The reverence contained 2.5 mls buffer, 0.3 mls of the substrate (adrenaline) and 0.2 mls of distilled water. The absorbance at 480 nm was monitored every 30 s for 150 s.

Catalase

The 0.5 mls homogenate was added to the reaction mixture, which contained 1 mls of 0.01 M phosphate buffer (pH of 7.4), 0.5 mls of 0.2 M of H_2O_2, 0.4 ml H_2O and incubated for different time period. The reaction was then terminated by adding 2 mls acid reagent (dichromate/acetic acid mixture), which was prepared by mixing 5 % potassium dichromate with glacial acetic acid (1:3 by volume). To the control, the enzyme was added only after the acid reagent had been added. All the tubes were heated for 10 mins and the absorbance was read at 610 nm. The catalase activity was expressed in terms of umoles of H_2O consumed/min/mg.

Estimation of Reduced Glutathione

The amount of reduced glutathione in the sample was estimated by the method of Boyne and Elman. 1 ml of the sample which was extracted was treated with 4.0 ml of metaphosphoric acid precipitating solution of (1.67 g of glacial metaphosphoric acid, 0.2 g of EDTA and 30 g of NaCl, all dissolved in 100 mL of water). Centrifugation was carried on the mixture, after which 2.0 ml of protein free supernatant was mixed with 0.2 ml 0f 0.4 M Na_2HPO4 and 1.0 ml of DTNB reagent (40 mg of DTNB in 100 ml of aqueous 1 % trisodium citrate). Absorbance was read at 412 nm with 2 min. GSH concentration was expressed as nmol/mg/protein.

Statistical analysis
Statistical analysis was carried out on each group, followed by ANOVA test (graph pad prism software) which was used to compare the mean value of each treatment. Significant differences were determined between the means of parameters using the Dunnian T test ($P < 0.05$).

3. RESULTS AND DISCUSSION

Phytochemical Screening
Tannins were detected in NMHE. Cardiac glycosides, flavonoids, alkaloids and reducing sugar were detected in both extracts while steroid was detected in only the ointment (Table 1). These compounds have been proven to be active against pathogens which include those that cause enteric infections (Owolabi *et al*., 2007); they exhibit antibacterial activities. Compounds such as alkaloids are antimalarial agents can act as stimulants and as analgesics, while glycosides moieties, such as saponins, antraquinones, cardiac glycosides and flavonoids can act as antiparasitic agent. They inhibit tumor growth and are used as antidepressant.

It is believed that the metabolites detected in these extracts are one of the major active constituents responsible for wound healing in the experimental rats and as a result would accelerate the rate of wound contraction. Tannins have antrigent properties. Alkaloids are known to have activities such as anticancer, analgesics, antimalarial, etc. It has been known that it acts as a starting material for many drugs in pharmaceuticals. Terpenoids play a major and important role in traditional and herbal medicines. Glycosides are used in medication. Phenols act as drugs, and from study are known to act as a good defense against infection (Ayoola, 2008). When these constituents are available in high concentration in the extract, could increase and accelerate wound healing in rats.

Antimicrobial activity
The result for the antimicrobial activity of AIHE and NMHE is shown on Tables 2 and 3. The seed extracts was found to effectively inhibit the growth of organisms at different concentrations, as compared to the negative and positive control. *P. aeroginosa* was found to be more susceptible to NMHE than *S. aureus* and *B. subtilis*. *P. aeroginosa*, which is more susceptible, has a minimum inhibitory concentration of 50 mg/mL, *B. subtilis* has 100 mg/mL and *S. aureus* has a MIC of 50 mg/mL. P. *aeroginosa* has a zone of inhibition diameter of 16.00 mm, *S. aureus* having 14.00 mm and *B. subtilis* 12.00 mm.

AIHE was found to effectively inhibit the growth of organisms at different concentration, as compared to the negative and positive control. *S. aureus* was slightly more susceptible than *P. aeroginosa. P. areoginosa* and *S. aureus* have minimum inhibitory concentration of 25 mg/mL, *B. subtilis* has MIC of 50 mg/mlL. *S. aureus* has a zone of inhibitory diameter of 16.00 mm, *B. subtilis*, has 18.00 mm and *P. aeroginosa* has a zone of inhibition diameter of 10.00 mm.

Table 1: Phytochemicals Constituents of the Seed Extract

Phytochemicals	*Azadirachta indica*	*Neocarya marcophylla*	Ointment
Flavonoids	+	-	-
Reducing sugar	+	+	-
Alkaloid	+	+	-
Carbohydrate	-	-	-
Cardiac glycoside	+	+	-
Phenols	-	-	-
Saponin	-	-	-
Steroid	-	-	+
Tannins	-	-	-
Terpenoids	+	+	-
Quinones	+	+	-

- indicates absence of phytochemical and + presence of phytochemical

Table 2: Antimicrobial activity of *N.macrophylla* against flora microorganism

Test organisms	Zone of inhibition diameter(mm)						
	Concentration of *N. macrophylla* seed extract(mg/mL)						
	-ve control	200	100	50	25	12.5	+ve control
S. aureus.		14	12	10			38
B. subtilis		12	10				36
P.aeroginosa		16	14	10			34

-ve control= hexane, +ve control = Getamicin

Table 3: Antimicrobial activity of *A. indica* seed extract against flora microorganism

Test organinisms	Zone of inhibition diameter (mm)						
	Concentration of *A. indica* seed extract (mg/mL)						
	-ve control	200	100	50	25	12.5	+ve control
S. aureus.	-	16	14	12	10	-	38
B. subtilis	-	18	14	10	-	-	36
P.aeroginosa	-	18	14	12	10	-	34

-ve control= hexane, +ve control = Getamicin

Minimum inhibitory concentration of the seed extracts

The extracts have good antimicrobial activities (Tables 4 and 5). AIHE inhibited the growth of all the microorganisms tested at 50 mg/mL. There was no inhibition of growth in any of the organism at a concentration of 200 mg/ mL and 100 mg/mL. NMHE showed good antibacterial activity. All microorganisms tested were inhibited at concentration of 100 mg/mL, 50 mg/mL and 25 mg/mL respectively. There was inhibition of *B. subtilis* at a concentration of 200 mg/mL, but no inhibition of *S. aureus* and *P. aeroginosa* at this concentration

Table 4: Minimal inhibitory concentration(MIC) of *A. indica* seed extracts

Test organisms	Concentration of A. *indica* seed extract(mg/mL)			
	200	100	50	25
S. aureus.	-	-	+	+
B. subtilis	-	-	+	+
P.aeroginosa	-	-	+	+

- indicates no growth of organism; + indicated growth of organism

Table 5: Minimal inhibitory concentration(MIC) *N. macrophylla* seed extract

Test organisms	Concentration of *N. macrophylla* seed extract(mg/mL)			
	200	100	50	25
S. aureus.	-	+	+	+
B. subtilis	+	+	+	+
P.aeroginosa	-	±	+	+

- no growth of organisms; +growth of organisms

Evaluation of wound healing activities

It was observed that rats in Group B had a reduced area faster than the rats in other groups, followed by those in Group D. Seed extract used on Group B was 20 % AIHE, while extract used on Group D was 15 % AIHE. Group C rats had a reduced area faster than Group E, extracts used in treating them were 20 % and 15 % NMHE. All these extracts had a reduced rate faster than negative and positive controls. Positive control had reduced wound area faster than the negative control (Table 6; Fig. 1). This is an indication that 20 % AIHE is more effective in the treatment and healing of wound in rats, followed by 15 % NMHE.

Wound contraction and Epitheliazation time

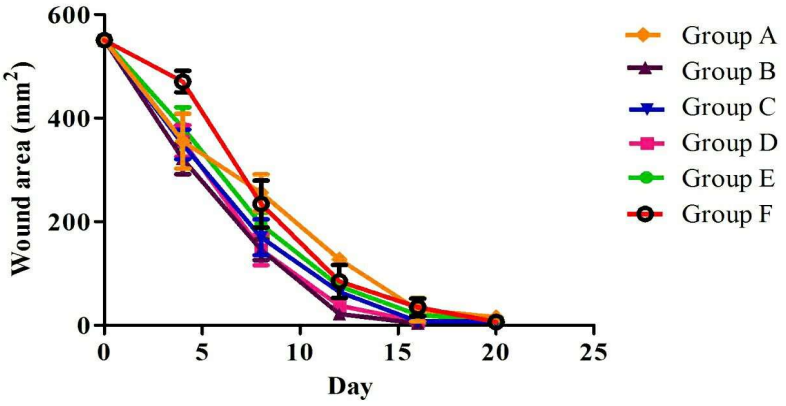
It was observed that when the extracts which served as medication were applied on the wound surface of the rats, the experimental rats in Group B had the fastest epitheliazation time compared to rats in the other groups, followed by rats in Group D (Table 7; Fig. 2). Seed extract used on Group B was 20 % AIHE, while extract used on Group D was 15 % AIHE. Rats in Group C had epitheliazation faster than those in Group E. Extracts used in treating them were 20 % and 15 % NMHE respectively. All the rats that were treated the seed extracts reached epitheliazation faster than negative and positive controls. Positive control had faster epitheliazation time than the negative control. 20 % AIHE seemed to be more effective in the treatment and healing of wound in rats followed by 15 % NMHE.

Group B rats treated with 20 % (v/v) AIHE had the fastest epitheliazation time (18.33±1.86), followed by Group D which was treated with 15 % (v/v) AIHE (18.67±2.16). Group C (20 % (v/v) NMHE had epitheliazation time of 19.29±1.25; Group E treated with 15 % (v/v) NMHE had epitheliazation time of 20.17±2.64 (Table 8; Fig 3). Group A (100 % ointment), which served as the positive control had epitheliazation time of 20.57±1.62. Group F (negative control), which was not treated at all, had epitheliazation time of 24.00±00. According to the definition of Chattopaddhya *et al*. (2001), healing of wound is complex in nature and dynamic process which involves tissue structure restoring back to its normal state. Healing depends upon the repairing ability of the tissue, type and extent of damage, and general state of the host's health (Madden *et al.,* 1968). From these values, it is clear that 20 % (v/v) *Azadirachta indica* and 15 % (v/v) *Azadirachta indica* extracts achieved epitheliazation faster than the others. Therefore they can heal wound faster than the other extracts.

Table 6 : Wound area of experimental animals

GROUP	DAY 4	DAY 8	DAY 12	DAY 16	DAY 20	
Group A	355.34±52.7^{b}	255.77±35.61^{a}	127.17±39.88^{b}	29.83±10.01^{a}	15.65±3.46	
Group B	321.24±29.34^{b}	145.59±52.00^{b}	21.95±4.01^{b}	3.53±1.24^{a}	0.00±0.00	
Group C	349.22±28.49^{b}	169.94±34.55^{ab}	54.04±30.02^{ab}	7.43±3.26^{a}	7.07±0.00	
Group D	355.30.48^{b}	145.70±30.06^{b}	37.90±14.60^{b}	7.33±3.21^{a}	0.00±0.00	
Group E	381.99±35.60^{ab}	194.50±223.43^{ab}	75.01±11.97^{ab}	20.49±6.83^{a}	8.10±3.64	
Group F	530.94±58.82^{b}	383.06±75.34^{ba}	158.56±64.50^{ab}	84.56±31.60.^{a}	17.45±2 .20	

A positive control", Group B 20 % (v/v) *Neem* extract, Group C 20 % (v/v) *Gingerbread*, Group D " 15 % (v/v) *Neem* extract, Group E 15 % (v/v) *Gingerbread* extract and Group F "the negative control".



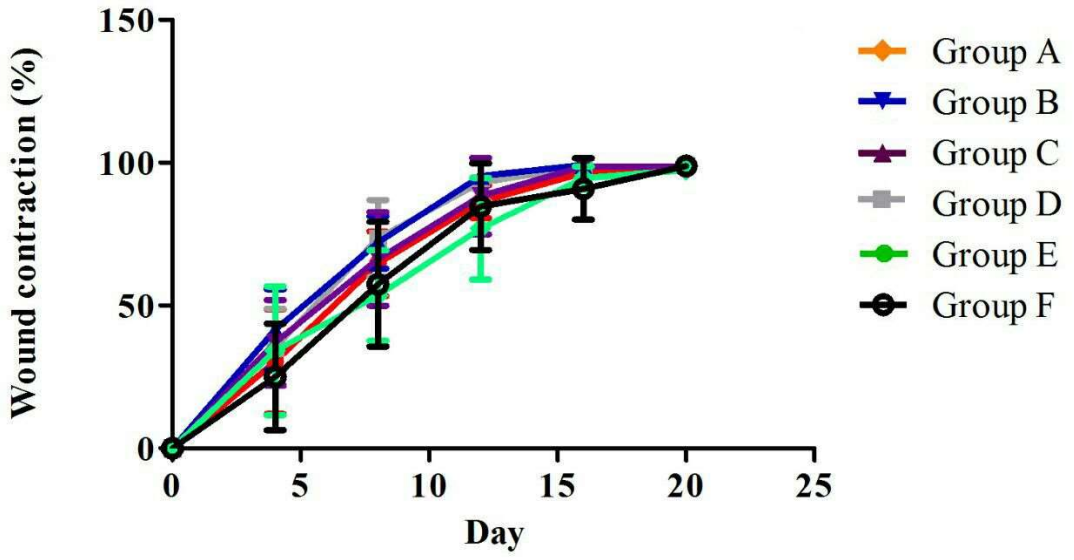
Group A positive control", Group B 20 % (v/v) *Neem* extract, Group C 20 % (v/v) *Gingerbread*, Group D " 15 % (v/v) *Neem* extract, Group E 15 % (v/v) *Gingerbread* extract and Group F "the negative control".

Figure1: Graph showing the rate of wound contraction in %

Table 7: Wound contraction of experimental animals

GROUP	DAY 0	DAY 4	DAY 8	DAY 12	DAY 16	DAY 20
Group A	550.00±00	34.17±22.54^{a}	53.50±15.86^{b}	76.88±17.76^{a}	94.58±4.07^{ab}	97.42±1.73
Group B	550.00±00	41.59±14.11^{a}	72.11±9.16^{ab}	95.29±1.70^{a}	99.18±0.75^{a}	100.00±0.00^{a}
Group C	550.00±00	37.03±14.94^{a}	66.33±16.34^{ab}	88.35±13.37^{ab}	98.64±1.32^{a}	98.71±0.00
Group D	550.00±00	35.33±13.58^{a}	73.51±13.39^{a}	93.10±6.49^{a}	98.65±1.30^{a}	100.00±0.00^{a}
Group E	550.00±00	30.38±18.28^{a}	64.64±11.27^{ab}	86.36±5.76^{ab}	96.49±2.83^{ab}	98.63±1.44
Group F	550.00±00	7.85±5.44^{a}	29.30±6.21^{ab}	62.70±15.20^{ab}	84.63±10.76^{b}	96.83±1.06

Group A positive control", Group B 20 % (v/v) Neem extract, Group C 20 % (v/v) Gingerbread, Group D " 15 % (v/v) Neem extract, Group E 15 % (v/v) Gingerbread extract and Group F "the negative control



Group A positive control", Group B 20 % (v/v) Neem extract, Group C 20 % (v/v) Gingerbread, Group D " 15 % (v/v) Neem extract, Group E 15 % (v/v) Gingerbread extract and Group F "the negative control".

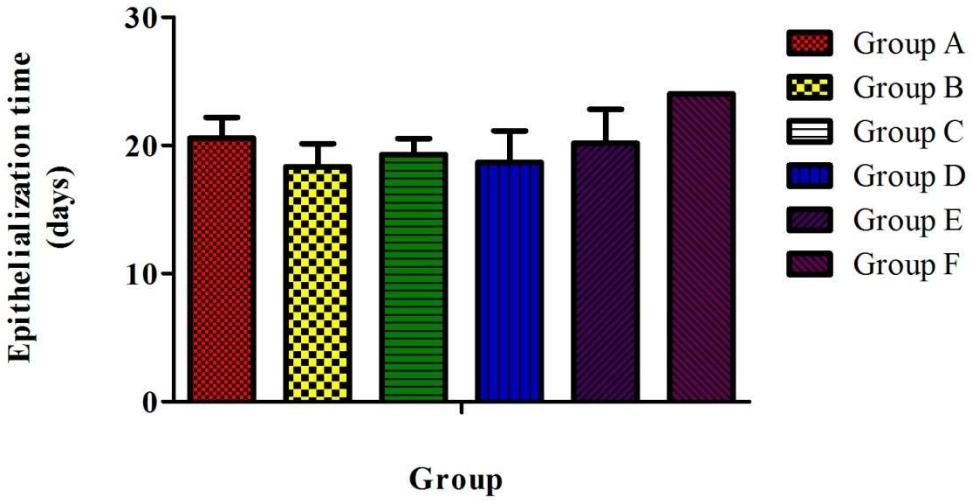
Figure 2: Graph showing the rate of wound contraction in %

TABLE 8: Epitheliazation time of the experimental rats

GROUP	EPITHELIZATION TIME

Group A	20.57±1.62[b]
Group B	18.33±1.86[b]
Group C	19.29±1.25[b]
Group D	18.67±2.16[b]
Group E	20.17±2.64[b]
Group F	24.00±0.00[a]

Group A positive control", Group B 20 % (v/v) AIHE, Group C 20 % (v/v) NMHE, Group D " 15 % (v/v) AIHE, Group E 15 % (v/v) NMHE and Group F "the negative control".



Group A positive control", Group B 20 % (v/v) Neem extract, Group C 20 % (v/v) Gingerbread, Group D " 15 % (v/v) Neem extract, Group E 15 % (v/v) Gingerbread extract and Group F "the negative control".

Figure 3: Histogram showing epitheliazation time in days

Endogenous enzymes

Endogenous enzymes are enzymes secreted in the body naturally. Enzymes in the body help in healing of wounds and body defense. They serve as antioxidant and scavengers, thereby inhibiting and controlling the activities of free

radicals in the body. It was observed that Group B and Group D have higher values of Superoxide Dismutate and Catalese (Table 9). These were the groups treated with 20 % and 15 % AIHE, and both have lower value of Reduced Gluthathione. These high values indicate that the extracts helped to increase the generation of these enzymes in higher rates than the other groups. This resulted in Group B and Group D having reaching epitheliazation time faster than the rest of the groups. Endogenous enzymes help to inhibit free radicals, which when in excess, are dangerous to health. Catalase converts hydrogen peroxide to water and molecular oxygen. Superoxide dismutase neutralizes superoxide ions by going through oxidative and reductive cycles.Low level of Superoxide Dismutate is an indication that only minimum quantity of it is needed by the body to function effectively, compared to other enzymes.

Table 9: Endogenous enzymes (µmol/g tissue)

	GSH	SOD	CAT
Group A	44.90	97.57	90.22
Group B	42.10	107.50	162.40
Group C	55.54	60.54	90.22
Group D	44.89	35.40	17.28
Group E	52.90	75.67	6.28
Group F	43.54	85.07	17.27

TABLE 10: Histological examination of the skin scar area

Group	Histological examination of the skin scar area
Group A	The epidermis (thick arrow) is intact and thick. The dermis (star) contains numerous hair follicles (thin arrows) enmeshed within dense fibrous connective tissue.
Group B	The epidermis (thick arrow) is thick. There are numerous mononuclear cells and hair follicles (arrows) in the dermis (star).
Group C	Epidermis is intact. Numerous hair follicles are present. Note the keratin pearl (arrow).
Group D	Epidermis (thick arrow) is thickened and appears to be regenerating. Note the discontinuous portion (star). There is paucity of hair follicles and numerous foci of mononuclear cells (arrows) in the dermis.
Group E	The epidermis (thick arrow) is intact and thin. The dermis (star) is reduced in size contains numerous hair follicles (thin arrows) and sebaceous glands enmeshed within dense fibrous connective tissue. Beneath the dermis is the thick muscle layer (blue arrow) and subcutis (red arrow).
Group F	The epidermis (thick arrow) is thin and intact. Dermis (star) appears normal with hair follicles and sebaceous glands (arrows).

Group A is the positive control; Group B is 20 % neem extract; Group C is 20 % Ginger bread extrct; Group D is 15 % neem extract; Group E is 15 % Ginger bread extract and Group F is the negative control

4. CONCLUSION

This study revealed that *A. indica* and *N. macrophylla* have wound healing properties. This may be as a result of their ability to scavenge free radicals, inhibit bacteria that might be on the wound and inhibit inflammatory pathway. This is seen in the rate of wound contraction, period of epitheliazation showed the wound healing effect of the seed extracts. *A. indica and N. macrophylla* seeds have potential in the medicinal industry.

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