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Wound Healing and Antimicrobial Effects of *Azadrachita indica* Leaves Extracts on *Eschericha coli* Infected Diabetic Wounds using Albino Rat Model

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The wound healing and antimicrobial effects of *Azadirachta indica* leaf extracts were evaluated on *Eschericha coli* infected diabetic wounds using albino rat model. The study was a cross-sectional work done between January and June, 2020. *Eschericha coli* was isolated and identified from 50 infected diabetic patients using Eosin methylene blue and standard biochemical tests. The crude extracts of *A. indica* were gotten using ethanol and water by soxhlet method. The phytochemical such as saponins, phenols, tannins, flavonoid and alkaloids were determined quantitatively and qualitatively using standard method. *In vitro* antimicrobial effect of extracts and their combination were evaluated. The wound healing effects of the extracts were done using six weeks old albino rat model. Out of 50 swab samples of infected diabetic wounds, 30 isolates were obtained which *E. coli* was 20% of the bacterial isolates from the infected wound. The phytochemical analysis of the extracts showed the presence of saponin, flavonoids, steroid, alkaloids, tannins and phenol. Among

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the leaf extracts analysed, *A. indica* ethanol extract has the highest inhibition zone against *E. coli* (10.67±1.15 mm) at 500 mg/ml and 1000 mg/ml concentration. The Minimum inhibitory concentration (MIC) was 250 mg/ml while the Minimum Bactericidal Concentration (MBC) was 1000mg/ml. Diabetic and healthy groups of albino rats were treated after 1 week of infection. Comparing the negative control rats witsh those treated with daily topical application of the leaf extracts, showed significant reduction in wound size and rapid healing (P = 0.05). Comparing with positive control, the leaf extracts have almost the same healing effect with the positive control (povidone iodine). The leaf extracts of *A. indica* possess antimicrobial properties for *E. coli* especially the ethanol extract which heals faster than aqueous extract and can be used as an alternative for healing infected non diabetic and chronic diabetic wounds.

Keywords: Diabetic wound; Azadirachta indica; antimicrobial and wound healing.

1. INTRODUCTION

Man has relied so much on medicinal plants for healing and food needs since the dawn of history. The traditional use of medicinal plants for curing and preventing illness, including the promotion of both physical and spiritual well-being among human beings have become paramount in almost every household [1]. Several plant species of medicinal plants have been identified to be naturally distributed. The traditional use of medicinal plants in addressing certain health problems has been handed down from generation to generation. Many of these plant species are known to majority as a source of medicine for treating a particular ailment, without the knowledge that two or more species could be mixed together to produce a more effective medicine. Example *Azadiracha indica* is commonly known for the treatment of malaria scourge, though it could also be used to treat ailments like hepatitis and intestinal problems when mixed with the bark and leaves of *Mangiferia indica* with some fruits of *Citrus aurantifolia* [1].

Azadirachta indica known as Neem, Maina in Hausa, Atu yabasi in Igbo, Dongoraro in Yoruba and Dongoyalo in Idoma is a widely distributed indigenous Indian plant and it is also grown in Nigeria [2]. It has been reported that every part of the neem tree has been used as traditional medicine for household remedy against various human diseases from ages [2]. *Azadirachta indica* leaf contains some phytochemicals found in medicinal plants which include tannins, flavonoids, alkaloids, phenols, steroids and saponins [3]. Many researchers have also studied the medicinal properties of neem leaf which include its antipyretic effect, antimalarial effect, antidiabetic effect, antifertility effect, cardiovascular effect, wound healing effect etc. [2]. Based on researchers' medical properties reported about the neem plant, this research work tends to determine the antimicrobial and wound healing effects of *A. indica* leaf extracts on *Escherichia coli* infected wounds using albino rat model.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in Anambra State, which is the 8th populous state in Nigeria, located in South-East geopolitical zone of Nigeria. It is located between latitude 6^0 20'00'' North and longitude 7^0 00''00'' East. Anambra state has a population of 2,061961.984. Anambra state has a tropical climate. The average temperature is 25.9℃ while the average rainfall in a year is 1386mm [4].

2.2 Study Population

The target population are diabetic patients with infected wounds.

2.3 Study Design

This work was a cross- sectional study (between January and June 2020) done to determine wound healing and antimicrobial effects of *Azadirachta indica* on infected wounds of diabetic induced albino rats. At enrolment, consent of the patients were obtained.

2.4 Sample Techniques

In this study, random sampling technique was used. Samples were collected from medical wards of Chukwuemeka Odumegwu Ojukwu Teaching Hospital, Amaku, Awka in Anambra State, Nigeria.

2.5 Isolation of Microorganisms

2.5.1 Sample collection

Fifty (50) wound swab of patients with infected wounds and are attending different clinics in selected hospitals were collected. Wound exudates were collected using sterile cotton tipped swab before antiseptic dressing. Sterile normal saline were used to moisten the swab stick before collecting the specimen [5].

2.5.2 Preparation of culture media

All culture media employed in this study were prepared according to the manufacturer's instructions. They were sterilized by autoclaving at 121^0 C, 15psi for 15 minutes.

2.5.2.1 Isolation and identification of bacteria

Each sample was inoculated onto Nutrient agar and MacConkey agar and incubated at 37^0 c for 24 hours. After the incubation, the different colonies were subcultured on Nutrient agar and MacConkey agar and incubated at 37^0 c for 24 hours to get pure cultures. Then the colonies were identified macroscopically, microscopically, using biochemical analysis and by culturing on Eosin Methylene Blue agar. Microscopic identification was by gram staining. Biochemical tests include: catalase test, oxidase test, motility test, methyl red test, Voges-proskaeur test, indole test, citrate test and sugar fermentation and assimilation tests [6].

2.6 Plant Collection and Identification

The leaves of *Azadirachta indica* were collected from the bush in Nnamdi Azikiwe University, Awka. The identification and authentication of the plant was done at Department of Botany, Nnamdi Azikiwe University, Awka with voucher specimen number NAUH/14^B. The leaves were washed with sterile water, shade dried, ground onto powdered form and store in an air tight container for future use.

2.7 Preparation of Crude Extract

One hundred (100) grams of the powdered material were extracted with 500ml water and 500ml of ethanol separately for 24hours using soxhlet method [5].

2.8 Phytochemical Screening

2.8.1 Qualitative and quantitative analysis of phytochemical constituents

This was carried out to determine the presence of saponins, tannins, flavonoids, phenols and alkaloids. This was done using standard method of [7,8].

2.8.1.1 Test for Saponins (Foam Test)

Two (2) g of the powdered sample boiled in 20 ml of distilled water in water bath and filtered the solution. Then 10ml of the filtrate was mixed with 5 ml of distilled water and shake vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shakes vigorously which leads to formation of emulsion; indicated presence of saponins.

2.8.1.2 Test for tannins (Ferric Chloride Test)

Two hundred (200) mg of plant extract was treated with few drops of 0.1% ferric chloride and observed for blue or black colouration, the formation of blue black colour confirmed the presence of tannins.

2.8.1.3 Test for Alkaloids (Wagner's Test)

Extraction solution, 0.5ml of extract solution was treated with 2-3 drops of Wagner's reagent (solution of iodine in potassium iodide) and the formation of reddish brown precipitation indicates the presence of alkaloids.

2.8.1.4 Test for Flavonoids (Alkaline Reagent Test)

To the extract solution few drops of sodium hydroxide was added, formation of an intense yellow colour, which turns to colourless on addition of few drops of concentrated H_2SO_4. The test tube was shaken well and allowed to stand for some time. The appearance of red colour in upper layer indicates the presence of sterol and formation of yellow colour at the lower layer indicating the presence of triterpenoids.

2.8.2 Quantitative determination of phytochemical constituents of the leaves

2.8.2.1 Tannin

Analytical method for quantitative determination of tannin was according to [9]. By dissolving 50g

of sodium tungstate (Na_2WO_4) in 37cm3 of distilled water, Folin-Denis reagent was made. To the reagent prepared above, 10g of phosphomolybdic acid ($H_3PMo_{12}O_{40}$) and 25cm_3 of orthophosphoric acid (H_3PO_4) were added. Two-hour reflux of the mixture was carried out, cooled, and diluted to 500cm_3 with distilled water. One gram of each fine leave powder (sample) in a conical flask was added to 100cm_3 of distilled water. This was boiled gently for 1hour on an electric hot plate and filtered using number 42 (125mm) Whatman filter paper in a 100cm_3 volumetric flask. Addition of 5.0cm_3 Folin-Denis reagent and 10cm_3 of saturated Na_2CO_3 solution into 50cm_3 of distilled water and 10cm_3 of diluted extract (aliquot volume) was carried out after being pipetted into a 100cm_3 conical flask for colour development. The solution was allowed to stand for 30 minutes in a water bath at a temperature of 25^{o}C after thorough agitation. With the aid of a Spectrum Lab 23A spectrophotometer optical density was measured at 700nm and compared on a standard tannic acid curve. Dissolution of 0.20g of tannic acid in distilled water and dilution to 200cm_3 mark (1mg/cm_3) was used to obtain tannic standard curve. Varying concentrations (0.2–1.0mg/cm_3) of the standard tannic acid solution was pipetted into five different test tubes to which Folin-Denis reagent (5cm_3) and saturated Na_2CO_3 (10cm_3) solution was added and made up to the 100cm_3 mark with distilled water. The solution was left to stand for 30minutes in a water bath at 25^{o}C. Optical density will be ascertained at 700nm with the aid of a Spectrum Lab 23A spectrophotometer. Optical density (absorbance) versus tannic acid concentration was plotted. The following formula was used in the calculation:

Tannic acid (mg 100g) = C×extract volume×100/Aliquot volume × weight of sample,

Where C is concentration of tannic acid read off the graph.

2.8.2.2 Determination of alkaloids

Quantitative determination of alkaloid was according to the methodology by [9]. Exactly 200cm_3 of 10% acetic acid in ethanol was added to each fine leave powder sample (2.50g) in a 250cm_3 beaker and allowed to stand for 4 hours. The extract was concentrated on a water bath to one-quarter of the original volume followed by addition of 15 drops of concentrated ammonium hydroxide drop wise to the extract until the precipitation is complete immediately after filtration. After 3 hours of mixture sedimentation, the supernatant was discarded and the precipitates was washed with 20cm_3 of 0.1M of ammonium hydroxide and then filtered using Gem filter paper (12.5cm). Using electronic weighing balance, the residue was dried in an oven and the percentage of alkaloid was expressed mathematically as

% Alkaloid= Weight of alkaloid /Weight of sample ×100.

2.8.2.3 Determination of flavonoid

Flavonoid determination was by the method reported by [10]. Exactly 50cm_3 of 80% aqueous methanol added was added to 2.50g of sample in a 250cm_3 beaker, covered, and allowed to stand for 24hours at room temperature. After discarding the supernatant, the residue was reextracted (three times) with the same volume of ethanol. Whatman filter paper number 42 (125mm) was used to filter whole solution of each leave sample .Each leave sample filtrate will later be transferred into a crucible and evaporated to dryness over a water bath. The content in the crucible was cooled in a desiccator and weighed until constant weight is obtained. The percentage of flavonoid was calculated as

% Flavonoid= Weight of flavonoid/ Weight of sample ×100.

2.8.2.4 Determination of saponin

Saponin quantitative determination was carried out using the method reported by [9]. Exactly 100cm_3 of 20% aqueous ethanol was added to 5 grams of each dried leave powder sample in a 250cm^3 conical flask. The mixture was heated over a hot water bath for 4 hours with continuous stirring at a temperature of 55^{o}C. The residue of the mixture will be reextracted with another 100cm^3 of 20% aqueous ethanol after filtration and heated for 4 hours at a constant temperature of 55^{o}C with constant stirring. The combined extract was evaporated to 40cm^3 over water bath at 90^{o}C. 20cm^3 of diethylether was added to the concentrate in a 250cm^3 separator funnel and vigorously agitated from which the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice. 60cm^3 of n-butanol was added and extracted twice with 10cm^3 of 5% sodium chloride. After discarding the sodium chloride layer the remaining solution was heated in a

water bath for 30minutes, after which the solution was transferred into a crucible and was dried in an oven to a constant weight. The saponin content was calculated as a percentage:

% Saponin= Weight of saponin /Weight of sample ×100.

2.8.2.5 Determination of cyanogenic glycoside

Cyanogenic glycoside quantitative determination methodology used in this research was that used by [9]. It was weighed into a 250cm^3 round bottom flask and about 200cm^3 of distilled water was added to one gram of each dry leaves powder sample and allowed to stand for 2 hours for autolysis to occur. Full distillation was carried out in a 250cm^3 conical flask containing 20cm_3 of 2.5% NaOH (sodium hydroxide) in the sample after adding an antifoaming agent (tannic acid). Cyanogenic glycoside (100cm_3), 8cm_3 of 6M NH_4OH (ammonium hydroxide), and 2cm^3 of 5% KI (potassium iodide) was added to the distillate(s), mixed, and titrated with 0.02M $AgNO_3$ (silver nitrate) using a microburette against a black background. Turbidity which was continuous indicates the endpoint.

Content of cyanogenic glycoside in the sample was calculated as

Cyanogenic glycoside (mg 100g) = Titer Value (Cm_3) × 1.08 × exact volume/ Aliquot volume (Cm_3) × sample weight (g) ×100.

2.8.2.6 Determination of phenols

Defatting of 2g leaves powder sample was carried out for 2 hours in 100cm^3 of ether using a soxhlet apparatus. The defatted sample (0.50g) was boiled for 15minutes with 50cm^3 of ether for the extraction of the phenolic components. Exactly 10cm^3 of distilled water, 2cm^3 of 0.1N ammonium hydroxide solution, and 5cm^3 of concentrated amyl alcohol was also added to 5cm^3 of the extract and left to react for 30 minutes for colour development. The optical density was measured at 505nm. 0.20g of tannic acid was dissolved in distilled water and diluted to 200mL mark (1mg/cm^3) in preparation for phenol standard curve. Varying concentrations (0.2–1.0mg/cm^3) of the standard tannic acid solution was pipetted into five different test tubes to which 2cm^3 of NH_3OH, 5cm^3 of amyl alcohol, and 10cm^3 of water was added. The solution was made up to 100cm^3 volume and left to react for 30 minutes for colour development. The optical density was determined at 505nm.

2.9 Reconstitution of Plant Extract

The extracts were reconstituted using the described by [10]. The dried extracts were reconstituted by dissolving 10g of extract in 10ml of Dimethyl sulfoxide (DMSO). This extract were considered as 1000mg/ml.

2.10 Preparation of Inoculum

This was done using [11] method. The isolate was aseptically scraped and transferred into 10ml of sterile water in a sterile bottle and agitated vigorously. The suspension was diluted serially tenfold and used for antibacterial screening.

2.11 *In Vitro* Antibacterial Activity Assessment of the Extracts on the Isolates

Test Microorganisms: Microbial culture of *E. coli* isolated from the wounds were used for determination of antimicrobial activity.

One (1) ml of 24 hours culture of the isolate which was adjusted to 0.5 MacFarland standard corresponding to approximately 1.0 ×10^8cuf/ml was introduced into sterile plates and then 19ml of molten Muller Hinton agar at 45℃ was added to the plate shaken gently so as to mix contents. Then the molten agar and the test organism were allowed to solidify on a flat bench for 30mins. A sterile cork borer of 6mm diameter was used to drill holes 4mm deep. The wells were filled with 0.5ml of each of the extracts concentration (62.5mg/ml-1000mg/ml) then standard antibacterial solution (gentamycin) and sterile water were used as positive and negative control respectively. The plates were incubated at 37^0 c for 24 hours. Antimicrobial activity was determined by measuring the zones of growth inhibition. The experiments were carried out in triplicates and results were calculated as mean ± SD.

2.12 Minimal Inhibitory Concentration and Bactericidal Concentration of Crude Extracts

The Minimum Inhibitory Concentration (MIC) of different extracts were determined by the tube dilution method according to [12].

Minimum Bactericidal Concentration (MBC) is the lowest concentration of the sample that prevents fungal and bacterial growth respectively after incubation required to kill the organisms. For MBC determination, 1ml of the broth culture was taken from the MIC test tube that showed no growth by visual inspections and inoculated on to the surface of a solidified nutrient agar and Potato dextrose agar contained in 90mm petri dishes using spread plate method and incubated at 37℃ for 48hrs. The lowest concentration with no visible growth on the agar plates indicated [12].

2.13 Gel Formation

One (1) g of the dried solvent extract was made up to 100g using Vaseline and a paste was made [13].

2.14 *In Vivo* Antimicrobial Assay

The *in vivo* antibacterial assay of the leaves extract on selected isolates was carried out using albino rats.

2.15 Animals Selection, Induction, Excision and Inoculation (Infection)

2.15.1 Animal selection

Twenty four (24), six weeks old healthy albino rats that weigh 300g were obtained from a Faculty of Pharmacy of Nnamdi Azikiwe University's animal house Agulu, Anambra State. Twelve (12) of the albino rats served as the healthy rat (the healthy rats were observed to make sure that they are sound by observing their activeness, their skin condition e. t. c) while the remaining twelve (12) rats served as diabetic rats. The rats were housed in a standard metal cage and were maintained on standard pellet diet and water throughout the experiment. The rats were kept at room temperature before and during the experiment.

2.15.2 Diabetes induction

One group of the rat models were induced with diabetic using insulin syringe with 0.1ml of Alloxan for 100mg/kg body weight intraperitonally without anesthesia as described by [14]. Before diabetic induction, the blood sugar level of the experimental animals were checked. After which the albino rats were observed for two weeks for the development of signs diabetics like frequent urination, before excision and infection of the wound with isolates as in healthy rats.

2.15.3 Excision and inoculation of wound on albino rats

Wound was created on the dorsal interscapular region of each animal by disinfecting with 70% (v/v) ethanol and shaved carefully using sterile surgical blades then wounds were created on that area using sterile scissors. After which the bacterial inoculums were prepared and were evenly applied topically on the injured site. The animals were carefully observed for one (1) week to develop sign of infection [15].

2.16 Antimicrobial and Wound Heling Effects

2.16.1 Antimicrobial and wound healing effects of the extracts on induced wound infection in healthy albino rats

The efficacy of the extracts in treating wound infection caused by the selected isolate in healthy albino rats were evaluated. Four (4) groups of rats (3 rats in each group (I, II, III and IV)) were taken. A full cutaneous injury were induced at the dorsal region in all groups and infected with the selected isolate. This was done directly at the site of the injury as described earlier. All the rats in group III infected with the inoculum of the selected isolate acted as negative controls. Rats in group I and II were infected and treated with a daily topical application of 0.5ml of the extracts concentration that are the MBC. In group IV, the rats were treated with daily topical application of 5% povidone iodine ointment which served as a positive control. All rats were monitored for 1 week for sign of infection before commencement of treatment [14].

2.16.2 Antimicrobial and wound healing effects of the extracts induced wound infection in diabetic albino rats

Efficacy of the extracts in treating wound infection caused by the selected isolates in diabetic albino rats were evaluated. Four (4) groups of rat (3 rats in each group (I, II, III and IV)) were taken. A full cutaneous injury was induced at the dorsal region in all groups and infected with the selected isolate. This was done directly on the site of the injury as described earlier after 1 week of injecting them with Alloxan. All the rats in group III infected with

inoculum of the selected isolate acted as negative control and were treated with Vaseline. In group I and II, the rats were injured, infected and treated with daily topical application of 0.5ml of the extracts concentrations that are the MBC. In group IV, the rats were treated with daily topical application of 5% povidone iodine ointment which served as a positive control group. All rats were monitored for I (one) week for any sign of infection before treatment starts [14]

2.17 Pre –Treatment

This was done to establish Koch's postulate in disease process as described by [16] Wound swab of the infected rats were carefully collected using swab stick from the infected area after (1) week of inoculation. This was inoculated immediately onto MacConkey agar and incubated aerobically at 37^0 c for 24 hours. Then the colonies were further identified microscopically, macroscopically and using biochemical tests.

2.18 Post-Inoculation Treatment

2.18.1 Treatment

The experimental animals were treated daily with the extracts. The positive controls were treated with topical 5% povidone iodine while the negative control were treated with Vaseline. The animals were carefully monitored for 3 weeks and wound sizes were measured till fully recovered [17].

2.18.2 Measurement of wound area

The measurement of wound size was done with the aid of a transparent tracing paper every 2days. The transparent paper was placed on $1mm^2$ grid graph sheet and wound area was recorded. The percentage of wound contraction (wound healing) was calculated using the formula below [17]:

Percentage of wound contraction = ((initial wound area – specific day wound area)/ initial wound area) ×100%.

2.19 Statistical Analysis

The data obtained were subjected to one-way ANOVA using Statistical Package for Social Sciences (SPSS) 21 for window evaluation. P-values < 0.05 was considered significant.

3. RESULTS AND DISCUSSION

Out of 50 swab samples of infected diabetic wounds, 30 isolates which include 6(20%) *Escherichia coli* were obtained.

Table 1. Morphology and biochemical characteristics of the isolate

Test	Organism
	Escherichia coli
Morphology	Large, thick, greyish white moist colony on nutrient agar
EMB agar	Green metallic sheen
Grams staining	Gram negative rod
Catalase	-
Oxidase	-
Urease	-
Methyle red	+
Voges-proskaeur	-
Indole	+
Motility	+
Citrate	-
Sucrose	+
Lactose	+
Maltose	+
Galactose	Varies
Fructose	Varies
Glucose	+

Key: +: positive
-: negative

3.1 Morphological and Biochemical Characteristics

Table 1 show the results of the morphological and biochemical characteristics of the bacterial isolate.

Table 2 shows the phytochemical constituent of *Azadirachta indica.* Both the aqueous and ethanol extracts. It was observed that the both extracts contain alkaloid, phenol, tannins, flavonoids, saponin, and steroid.

Table 2. Phytochemical constituent of *Azadriachta indica* leaves extract

Constituents	Aqueous extract	Ethanol extract
Saponin	+	+
Flavoids	+	+
Steroids	+	+
Alkaloids	+	+
Tannins	+	+
Phenol	+	+

KEY: +: positive
-: negative

3.2 Antimicrobial Effects of the Crude Extracts on the Isolates

Table 3 shows the antimicrobial effects of *Azadirachta indica* extracts (ethanol and aqueous) at different concentration on *E. coli* using agar well diffusion method. *Azadrichta indica* ethanol extract showed inhibitory responds of 10.00mm, 10.33mm, 10.67mm and 10.67mm at 125mg/ml, 250mg/ml, 500mg/ml and 1000mg/ml concentration while *Azadriachta indica* aqueous extract showed no inhibitory response to *E. coli*.

Table 4 shows the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the *Azadirachta indica* ethanol and aqueous extracts.

3.3 Wound Healing Studies in Healthy and Diabetic Albino Rats

The ability to heal wound in diabetics albino rats were compared based on pathological changes. After one (1) week of wound inoculation, the wounds swell with abscess formation. By 20 days, there are wound healing in some of the experimental albino rats. The wound healing was determined based on the macroscopic closure of the wound interface and restoration of the epithelial cover.

Table 3. Antimicrobial Effect Of The Extracts On *E. coli*

Concentration (mg/ml)	Mean zone of inhibition ± SD(mm)	
	Aqueous extract	Ethanol extract
62.5	0.00±0.00	0.00±0.00
125	0.00±0.00	10.00±0.00
250	0.00±0.00	10.00±0.58
500	0.00±0.00	10.67±1.15
1000	0.00±0.00	10.67±1.15
+control	27.0±0.00	27.0±0.00
-control	0.00±0.00	0.00±0.00

Positive control: Gentamicin: 27.00±0.00mm
Negative control: sterile distilled water: 0.00mm,
Result represented as Mean±SD

Table 4. Minimum inhibitory Concentration and Minimum bactericidal concentration

Isolates	Aqueous extract		Ethanol extract	
	MIC (mg/ml)	MBC	MIC (mg/ml)	MBC
E. coli	250	1000	250	1000

Tables 5 and 6 show percentage contraction of wounds infection treated with *Azadirachta indica* aqueous extract and *Azadirachta indica* ethanol extract and controls for the selected isolates contaminated wound infection from days 1 to days 28 both in non- diabetic and diabetic albino rats.

Percentage of wound contraction = ((initial wound area – specific day wound area)/ initial wound area) ×100%.

The result of the phytochemical analysis of *A. indica* aqueous and ethanol extracts shows the presence of saponin, flavonoids, steroid, alkaloids, tannins and phenol. This result is in concordance with [3]. Innocent reported that flavonoid is an antioxidant that provides anti-inflammatory activity and that this may be the reason the extracts are used for treatment of wounds.

In this study, the *in vitro* antimicrobial activities of *A. indica* extracts (aqueous and ethanol) were done on the isolates separately and combined. The result of the antimicrobial effect shows that *A. indica* aqueous extract has no inhibitory effect on *E. coli* but *A. indica* ethanol extract shows

inhibitory effect starting from 125mg/ml (10.00mm), 250mg/ml (10.33mm), 500mg/ml (10.67mm) and 1000mg/ml (10.67mm) as shown in table 3. This agreed with the work of [8] for *A. indica* ethanol extract but disagrees with the work for *A. indica* aqueous extracts which have no inhibitory effect on *E. coli*. [7], revealed that different parts of neem plant contain biological compounds responsible for antibacterial, antiviral and antifungal activities. It is considered as safe medicinal plants and modulates the numerous biological processes without any adverse effect. [8] reaveled that *A. indca* extracts both aqueous and ethanol has no effect on *E. coli* and this is also in concordance with the result of this work but differs where my work showed that *A. indica* ethanol has inhibitory effect on *E. coli*.

The minimal inhibitory concentration (MIC) of the extracts on *E. coli* is 250mg/ml while the MBC is 1000mg/ml. Therefore, the extracts show equal activity of 1000mg/ml against the test isolates. [12] revealed that there is correlating increase in antimicrobial activity with increase in extract concentration.

This work involved an attempt to use aqueous and ethanol extracts of *A. indica* leaves as topical antimicrobial and wound healing agents for the treatment and healing of wound infection caused by *E. coli* in diabetic and non-diabetic albino rats. The topical application extracts is an efficient therapeutic method of destroying microbial populations because the availability the drug at the infected wound site leads to enhanced wound healing activity. The virulence capacity of microorganism, infection dose and host immune response are important factors that can cause massive damage during infection [14]. From this study, it appear that the extracts exhibit

Table 5. Percentage wound healing of the Diabetic *E. coli* infected wound

Days	Percentage wound healing by the extracts (%)			
	Povidone iodine	**Vaseline**	**Aqueous extract**	**Ethanol extract**
0	0	0	0	0
2	-26.3165	7.40778	12.96333	20.75442
4	31.57911	18.51889	18.51889	32.07522
6	63.15823	25.92556	29.63	54.71681
8	84.21044	22.2222	66.66667	62.26439
10	100	25.92556	85.18556	81.13163
12	100	18.51889	100	100
14	100	27.77778	100	100
16	100	29.63	100	100
18	100	31.48111	100	100
20	100	29.63	100	100
22	100	33.33333	100	100
24	100	35.18556	100	100
28	100	40.74111	100	100

Table 6. Percentage wound healing of non-diabetic *E. coli* infected wound

Days	Percentage wound healing by the extracts (%)			
	Povidone iodine	**Vaseline**	**Aqueous extract**	**Ethanol extract**
0	0	0	0	0
2	30	0	14.81444	23.63664
4	30	0	18.51889	38.18168
6	56.667	0	37.03667	56.3638
8	91.667	8.33375	66.66667	85.45496
10	100	8.33375	85.18556	92.72694
12	100	8.33375	92.59222	100
14	100	12.5	96.29667	100
16	100	18.75	100	100
18	100	22.91625	100	100
20	100	25	100	100
22	100	25	100	100
24	100	25	100	100
28	100	100	100	100

favorable antimicrobial activities against the test organism and the concentration that gave a good result is 1000mg/ml. DMSO is used as the solvent in all the analysis and Vaseline is used in the formation of gel. These findings are in line with the work of [14] on the wound healing potentials of *Elaeis guinensis* jaeq leaves on infected albino rat model. Vaseline jelly was used in the ointment formulation at ratio of 4:1. Vaseline was used as negative control while povidone iodine is used as positive control.

The process of wound healing involves stages like wound contraction, granuloma formation and collagen maturation. The contraction of wound plays a significant role in healing of excision wound while granuloma formation plays a role in healing of sutured incision and dead space wound. The result of my work observed that the extract formulation treated albino rat showed a higher percentage closure of wound size and rate of wound contraction compared to the value obtain for standard drug- treated and negative control.

The result of my work demonstrated that in wound induced infection with *E. coli*, in diabetic rats, the extracts exhibit wound healing capacity and healed wounds properly at day 12 of the treatment (100%). When compare with the positive control, with *A. indica* aqueous extract having the highest wound healing capacity of 85.19% on the day 10 while *A. indica* ethanol extract has 81.13% wound healing capacity. When compared with the negative group, there is high significant difference (P= 0.05). In healthy rats *A. indica* ethanol extract has 92.73% wound healing capacity on the day 10 *A. indica* aqueous extract 85.19% as shown in Tables 5 and 6 below, there is significant value when compared with untreated group (P< 0.05).

This result agreed with the work of [18] which revealed that *A. indica* extracts showed significant wound healing process by enhancing proliferation phase and this could be as a result of presence of excellent amount of amino acid, vitamin and mineral that are important in wound healing process in proliferation phase.

4. CONCLUSION

The leaf extracts of *A. indica* possess antimicrobial properties for *E. coli* especially the ethanol extract which heals faster than aqueous extract and can be used as an alternative for healing infected non diabetic and chronic diabetic wounds. Therefore, plant extracts can be used for production of topical ointments for wound treatment. Though more research need to be carried out on other pathogens that cause wound infection and other medicinal plants with both antimicrobial and wound healing capacity.

5. LIMITATION

Due to Covid-19 pandemic most patients were out patients and also there were some hospital restrictions on the visit to the medical wards.

ETHICAL CONSIDERATION

Ethical approval was gotten from ethical committee of Amaku Teaching Hospital, Awka with Ref: COOUTH/CMACTH.C/VOl. 1/0090 and also animal ethical approval was gotten from Animal ethical committee of Nnamdi Azikiwe University, Awka with Ref: NAU/ARCE/2021/ 00006.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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