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In Vitro Antidiabetic Evaluation and Wound Healing Activity of Root Bark Extracts of *Gardenia angustifolia* in Streptozotocin-induced Diabetic Rats

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Abstract

This research investigates the glucose-lowering activity and wound-healing effect of the methanol extract of *G. angustifolia* root bark (MEGR) and ethyl acetate extract of *G. angustifolia* root bark (EAGR) on streptozotocin-induced diabetic rats. Qualitative phytochemical analysis of the extracts was conducted according to standard procedures. The glucose-lowering ability of the extracts was assayed by *in vitro* inhibition of α-amylase and α-glucosidase activities. The excisional wound was adopted to evaluate the tissue repair ability of the extracts on diabetic rats. The qualitative phytochemical test identified alkaloids, flavonoids, saponins, total phenols, terpenoids, tannins, and cardiac glycosides in EAGR. The MEGR contains all the aforementioned phytochemicals except tannins and terpenoids. Glucose lowering ability of the extracts studied *in vitro* shows that with a significantly lower ($p < 0.05$) IC_{50} value (34.54 ± 1.31 µg/mL), EAGR has a higher ability to impair the activity of α–glucosidase compared to MEGR (54.03 ± 1.31 µg/mL). Furthermore, with IC_{50} values of 119.59 ± 1.20 µg/mL and 128.13 ± 0.35 µg/mL for EAGR and MEGR, respectively, there is no significant difference ($p > 0.05$) in the ability of both extracts to impair α-amylase activity. On the 20th day post-wounding, ointment formulation from the extracts improved the tissue repair process by promoting the wound contraction rate with a percentage of 81.09 ± 1.24 % and 79.92 ± 1.64 % for EAGR and MEGR treated groups, respectively. Furthermore, the total protein content of regenerating tissues was significantly improved ($p < 0.05$) in the groups treated with EAGR and MEGR, respectively (1.60 ± 0.12 mg/g and 1.57 ± 0.14 mg/g) compared to the diabetic control group (0.68±0.04 mg/g). The ointment formulation from the EAGR and MEGR significantly decreased the period of epithelialization (21.50 ± 0.50 days and 22.25 ± 0.85 days, respectively) compared to the diabetic control group (30.50 ± 0.50 days). The time taken for 50% wound closure was 11.72 days and 12.54 days for groups treated with EAGR and MEGR, respectively. This indicates a shorter time than the diabetic control group (21.24 days). In conclusion, this research indicates the glucose-lowering effect of MEGR and EAGR by impairing the activities of α-amylase and α-glucosidase. Furthermore, the extracts can promote the tissue repair process in the excision wound model of diabetic rats.

Keywords: Diabetes; Excision; Gardenia angustifolia; Ointment; Phytochemicals; Wound healing.

1. Introduction

Diabetes mellitus is linked to decreased insulin production by beta cells in the pancreas or reduced response of the cells to insulin [1]. In the long term, poorly controlled case of diabetes mellitus is linked to some problems, such as damage to the eyes, nerves, and

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kidneys and impaired healing of wounds. Prolonged high blood sugar delays the healing of wounds by impairing the immune system, thereby increasing the chances of infection by microbes and promoting damage to the cells [2]. The current strategies in the management of diabetes mellitus include the use of oral hypoglycemic medications and insulin therapy. Furthermore, surgery, hyperbaric oxygen therapy, and antibiotics are beneficial in managing chronic diabetic wounds [3]. Unfortunately, most of these therapeutic strategies have limitations ranging from high cost, development of allergic reactions, and the potential for wound-associated bacteria to become resistant because of prolonged use of antibiotics. Due to these limitations, alternative therapy is sourced from natural products believed to be more accessible, affordable, and with minimal or no side effects [4].

Gardenia angustifolia (*G. angustifolia*) is a flowering shrub that belongs to the Rubiaceae family, and about 200 species are identified in the genus Gardenia. It is commonly called Cape jasmine and is well known as 'Ikaga' by the Igala tribe of the middle region of Nigeria, West Africa [5]. There are various documented literature reports on the medicinal uses of variants of Gardenia. Koudouvo *et al*. [6] demonstrated the antipyretic effects of *Gardenia ternifolia* leaves. The literature has documented that methanol extracts of the leaves, fruits, and roots of *Gardenia angustifolia* possess wound healing and anti-ulcerogenic effects. Traditional medicine uses herbal preparations from the plant's parts to alleviate diabetes, cough, infertility, and chicken pox [5]. Other documented studies on the *Gardenia angustifolia* plant give details on the *in vivo* antidiabetic effect of the plant's parts; this study differs because it gives a comparative study on the *in vitro* antidiabetic effect of methanol and ethyl acetate extracts of the root bark. Moreover, the wound-healing effect of *Gardenia angustifolia* root bark on diabetic models of experimental rats is yet to be explored. In furtherance, this study aims to investigate the *in vitro* hypoglycemic effect and tissue repair ability of methanol and ethyl acetate extracts of *Gardenia angustifolia* root bark on streptozotocin-induced diabetic rats.

2. Materials and Methods

2.1. Chemicals

Methanol and Ethyl acetate (BDH England), α–glucosidase, α-amylase, ρ- nitrophenyl glucopyranoside (ρNPG), starch, acarbose, Streptozotocin, dinitro salicylic acid (Sigma Aldrich Co. LLC, USA).

2.2. Procurement and Authentication of Plant

Roots of the *Gardenia angustifolia* plant were procured from a farm in Abocho, Dekina local government area of Kogi State, Nigeria. A taxonomist in the Department of Plant Science and Biotechnology, Prince Abubakar Audu University, Anyigba, Nigeria, identified and authenticated the plant and deposited a voucher number of PT-B-152.

2.3. Choice of Solvents for Crude Extraction

Methanol and ethyl acetate were the choice solvents for crude extraction. The selection of solvents was based on their difference in polarity, which would impact the yield obtained and the phytonutrients extracted.

2.4. Crude Extraction Process

The crude extracts were obtained by cold maceration technique according to the protocol outlined by Larayetan *et al.* [7]. The roots of the *Gardenia angustifolia* plant were cleaned, washed, and shade-dried at room temperature. The dried root bark was pulverized using an electric blender. The pulverized root bark (1000g each) was soaked separately in methanol and ethyl acetate solvents for 72 hours. The obtained filtrate from the mixture was concentrated over a water bath to obtain the crude extracts.

2.5. Ethical Approval

The use of experimental animals in this experiment was approved by the Directorate of Research and Innovation of Prince Abubakar Audu University, Anyigba, Nigeria, under the Helsinki protocol and the World Medical Association (WMA) statement on animal use in Biomedical Research (PAAU/DRI07.07.2022).

2.6. Qualitative Phytochemical Analysis

The qualitative determination of phytochemicals in the methanol and ethyl acetate extracts of G. angustifolia root bark was conducted according to standard procedures outlined in **Table 1** [8-10].

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Table 1: Procedures for Qualitative Phytochemical Analysis

Phytochemical	Method
Alkaloids	2 mL of dilute HCl was added to 2.0 mg of extract. The filtered mixture was treated with 3-5 drops of Wagner's reagent. The Formation Reddish-brown precipitate indicates alkaloids.
Flavonoids	5 mL of distilled water was added to 0.5 mg of extract and filtered. 20% sodium hydroxide was added dropwise to 2 mL of the filtrate. The formation of a deep yellow color, which turns clear upon adding dilute hydrochloric acid, indicates flavonoids.
Tannins	5 mL of distilled water was added to 0.5 mg of extract and filtered. To 2 mL of the filtrate, 10 % ferric chloride solution was added. A bluish or greenish color indicates tannins.
Saponins	5 mL of distilled water was added to 0.5 mg of extract and filtered. To 2 mL of the filtrate, 6 mL of water was mixed. Continuous froth observed upon rigorous shaking indicates saponins.
Phenols	5 mL of distilled water was added to 0.5 mg of extract and filtered. A deep blue color obtained upon adding aqueous 5 % ferric chloride to 2 mL filtrate indicates phenols.
Terpenoids	5 mL of distilled water was added to 0.5 mg of extract and filtered. To 2 mL of the filtrate, 1 mL of chloroform was added. Further, the dropwise addition of concentrated sulphuric acid with the formation of a reddish-brown precipitate indicates terpenoids.
Cardiac glycosides	5 mL of distilled water was added to 0.5 mg of extract and filtered. To the filtrate, 2 mL of glacial acetic acid was added dropwise to the Ferric chloride solution. 1 mL of concentrated sulphuric acid was slowly added to the resultant mixture. The formation of a brown ring along the interface indicates a positive test.

2.7. In vitro Antidiabetic Assays

2.7.1. Inhibition of α- Amylase Activity

The inhibition of α – amylase activity by the root bark extracts of *G. angustifolia* was conducted according to the method described by Oboh *et al.* [11]. 500 µL of various dilutions of the extracts were mixed with 500 µL of 0.5 mg/ml of pancreatic α – -amylase in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). Thereafter, 1% (500 µL) starch was mixed and incubated at 25ºC for 10 minutes. 1.0 mL of the color reagent dinitro salicylic acid (DNSA) was added to stop the reaction. In boiling water, the mixture was incubated and cooled to room temperature. Distilled water was added to make up the volume of the reaction mixture to 10 mL, and the absorbance of the sample was measured at 540 nm using a spectrophotometer. The α-amylase inhibitory activity of the extracts was calculated and expressed as percentage inhibition as described in the formula below:

$$\% \text{ Inhibition} = [\,(Abs_{Control} - Abs_{Sample}) / Abs_{Control}\,] \times 100$$

2.7.2. α–glucosidase Inhibition Assay

The inhibition of α- glucosidase activity by the root bark extracts of *G. angustifolia* was conducted according to the method described by Oboh *et al.* [11]. 50 µL of various dilutions of the methanol and ethyl acetate extracts of *G. angustifolia* root bark were mixed with 100 µL of α-glucosidase solution and incubated at 25 ^{0}C for 10 minutes. In the reaction mixture, 50 µL of *p*–nitrophenyl -α- D- glucopyranoside solution (500 mmol/l) was added and further incubated at 25 ^{0}C for 10 minutes. The absorbance of the mixture was read at 405 nm using a spectrophotometer. The α-glucosidase inhibitory activity of the extracts was calculated and expressed as percentage inhibition as described in the formula below:

$$\% \text{ Inhibition} = [\,(Abs_{Control} - Abs_{Sample}) / Abs_{Control}\,] \times 100$$

2.8. Induction of Diabetes Mellitus

The protocol described by Daye *et al.* [12] was adopted to induce diabetes mellitus. Twenty-five (25) albino rats weighing 180 – 200 g were procured from the Department of Biochemistry, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria. Diabetes mellitus was induced by injecting a single dose of 60 mg/kg body weight of streptozotocin intraperitoneally [12]. Streptozotocin is a choice agent to induce type 1 diabetes due to its ability to induce oxidative stress and promote damage to the pancreatic beta cells at the high dose of 60 mg/kg. The damage was induced by streptozotocin results in low insulin levels and subsequent high blood glucose levels [13]. The streptozotocin was dissolved in 0.1ml fresh cold citrate buffer pH 4.5 and was administered to rats after being fasted for 16 hours. 72 hours after injecting streptozotocin to induce hyperglycemia, blood samples were obtained by pricking the tail artery of rats with a sterile lancet to determine blood glucose levels using

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Accu-check glucometer/strips. The experimental rats with blood glucose levels greater than 200mg/dl were considered diabetic and selected for the study.

2.9. Wound Healing Activity

2.9.1. Excisional Model

The protocol outlined by Morton and Malone [14] was adopted for the excision wound model. The diabetic experimental rats were placed under light ether anesthesia. The skin on the dorsal region of the rats was excised to full thickness, and a wound area of 400 mm^2 was obtained. In order to obtain the decrease in wound area, a 1 mm^2 graph paper was used in tracing the margin of the wound. The wound area was measured on the day of wounding and subsequently on the 4th, 8th, 12th, and 16th day post-wounding. The wound contraction rate and the period of epithelialization were determined. The percentage of wound contraction is determined using the formula below:

% Wound contraction =

$$\frac{Wound\ area\ on\ day\ 0 - Wound\ area\ on\ test\ day}{Wound\ area\ on\ day\ 0} \times 100$$

The wound epithelialization time is estimated to be the number of days taken for the scar tissue on the wound to detach without leaving an open wound [15].
The period taken for 50% wound closure (WC_{50}) was obtained by plotting a graph of percentage wound closure against the number of days [16].

2.9.2. Ointment Preparation for Topical Application

Esimone et al. [17] outlined the protocol for preparing the ointment for topical application. A 50% (w/w) of the ethyl acetate and methanol extract ointment of *G. angustifolia* root bark was formulated using a soft paraffin base. The mixture was continuously and gently stirred until a homogenous product was achieved. The semi-solid mixture obtained was packed into a clean ointment jar and labeled.

2.9.3. Experimental Design for Excision Wound Model

Twenty (25) experimental animals were divided into five (5) groups of four (5) rats each.
Group 1: Non-diabetic control treated topically with paraffin base
Group 2: Diabetic control treated topically with paraffin base
Group 3: Diabetic standard treated topically with povidone-iodine
Group 4: Diabetic and treated topically with ethyl-acetate extract of *G. angustifolia* root-bark
Group 5: Diabetic and treated topically with methanol extract of *G. angustifolia* root-bark extract.
All extracts were applied topically on the experimental rats for 24 days.

2.9.4. Estimation of Wound Surface Total Protein

At days 4, 8, 12, and 16 post-wounding, the amount of total protein in the regenerated tissues from the wound sample has healed lesions was measured according to the protocol described by Lowry *et al*. [18].

2.9.5. Determination of NF-kβ p65 Activity

The wound tissue homogenate was evaluated for NF-kβ p65 activity using NF-kβ p65 transcription factor assay ELISA kit according to the protocol as described by Juan *et al*. [15]. Absorbance was read at 450 nm in a microplate reader. The concentration of NF-kβ in the sample was expressed as pg/mL.

2.9.6. Determination of Hydroxyproline Content

In determination of the hydroxyproline content, granulation tissues obtained from the wound were dried for 72 hours at a steady temperature of 60ºC in the oven. 5 mL of 6 N HCl was added to the tissue and kept for 24 hours at 110ºC. The obtained hydrolysate was neutralized to pH=7 and used to determine hydroxyproline content in the healed lesions' regenerated tissues based on Woessner's protocol [19].
The assay results calculated the collagen content by assuming a hydroxyproline content of 13.5%.
Collagen content (µg/mL) = Hydroxyproline level (µg/mL) x 100 / 13.5

2.9.7. Microbial load of Wound Swabs

The protocol described by Sunday *et al.* [20] was used to determine the wound swabs' microbial load assay. Microbial load was determined on the 4th, 8th, 12th, and 16th days post-wounding. Aseptically, wound swabs from each animal were collected in triplicates using swab sticks. Each infected swab was inoculated into the sterile nutrient broth and homogenized to obtain stock solutions for 10-fold serial dilutions using sterile syringes. Aliquots of 1 ml of dilutions (10^{-3}) were introduced into

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sterile petri dishes, after which molten sterile nutrient agar and potato dextrose agar were added and swirled gently. Inoculated plates were allowed to stand for 30 minutes before incubating at 37ºC for 24 hours and 30ºC for 72 hours for nutrient agar plates and potato dextrose agar plates, respectively. After incubation, colonies were counted, and the results were expressed as colony-forming units per milliliter (cfu/mL) of swab stick homogenate.

2.10 Statistical analysis

Results were presented as Mean ± SEM of replicate values. One–way Analysis of Variance (ANOVA) was used to compare data on Graph Pad Prism Windows 10.2.0 (392) (Graph Pad Software, Inc), followed by Tukey's multiple comparison test. A p-value lower than 0.05 was considered significant.

3. Results and Discussion

3.1. Phytochemical Composition of Methanol and Ethyl acetate Extracts of Gardenia angustifolia Root Bark

Phytoconstituents in plants, such as tannins, flavonoids, and phenolics, have been associated with various pharmacological effects, including free radical scavenging activity, glucose-lowering effects, protective effects against microbes, inflammation, and cancer (**Table 2**) [21].

Table 2: Qualitative Phytochemical Contents of Methanol and Ethyl acetate Extracts of *Gardenia angustifolia* Root Bark.

Phytochemicals	EAGR	MEGR
Flavonoids	+	+
Saponins	+	+
Tannins	+	-
Cardiac glycosides	+	+
Alkaloids	+	+
Total phenols	+	+
Terpenoids	+	-

MEGR – Methanol Extract of *Gardenia angustifolia* Root Bark; EAGR – Ethyl acetate Extract of *Gardenia angustifolia* Root Bark; + = Present; - = Absent.

3.2. Inhibition of α-amylase and α-glucosidase activities by Extracts of Gardenia angustifolia Root Bark

In this study, the capacity of MEGR and EAGR to inhibit the carbohydrate digestive actions of α- amylase and α-glucosidase was used to assess the extracts' *in vitro* hypoglycemic potential. The inhibitory effect of acarbose and the extracts on α-amylase and α-glucosidase activity was dose-dependent. Moreover, Acarbose with the lowest IC_{50} of 11.81 ± 0.99 µg/mL is a more potent inhibitor of α-amylase activity compared to the EAGR and MEGR with IC_{50} values of 119.59 ± 1.20 µg/mL and 128.13 ± 0.35 µg/mL respectively (**Figure 1**).The EAGR with a significantly lower IC_{50} value (34.54 ± 1.31 µg/mL) is a more potent inhibitor of α–glucosidase activity compared to the MEGR (54.03 ± 1.31 µg/mL) (**Figure 2**).

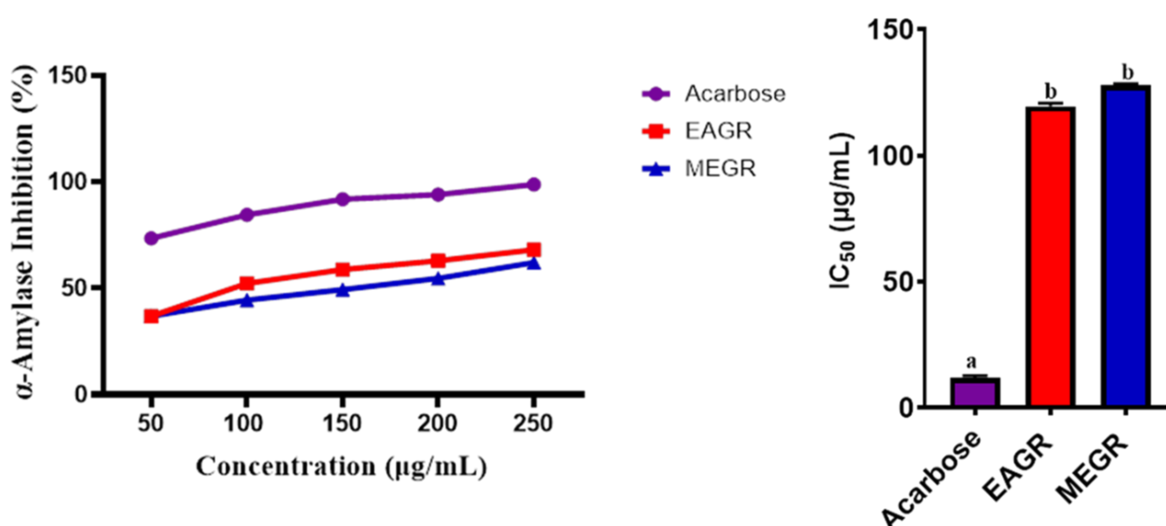

Figure 1: Inhibition of α–amylase activity by methanol and ethyl acetate extracts of *G. angustifolia* Root bark.
MEGR – Methanol Extract of *G. angustifolia* Root Bark
EAGR – Ethyl acetate Extract of *G. angustifolia* Root Bark

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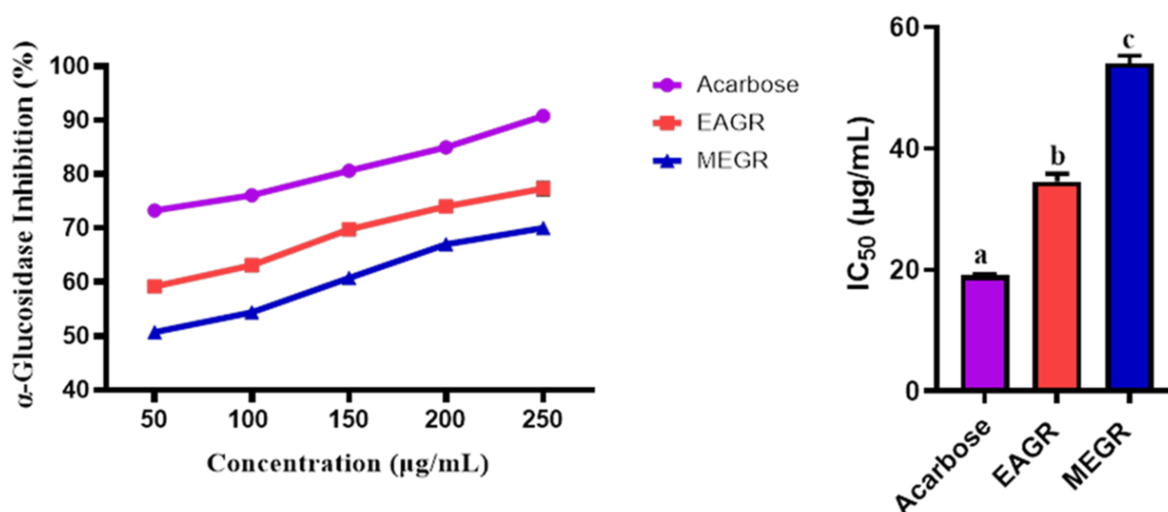

Figure 2: Inhibition of α–glucosidase Activity by methanol and ethyl acetate extracts of *G. angustifolia* Root bark.
MEGR – Methanol Extract of *G. angustifolia* Root Bark
EAGR – Ethyl acetate Extract of *G. angustifolia* Root Bark

The higher effectiveness of EAGR in inhibiting α-glucosidase activity compared to the MEGR could be attributed to the presence of tannins in the EAGR, which is absent in the MEGR. Tannins can bind α–glucosidase, altering its structure by lowering its hydrophobicity and decreasing its catalytic ability to hydrolyze starch [22]. The hydrophobic property of an enzyme limits its association with water, which is important for its stability and proper folding of protein structure, thereby ensuring proper enzyme function [23]. Furthermore, the collective therapeutic effect of the phytoconstituents present in the EAGR can influence its effectiveness in inhibiting α–glucosidase activity [24]. The study conducted by Heba *et al.* [25] also stated that the ethyl acetate extract of *Commiphora myrrha* is a better inhibitor of α–glucosidase activity when compared to the methanol extract.

Impairing the ability of α-amylase and α-glucosidase to digest carbohydrates is important in regulating post-prandial glucose concentrations, which helps to control hyperglycemia. Inhibitors of these carbohydrate hydrolyzing enzymes decrease the rate at which α-amylase converts starch to simple sugars by hydrolyzing the α-1,4-glycosidic bond in starch to give disaccharides and impair the ability of α-glucosidase to convert disaccharides into single glucose units [26]. Literature reports indicate that many plants are natural reservoirs of substances that can impair the activities of α-amylase and α-glucosidase. Therefore, plants with such attributes are valuable in lowering post-prandial glucose concentrations [27]. A diabetic patient with insulin deficiency or insulin resistance lacks the proper mechanism to control the blood sugar level. Therefore, regulating the concentration of post-prandial glucose by preventing the hydrolysis of carbohydrates is key in managing the diabetic condition. The ability of medicinal plants to lower high blood glucose levels can be attributed to phytonutrients such as tannins, total phenols, flavonoids, and terpenoids [28]. The outcome of this study indicates that the carbohydrate hydrolyzing ability of the MEGR and EAGR could be linked with the presence of the aforementioned phytonutrients. The ability of tannins to bind carbohydrates and proteins makes them effective inhibitors of α-amylase activity [29]. This study's result agrees with the study of Gladis and Chellaram [29], which also indicates a dose-dependent inhibition of the functions of α- amylase and α-glucosidase by aqueous extract of *Salacia obolonga*.

3.3. Excision Wound Model

3.3.1. Biophysical Changes of Excision Wound on Diabetic Rats

The biophysical changes studied include wound contraction, time taken for 50% wound closure (WC_{50}), and period of epithelialization. On the 20th day post-wounding, the wound contraction rate was significantly

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higher ($p < 0.05$) in the groups treated with ointment formulated from EAGR and MEGR (81.09 ± 1.24 % and 79.92 ± 1.64 %, respectively) when compared to the diabetic control group (46.17 ± 2.23 %) (**Table 3, Figure 3**). A medicinal agent that facilitates the wound closure rate will promote faster wound healing. Therefore, the increase in wound contraction rate can be attributed to the ability of the extracts to mobilize macrophages for wound repair and improve the synthesis and expansion of keratinocytes [30].

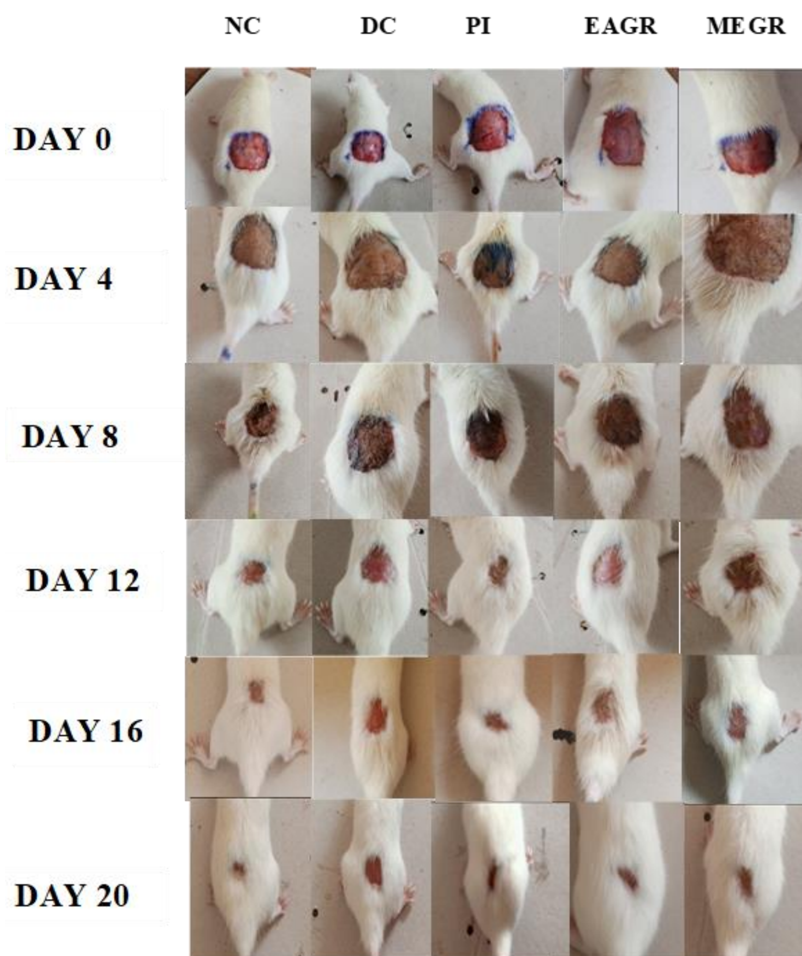

Figure 3: Morphological representations of wound contraction treated topically with EAGR (Ethyl acetate Extract of *G. angustifolia* Root Bark), MEGR (Methanol Extract of *G. angustifolia* Root Bark) and PI (Povidone Iodine). NC – Nondiabetic control, DC – Diabetic control.

Table 3: Effect of Topical Application of Ethyl Acetate and Methanol Extracts of *G. angustifolia* Root bark on Percentage Wound Contraction (%) in Streptozotocin-induced Diabetic Rats.

Days Post wounding	Non-diabetic Control (%)	Diabetic Control (%)	PI (%)	EAGR (%)	MEGR (%)
4	14.00 ± 0.25	1.00 ± 0.41^{a}	7.75 ± 0.38^{ab}	6.00 ±0.28^{ab}	3.00 ± 0.25^{ab}
8	32.60 ± 1.97	9.81 ± 3.62^{a}	31.44 ± 3.31^{b}	30.40 ± 2.24^{b}	27.41±2.00^{ab}
12	66.00 ± 0.38	11.34 ±2.00^{a}	64.89 ± 1.37^{ab}	61.12 ± 1.90^{ab}	55.78±2.47^{ab}
16	84.01 ± 0.91	41.12 ±0.93^{a}	80.67 ± 3.50^{ab}	78.34 ± 0.78^{ab}	70.67±0.81^{ab}
20	96.09 ± 2.24	46.17 ±2.23^{a}	85.00 ± 3.00^{ab}	81.09 ±1.24^{ab}	79.92±1.64^{ab}

Data are presented as Mean ± SEM. (n =4). Superscripts ^{a,b} are significantly different at $p < 0.05$ compared to the non-diabetic control and diabetic control, respectively.
PI – Povidone-iodine, EAGR: Ethyl acetate extract of *G. angustifolia* Root-bark; MEGR: Methanol extract of *G. angustifolia* Root-bark.

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Some literature studies have documented the wound-healing ability of some phytonutrients in plants [31]. The wound-healing qualities of terpenoids are attributed to their free radical scavenging ability and antimicrobial effects. The various stages of wound healing include inflammatory, fibroblastic, and maturation (remodeling) stages. Prostaglandins and arachidonic acid, two inflammatory mediators, are synthesized more frequently in chronic wounds. They hinder wound healing by delaying the transition from the wound-healing phase to the maturation phase [30]. Alkaloids, flavonoids, and steroids possess anti-inflammatory characteristics, which could make them beneficial in the healing of wounds [32]. Moreover, reduced collagen synthesis is linked to a direct impairment in the healing process of wounds [16].

The time taken for 50% wound closure was significantly shorter in the groups treated with the ointment formulated from the extracts compared to the diabetic control group (**Table 4**).

Table 4: Effect of Topical Application of Ethyl Acetate and Methanol Extracts of *G. angustifolia* Root bark on time taken for 50% wound closure (WC_{50}) in Streptozotocin-induced Diabetic Rats.

Groups	WC_{50} (Days)
Non-diabetic Control	10.42
Diabetic Control	21.24
Povidone - iodine (5% solution)	11.22
EAGR	11.72
MEGR	12.54

Data are presented as Mean (n =4). PI - Povidone - iodine; EAGR - Ethyl acetate extract of *G. angustifolia* Root-bark; MEGR -Methanol extract of *G. angustifolia* Root-bark. Each value is the mean wound closure time (WC_{50}).

Furthermore, a decreased period of epithelialization was noted in the groups treated with EAGR and MEGR (21.50 ± 0.50 days and 22.25 ± 0.85 days, respectively) compared to the diabetic control group (30.50 ± 0.50 days) (**Table 5**). The ability of an extract or ointment to decrease the epithelialization period could be attributed to its influence on the growth of epithelial cells, thereby decreasing wound size and facilitating healing [33].

Table 5: Effect of Topical Application of Ethyl Acetate and Methanol Extracts of *G. angustifolia* Root bark on Period of Epithelialization in Excision Wound Model in Streptozotocin-induced Diabetic Rats.

Groups	Period of Epithelialization (Days)
Non-diabetic Control	18.00 ± 0.41
Diabetic Control	30.50 ± 0.50^{a}
Povidone – iodine (5% Solution)	20.75 ± 0.48^{ab}
EAGR	21.50 ± 0.50^{ab}
MEGR	22.25 ± 0.85^{ab}

Data are presented as Mean ± SEM. (n =4). Superscripts ^{a,b} are significantly different at
$p < 0.05$ compared to the non-diabetic control and diabetic control, respectively.
Povidone – iodine (5% Solution). EAGR: Ethyl acetate extract of *G. angustifolia* Root bark; MEGR: Methanol extract of *G. angustifolia* Root bark.

3.3.2. Biochemical Analysis on Granulation Tissue of Excision Wound in Diabetic Rats

This study demonstrates that MEGR and EAGR can enhance the production of protein in the wound tissue of diabetic rats. On the 16th day post-wounding, the total protein content of wound tissue for groups treated with the ointment formulation from MEGR (1.57 ± 0.14 mg/g) and EAGR (1.60 ± 0.12 mg/g) was significantly higher ($p < 0.05$) than the diabetic control group (0.68 ± 0.04 mg/g) (**Table 6**). Consequently, the collagen and hydroxyproline content in the wound tissue of diabetic experimental animals treated with the extract formulation was also significantly higher ($p < 0.05$) than in the diabetic untreated animals (**Table 7**). The improved collagen concentration in a wound indicates a rapid increase in the wound-healing process due to a concomitant increase in tensile strength and enhanced tissue matrix, which facilitates the healing of wounds. Chronic diabetic wound is associated with decreased protein concentration and cellular proliferation of wound tissues [34, 35]. Moreover, a decrease in collagen content in chronic wounds delays the progression from the fibroblastic phase to the maturation phase. The level of expressed Nuclear Factor Kappa β p65 (NFkβ p65) at the site of tissue repair in diabetic untreated experimental animals (46.44 ± 3.04 pg/mL) was significantly higher ($p < 0.05$) in comparison to the diabetic experimental animals treated with EAGR (40.84 ± 0.54 pg/mL) and MEGR (43.88 ± 2.40 pg/mL) (**Table 7**).

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Table 6: Effect of Topical Application of Ethyl Acetate and Methanol Extracts of *G. angustifolia* root Bark on Total Protein Content of Wound Tissue in Streptozotocin-induced Diabetic Rats.

Days Post wounding	Non-diabetic control (mg/g)	Diabetic control (mg/g)	PI (mg/g)	EAGR (mg/g)	MEGR (mg/g)
4	1.81±0.15	0.48± 0.21[a]	1.41± 0.13[ab]	1.11 ±0.08[ab]	0.87±0.07[ab]
8	1.92 ± 0.20	0.59 ± 0.09[a]	1.86±0.16 [b]	1.47±0.10 [ab]	1.46±0.14[ab]
12	1.98±0.14	0.62±0.04[a]	1.88±0.22[b]	1.56±0.05 [ab]	1.50±0.24[ab]
16	2.01±0.25	0.68±0.04[a]	1.94±0.15[b]	1.60±0.12 [ab]	1.57±0.14[ab]

Data are presented as Mean ± SEM. (n =4). Superscripts [a,b] are significantly different at $p < 0.05$ compared to the non-diabetic control and diabetic control, respectively.
PI – Povidone-iodine; EAGR- Ethyl acetate extract of *G. angustifolia* Root bark; MEGR - Methanol extract of *G. angustifolia* Root bark.

Table 7: Effect of Topical Application of Ethyl Acetate and Methanol Extracts of *G. angustifolia* Root-bark on Collagen Content, Hydroxyproline Content and NF-kβ p65 Activity**.**

Groups	Collagen (µg/mL)	Hydroxyproline (µg/mL)	NF-kβ p65 (pg/mL)
Non-diabetic Control	40.29 ± 3.68	5.43 ± 0.63	25.17 ± 2.33
Diabetic Control	24.08 ± 1.40[a]	3.25 ± 0.19[a]	46.44 ± 3.04[a]
Povidone – iodine	37.83 ± 1.26[b]	5.11 ± 0.17[b]	37.96 ± 1.69[ab]
EAGR	31.31 ± 1.66[ab]	4.23 ± 0.22 [ab]	40.84 ± 0.54[ab]
MEGR	28.55 ± 2.33[ab]	3.85 ± 0.45 [ab]	43.88 ± 2.40 [ab]

Data are presented as Mean ± SEM. (n =4). Superscripts [a,b] are significantly different at $p < 0.05$ compared to the non-diabetic and diabetic control respectively. PI – Povidone-iodine; EAGR - Ethyl acetate extract of *G. angustifolia* Root bark; MEGR - Methanol extract of *G. angustifolia* root bark.

NFkβ p65 is a pro-inflammatory cytokine necessary in the inflammatory stage of wound healing. Previous research has revealed that delay in the healing of diabetic wounds can occur due to the upregulation of NFkβ p65 and other cytokines, causing the destruction of tissues, which leads to the development of chronic wounds [36]. The result follows previous studies by Abubakar *et al.* [37] and Juan *et al.* [15], which indicated that decreasing the production of cytokines that promote inflammation, e.g., NFkβ p65, promotes the healing of excisional wounds. Therefore, the wound-healing effect of MEGR and EAGR is also associated with the inactivation of the pathways involved in the expression of the transcription factor NFkβ p65.

3.3.3. Microbial Load Assay of Excision Wound

On the 4th, 8th, 12th, and 16th day post–wounding, topical application of the extracts produced a significant progressive decrease in the total fungal and bacterial count compared to the group of experimental animals that were diabetic and untreated (**Tables 8 & 9**).The healing of a wound can be delayed by the assemblage of bacteria and their lipopolysaccharides at the site of a wound. This assemblage can prolong or impair phagocytosis, delaying the transition from inflammatory to proliferation phases [38]. Another mechanism by which bacteria and their endotoxins slow down the healing of wounds is to increase the synthesis of some pro-inflammatory cytokines, thereby decreasing the proliferative activity of fibroblast and epithelial cells, consequently prolonging the period of epithelialization [39]. Microorganisms destroy the protective barrier in open wounds, thereby making the wound prone to infection. Therefore, the extracts of *G. angustifolia* root bark may promote wound healing by serving as a protective barrier for the wound, decreasing necrotic tissue, and facilitating wound repair [40]. Literature reports have documented that some phytonutrients such as phenols, flavonoids, tannins, and alkaloids exhibit antimicrobial ability, which has been associated with the wound-healing potential of some plants [41]. The phytonutrients in plant extracts make them promising therapeutic targets for treating various ailments such as high blood sugar and healing diabetic foot ulcers. Despite the pharmacological attributes of plant extracts, some limitations are associated with their usage, which include contamination of extracts, herb-herb interaction, toxicity to body organs, and lack of quality assurance. In furtherance of research, focus should be placed on standardizing extracts from plants in order to make them alternatives to conventional medications [42].

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Table 8: Effect of Topical Application of Ethyl Acetate and Methanol Extracts of *G. angustifolia* Root bark on Total Bacterial Count (TBC) of Wound Tissue in Streptozotocin-induced Diabetic Rats.

Days post-wounding	NDC (x10³ CFU/mL)	DC (x10³ CFU/mL)	PI (x10³ CFU/mL)	EAGR (x10³ CFU/mL)	MEGR (x10³ CFU/mL)
4	387.50 ± 2.60	472.75± 3.30[a]	336.75± 2.56[ab]	346.25 ± 1.65[ab]	365.75± 2.56[ab]
8	68.25 ± 2.17	205.75 ±3.33[a]	55.75±2.17[b]	147.25 ±1.80[ab]	166.25 ±2.17[ab]
12	34.50 ± 1.85	147.50 ± 2.97[a]	26.00 ± 2.58[ab]	59.00 ± 3.72[ab]	56.50 ±2.75[ab]
16	11.25 ± 1.31	103.25 ± 2.93[a]	NC	31.50 ± 2.50[ab]	39.00 ± 3.24[ab]

Data are presented as Mean ± SEM. (n =4). Superscripts [a,b] are significantly different at $p < 0.05$ compared to the non-diabetic control and diabetic control respectively.
PI – Povidone-iodine; EAGR- Ethyl acetate extract of *G. angustifolia* Root bark; MEGR -Methanol extract of *G. angustifolia* Root bark; NC – No Count

Table 9: Effect of Topical Application of Ethyl Acetate and Methanol Extracts of *G. angustifolia* Root bark on Total Fungal Count (TFC) of Wound Tissue in Streptozotocin-induced Diabetic Rats.

Days post-wounding	NDC (x10³ CFU/mL)	DC (x10³ CFU/mL)	PI (x10³ CFU/mL)	EAGR (x10³ CFU/mL)	MEGR (x10³ CFU/mL)
4	14.50 ± 0.63	20.75 ± 1.25[a]	11.25 ±1.11[ab]	13.75±0.63[ab]	16.00 ± 1.68[a]
8	11.25 ± 1.11	17.75 ±0.85[a]	8.25 ± 0.85[b]	12.25±1.38[ab]	13.50 ± 1.19[ab]
12	3.25 ± 1.25	12.75 ± 1.11[a]	5.25 ± 1.11[ab]	6.75 ±1.03[ab]	8.75 ±1.49[ab]
16	NC	9.25 ±1.11[a]	NC	NC	2.50 ±0.96[ab]

Data are presented as Mean ± SEM. (n =4). Superscripts [a,b] are significantly different at $p < 0.05$ compared to the non-diabetic control and diabetic control respectively.
PI – Povidone-iodine; EAGR- Ethyl acetate extract of *G. angustifolia* Root bark; MEGR -Methanol extract of *G. angustifolia* Root bark; NC – No Count.

Conclusion

This study justifies the glucose-lowering efficacy of the methanol and ethyl acetate extracts of *G. angustifolia* root bark. Additionally, the ointment formulation obtained from the extracts accelerated tissue repair in excisional wounds of diabetic rats. Therefore, the therapeutic effect of the extracts can be associated with the single or co-acting interactions between the plant's phytonutrients.
The methanol and ethyl acetate crude extracts of *G. angustifolia* root bark have promising therapeutic applications in treating an open sore in a diabetic patient. The extracts also can decrease post–prandial glucose, with the ethyl acetate extract being more effective than the methanol extract. However, more extensive research is needed using *in vivo* models of diabetic rats.

Recommendation

Gas-chromatography mass-spectrometry (GC-MS) analysis and molecular docking studies should be conducted on the extracts to elucidate further their components *in silico* interactions with α – amylase and α–glucosidase.

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Conflict of interest

The authors declare no conflict of interest.

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Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

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