

**Medicinal chemistry** 

# Wound Healing and Anti-Infective Properties of *Myrianthus arboreus* and *Alchornea cordifolia*

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### Abstract

Myrianthus arboreus P. Beauv. (Cecropiaceae) and Alchornea cordifolia (Schum. & Thonn.) Muel. Arg. (Euphorbiaceae) are tropical plants used for the treatment of ailments such as diarrhoea, malaria, boils, dysentery, wounds and skin infections. The study investigated the antimicrobial, antioxidant and wound healing properties of methanol leaf extract of M. arboreus (MLMA), aqueous (AqLAC) and ethanol leaf extracts (ELAC) of A. cordifolia. The antimicrobial activity of the extracts was examined using the agar diffusion and micro-dilution methods against Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 4853, Staphylococcus aureus ATCC 25923, Bacillus subtilis NTCC 10073 and clinical strain of Candida albicans. Antioxidant property of the extracts was determined by DPPH method and wound healing property of the extracts determined using excision wound model. MLMA exhibited activity against S. aureus, B.subtilis, E. coli, P. aeruginosa and C. albicans with MIC values of 8.0, 6.0, 8.0, 10.0 and 6.0 mg/mL, respectively. ELAC showed good antimicrobial activity against S. aureus, B. subtilis, E. coli, P. aeruginosa and C. albicans with MICs of 3.0, 4.0, 6.0, 4.0 and 4.0 mg/mL respectively; and AqACL had MICs of 2.5, 3.0, 10.0, 4.0 and 3.0 mg/mL, respectively. The IC  $_{50}$  of MLMA, AqACL and ELAC were 2.68, 0.79, 0.78 µg/mL respectively. The extracts (5% w/w AqACL and 10% w/w MMAL extract creams) showed potent wound healing capacity with better wound closure (p<0.05) at day 1 and day 9 (p<0.001) compared with untreated wounds. Histological investigations showed enhanced wound tissue proliferation, fibrosis and re-epithelization compared with the untreated wound tissues. Phytochemical screening of extracts revealed the presence of tannins, alkaloids, glycosides, terpenoids and flavonoids. The biological activities of the extracts from the two plants may justify their uses in treatment for microbial infections and wounds.

Keywords: Antibacterial; Antifungal; Wound contraction; Excision wound model

#### Introduction

Various types of wounds including injuries, cuts, pressure, diabetic, gastric and duodenal ulcers continue to exert tremendous impact on the cost of health care delivery systems all over the world and to patients and their dependents, especially in the developed countries with increasing aging population. A wound may be defined as a cut or break in the continuity of any tissue which may arise due to chemical, physical or microbial agent. Wound also include a breakdown in the protective function of the skin, the loss of continuity of epithelium with or without loss of underlying connective tissue following injury to the skin [1]. Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers of the skin or other organs of the body after injury. The wound healing process involves a complex series of interaction between different cell types, cytokine mediators and the extracellular matrix [2]. The dynamic process of wound healing consist of four continuous, overlapping, and precisely programmed phases, namely; haemostasis, inflammation, proliferation and tissue remodeling. The event of each phase must happen in a precise and regulated manner. Interruptions, aberrancies, or prolongation in the process can lead to delayed wound healing or non-healing chronic wound [3].

Wounds that exhibit impaired healing, including delayed acute wounds and chronic wounds, generally have failed to progress through the normal stages of healing. Such wounds frequently enter a state of pathologic inflammation due to a postponed, incomplete, or uncoordinated healing process. Most chronic wounds are ulcers that are associated with ischemia, diabetes mellitus, venous stasis disease, or pressure. The principal goals of wound management are to achieve rapid wound closure and a functional and aesthetic scar [4]. At the site of wound closure a flexible and fine scar with high tensile strength is desired. Once skin is injured, microorganisms that are sequestered at the skin surface obtain access to the underlying tissues and cause infections. Bacteria colonize wounds within 48 hours after injury. The major problem associated with wounds has to do with the high risk of microbial infection. Common bacteria that are normally present in infected and clinically non-infected wounds include *Pseudomonas aeruginosa, Staphylococcus aureus* and  $\beta$ -heamolytic *Streptoccocus sp* [5,6]. The presence of these organisms results in delayed wound healing by prolongation of the inflammatory phase of the healing process [3]. For this reason, suitable antimicrobial agents (either systemic or topical) are needed to reduce the risk of infection and at the long run, reduce the overall time for wound healing.

Medicinal plants are used to treat diseases all over the world especially in developing countries. Their use as therapeutic alternatives is due to their relatively cheaper cost, widespread availability and minimal or no side effects [7].

*Myrianthus arboreus* P. Beauv belongs to the family Cecropiaceae and it is a dioecious shrub or tree which grows up to 20 m tall. It is found growing in forest zones of tropical Africa including Guinea, Sierra Leone, Sudan, Ethiopia, southern part of DR Congo, Tanzania and Angola. It called 'nyankama' in Asante-Twi language in Ghana.

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Extracts of the leaves and leafy shoots of *M. arboreus* are used in the treatment of dysentery, diarrhoea, wounds, boils, dysmenorrhoea and incipient hernia and vomiting. A bark decoction is usually used to treat malaria, fever, cough, muscular pains, fractures and haemorrhoids [8-10]. The oil from the leaves is mainly made up of linoleic acids. Several pentacyclic triterpenoids have been isolated from the wood and the roots. Euscaphic acid, myrianthic acid, tormentic acid, ursolic acid and a derivative of ursenoic acid have been isolated from stems. Myrianthinic acid was isolated from the bark. The wood also contains myrianthiphyllin, a lignan cinnamate. Bark extracts of *M. arboreus* exhibited antiplasmodial, antimycobacterial and antitrypanosomal activities [8].

Alchornea cordifolia (Schum. &Thonn.) Muell. Arg. of the family Euphorbiaceae is an evergreen dioecious shrub which grows up to 8 m tall and it grows in the eastern part of Senegal to Kenya and Tanzania and throughout Central Africa to Angola. It is also cultivated in DR Congo for its medicinal purposes [11]. A. cordifolia is known in Asante-Twi dialect as 'agyama'. The leaves are normally used as infusions for the treatment of respiratory problems such as sore throat, cough and bronchitis, and also for management of intestinal problems such as gastric ulcers, diarrhoea, amoebic dysentery and worms. The poultice of the leaves is used for the treatment of wounds. The leaves and root bark of A. cordifolia are externally applied to treat leprosy and as antidote to snake venom [9-11]. The leaves, roots and stem bark have been found to contain terpenoids, steroid glycosides, flavonoids, tannins, saponins, carbohydrate and imidazopyrimidine alkaloids, alchorneine, alchornidine and several guanidine alkaloids. The leaves also contain a range of hydroxybenzoic acid, namely, gallic acid and its ester, anthralinic acid, protocatechuic acid and ellagic acid. Alchornoic acid has been also found in the seed oils [11]. Different extracts of the leaf, stem bark and root of A. cordifolia have been shown to have significant antibacterial and antifungal activity. The ethanol extracts of the leaves and fruits have shown significant trypanocidal, anthelminthic, amoebicidal and antimicrobial activities [11]. The study is designed to investigate the wound healing, antioxidant, antimicrobial properties of the methanol, ethanol and aqueous leaf extracts of M. arboreus and A. cordifolia.

# **Materials and Methods**

# Plant materials and chemicals

The fresh leaves of *M. arboreus* and *A. cordifolia* were collected from Krofrom in Atwima-Kwanwoma district of the Ashanti Region, Ghana in July, 2012 by Nana Yaw Atefa and authenticated by Dr. Alex Asase, Ghana Herbarium, Department of Botany, and University of Ghana, Legon, Accra, Ghana. Voucher specimens of the *M. arboreus* and *A. cordifolia* with voucher specimen numbers of AA 119 and AA 124 respectively, have been deposited at the Ghana Herbarium, University of Ghana, Legon, Accra, Ghana. The collected parts of the medicinal plants were dried at room temperature (28-30°C) for two weeks. The dried plants parts were then milled into powdered materials. All the chemicals and reagents were purchased from Sigma-Aldrich, St Louis, Missouri, USA, unless otherwise indicated.

# Extraction of plant materials

Twenty grams of dried powdered material of leaves of *M. arboreus* were weighed and added to 300 mL of 70% v/v methanol. Ultra-turrax<sup>\*</sup> T25 (homogeniser) (IKA, Wilmington, North Carolina, USA) was used to mix thoroughly the solvent and powdered plant material with a speed of 24000 m/min for 2 to 5 min under ice-cooling. The resultant mixture was filtered and rotary vapour was then used to concentrate the extracts at 40°C and lyophilized. The extraction procedure was

repeated for dried powdered leaves of *A. cordifolia* using sterile distilled water to obtain the aqueous extract, and 70% v/v ethanol to obtain the ethanol extract. The dried solid extracts were then weighed and yield determined.

#### Phytochemical screening of extracts

Preliminary phytochemical screening for secondary metabolites in the extracts was performed according the methods described by Evans, Safowora, and Harborne [12-14]. The amount of tannin in the extracts was determined according the method described by Agyare et al. [15].

### HPLC finger-printing of extracts

The HPLC chromtograms of the leaf extracts of *M. arboreus* and *A. cordifolia* were determined according to the method described by Agyare et al. [15]. Below HPLC conditions were used on a Thermo Finnigan HPLC system with Hypersil Gold C<sub>18</sub> reversed-phase (150x4.6 mm) as the column. The concentration of extracts used for the analysis was 10 mg/mL. The optimum HPLC conditions for the analysis were: injection volume: 10  $\mu$ L, detection wavelength: 260 nm, mobile phase: 0.1% acetic acid: acetonitrile (60:40 v/v, isocratic condition), temperature: 22°C, pump pressure: 28 MPa, flow rate: 1mL/min and running time: 10 min. The retention times for the various peaks for the extracts were determined.

Determination of antimicrobial activity: The activity of the extracts against the test organisms was determined according to the modified method described by Agyare et al. [16]. Twenty mililiters of nutrient agar (Oxoid, UK) was melted and stabilized at 45°C for 15 min. The stabilized agar was then seeded with 100 µL of 10<sup>6</sup> colony forming unit (cfu)/mL of a 24 h culture of each test organism (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 4853, Staphylococcus aureus ATCC 25923, Bacillus subtilis NTCC 10073 and clinical strain of Candida albicans). The seeded agar was then poured aseptically and allowed to set in sterile 90 mm petri dish. In each plate, 4 wells were made with a sterile cork borer with internal diameter of 8 mm and labelled appropriately. Each cup was filled to about 100 µL of the different concentrations of the extracts (12.5, 25.0, 50.0, 100.0, 200.0 and 300.0 mg/mL for the M. arboreus leaf extracts and 12.5, 25.0, 50.0 and 100.0 mg/mL for A. cordifolia leaf extracts) and 10.0 µg/mL and 100.0 µg/mL of ciprofloxacin and ketoconazole as the reference antibacterial and antifungal agents, respectively. The plates were allowed to stand for an hour on the bench to ensure adequate diffusion of the extracts and reference compounds. The plates with bacteria were then incubated at 37°C for 24 h and that of C. albicans was incubated at 30°C for 3 days. The experiments were performed in triplicate. The zones of growth inhibition were measured and mean zones of inhibition determined.

Determination of minimum inhibitory concentration (MIC): The MICs of the extracts against the test organisms were determined according to the modified methods described by Agyare et al. [16] and Eloff [17]. The calculated volumes of the test solutions (extracts) in the wells were serially diluted 50% (50 µL) with double strength nutrient broth (Oxoid, UK) and 10 µL of a 24 h old culture (107 cfu/mL) of S. aureus added to each well in 96-well plate. The required volume of the sterile water was then added to each well to make it up to the required volume of 100 µL and to obtain the required concentrations (2.5, 5.0, 7.5 and 10.0 mg/mL). The two plates were incubated at 37°C for 24 h. To indicate bacterial growth, 10 µL of 125 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-dipheylteatrazlium bromide) were added to each well and incubated at 37°C for 10 min. After MTT addition, wells with bacterial growth changed colour from yellow to blue/purple whiles those with no bacterial growth retained the yellow colour of the MTT. The above procedure was repeated for B.

*subtilis, E. coli, P. aeruginosa* and *C. albicans*, respectively. The wells with minimum concentrations that inhibited microbial growth were recorded as the MIC. The experiments were made in triplicate.

#### Preparation of aqueous cream of extracts

Various concentrations of aqueous creams of extracts (5.0 and 10.0% w/w) of *M. arboreus* and *A. cordifolia* and emulsifying ointment BP (as the base for the aqueous creams) were prepared according to the methods described in British Pharmacopoeia [18] without the addition of methyl paraben as preservative.

**Determination of antimicrobial activity of formulated creams of different extracts:** The agar well diffusion methods described by Agyare et al. [16] and Ugboduma et al. [19] as earlier described (section 2.5.1) were used for the determination of antimicrobial activity of the extracts was again used in evaluating the activity of the formulated aqueous creams of the different concentrations (5.0 and 10.0% w/w) of extracts against the test organisms.

# Determination of antioxidant activity

The antioxidant activity of the extracts was carried out according to the modified method described by Chizzola et al. [20] using 1,1-diphenyl-2-picryl-hydrazyl (DPPH). Solution of 0.002%w/v of DPPH in methanol was prepared and 3 mL of this solution were added to 1 mL of extracts in test tubes at different concentrations of 25.0, 12.5, 6.25, 3.125, 1.563, 0.781 and 0.391 µg/mL. Different concentrations of  $\alpha$ -tocopherol (vitamin E) were also prepared in 70% v/v methanol and 1 mL each was added 3 mL of DPPH in test tubes to serve as the reference antioxidant. The tubes were shaken and allowed to stand in the dark for 30 min after which the absorbances were measured at 517 nm. Absorbance at 517 nm for 1mL methanol (70% v/v) and 2 mL DPPH served as the control (A<sub>0</sub>); whiles the absorbance for 70% methanol alone represented the blank. Inhibitions of free radical scavenging activities were calculated using the formula:

(%) Inhibition= ((A0 – A1)/A0 x 100)

Where, A0=absorbance of the control and A1= absorbance of the test sample at 517 nm.

#### Ethical approval for animal work

The animals were given enough water and maintained under laboratory conditions (i.e. room temperature, 24 to 28°C, relative humidity, 60-70% and 12 h light/dark cycle). The rats were kept in the laboratory for one week before the experiment. This was done to reduce the stress of experimenter handling and conditions. Techniques and methods used in this study were performed in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (National Institutes of Health (NIH), US, Department of Health Services Publication no. 83-23, revised 1985). The protocols for the study were approved (Pharm/EthC/X8122013) by the Department of Pharmacology Ethics Committee, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Rate of wound healing (excision wound model): Forty two female Sprague-Dawely rats weighing averagely 115 g were anaesthetized with ketamine (120 mg/kg) per body weight subcutaneously prior to the creation of the wounds. The dorsal fur of the animals were shaved to a circular diameter of about 40 mm by means of razor blades and the anticipated area of the wound to be created were outlined on the shaved skin. These areas were cleaned with 70% v/v ethanol before the excision wounds were created. The entire wounds were left opened and the animals divided into six (6) groups of seven animals each. The first group was topically treated with 1% w/w silver sulphadiazine ointment (Arytons Drugs, Ghana). The second group treated with aqueous cream as a base or vehicle (negative control) and third animals was left untreated and allowed for natural wound healing to take place. The forth group was treated with concentration of 10% w/w of methanol leaf extract of *M. arboreus*. The fifth and sixth groups were treated with concentration of 5% w/w aqueous extract of A. cordifolia and 5% w/w ethanol leaf extract of A. cordifolia respectively. Wound treatment commenced on the 2<sup>nd</sup> day of wound creation. The concentrations of the aqueous cream extracts for the wound healing assay were based on their antimicrobial activities. The reference drug and extracts were topically applied to the wounds 24 hourly for 11 days. In the course of treatment, scaled photographs of the wound areas were taken (by means of high resolution digital camera Olympus digital camera, Cameron Sino, Hong Kong) alongside a millimetre scale measurement every 48 h starting from the 1st day of wound treatment. Wound size or contractions were measured in 3 days interval, until complete wound healing. The wound areas were determined.

# Histological investigations

Wound tissues were taken from both the treated and untreated groups on day 10 after creation of wounds for histological studies. Sectioning of the tissues was done in the grossing room and the tissues placed in a cassette. The tissues were then dehydrated using a sequence of ethanol-xylene series of solutions. The tissues were embedded with paraffin at 60°C, and then trimmed with the microtome at 25  $\mu$  and then sectioned at 4  $\mu$ . The processed sections were then stained with haematoxylin and eosin and then observed under the microscope.

# Statistical analysis

GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. Data are presented as mean  $\pm$  SEM (*N*=5) and analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. \*p<0.05, \*\*p<0.01 and \*\*\*p<0.001.

# Results

# Phytochemical screening of extracts

All the extracts (MMAL, AqACL and EACL) contained flavonoids, alkaloids, terpenoids, glycosides and tannins with varying amounts. Sterols were not found in MMAL extract (Table 1).

# HPLC profile of extracts

HPLC profile of the extracts revealed that they contained high

Extract	Yield								
	%w/w	%TC	Tannins	Saponins	Glycoside	Flavonoids	Alkaloids	Sterols	Terpenoids
MMAL	10.01	0.72	+	+	+	+	+	-	+
AqACL	12.53	0.81	+	+	+	+	+	+	+
EACL	16.48	0.98	+	-	+	+	+	+	+

Legend: MMAL=methanol leaf extract of *M. arboreus*; AqACL=Aqueous leaf extract of *A. cordifolia*; EACL=ethanol leaf extract of *A. cordifolia*; TC % w/w: percentage of tannin content (related to the dried plant material); + = present, - = absent of secondary metabolites

Table 1: Phytochemical screening of the extracts.

amounts (area under curve) of polar components (peaks) which eluted within the first 6 min (Figures 1-3). These chromatograms can also be used for identification purposes for the individual extracts.

# Antimicrobial activity of extracts

The three extracts (MMAL, AqACL and EACL) were active against all the test organisms within the concentration range of 25.0 to 100.0







Extracts(mg/mL)	S. aureus	B. subtilis	E. coli	P. aeruginosa	C. albicans
MMAL					
12.5	13.33 ± 0.00	0.0	11.33 ± 0.33	0.0	14.00 ± 0.58
25.0	14.33 ± 0.67	0.0	12.33 ± 0.33	0.0	16.33 ± 0.88
50.0	17.67 ± 0.33	0.0	13.67 ± 0.67	0.0	19.67 ± 0.33
100.0	20.33 ± 0.33	0.0	15.33 ± 0.88	0.0	22.67 ± 0.33
200.0	nd	17.67 ± 0.33	nd	0.0	nd
300.0	nd	21.33 ± 0.33	nd	13.33 ± 0.88	nd
AqACL					
12.5	0.0	0.0	0.0	0.0	0.0
25.0	14.33 ± 0.33	0.0	0.0	0.0	0.0
50.0	21.00 ± 0.57	21.67 ± 0.88	0.0	14.67 ± 0.33	24.67 ± 2.60
100.0	24.67 ± 1.45	24.67 ± 0.33	15.00 ± 0.00	25.33 ± 1.45	29.00 ± 2.08
EACL					
12.5	22.33 ± 1.45	0.0	0.0	0.0	0.0
25.0	20.67 ± 1.67	15.33 ± 0.88	0.0	15.67 ± 0.33	20.33 ± 0.33
50.0	23.33 ± 0.67	20.33 ± 0.33	11.33 ± 0.33	17.00 ± 2.89	24.00 ± 1.00
100.0	25.67 ± 0.67	25.00 ± 0.00	16.00 ± 0.58	21.00 ± 0.58	30.67 ± 0.67
Cipro (10µg/mL)	24.33 ± 0.67	30.67 ± 0.67	26.67 ± 0.88	26.67 ± 0.88	nd
Keto (100µg/mL)	nd	nd	nd	nd	20.67 ± 0.33

MMAL=Methanol leaf extract of *M. arboreus*; AqACL=Aqueous leaf extract of *A. cordifolia*; EACL=Ethanol leaf extract of *A. cordifolia*; Cipro=Ciprofloxacin; Keto=Ketoconazole; diameter of well: 8 mm; N=3; SEM=Standard Error Mean; nd=not determined

Table 2: Antimicrobial activity of M. arboreus and A. cordifolia extracts.

Organisms	MMAL (mg/mL)	AqACL (mg/mL)	EACL (mg/mL)		Ketoconazole (µg/mL)
S. aureus	8.0	2.5	3.0	0.25	nd
B. subtilis	6.0	3.0	4.0	0.10	nd
E. coli	8.0	10.0	6.0	0.13	nd
P. aeruginosa	10.0	10.0	4.0	0.25	nd
C. albicans	6.0	3.0	4.0	nd	5.0

MMAL=Methanol leaf extract of A. arboreus; AqACL=Aqueous leaf extract of A. cordifolia; EACL=Ethanol leaf extract of A. cordifolia; nd=not determined

Table 3: Minimum inhibitory concentrations (MIC) of extracts.

(Figure 5).

mg/mL. MMAL extract even at high concentration of 200 mg/mL was not active against *P. aureginosa* (Table 2) according to the agar diffusion method. The extracts had MIC range of 2.5 to 8.0 mg/mL against the Gram-positive bacteria and 3.0 to 10.0 mg/mL against the Gram-negative bacteria with *P. aureginosa* having the highest MIC of 10.0 mg/mL (Table 3). *M. arboreus* (10.0% w/w) and 5.0% w/w of *A. cordifolia* aqueous extract creams exhibited moderate activity against all test organisms (data not provided). Hence, these concentrations of extracts were selected for the *in vivo* wound healing studies.

#### Antioxidant activity

Both extracts of *A. cordifolia* (AqACL and EACL) exhibited strong free radical scavenging activity with  $IC_{50}$  of 0.79 and 0.78 µg/mL respectively, with the MMAL having  $IC_{50}$  of 2.67 µg/mL (Figure 4).

#### Rate of wound contraction

The aqueous creams of methanol leaf extract of *M. arboreus* (10% w/w) and aqueous leaf extract *A. cordifolia* (5% w/w) had significant influence on the rate of wound closure at day 1 (p<0.01), 9 (p<0.001) and 11 (p<0.05) compared with the untreated wounds. The ethanol leaf extract of *A. cordifolia* (5% w/w) did show any significant increase in wound contraction compared with the untreated wounds (Table 4).

#### Histological investigations

Both treated and untreated wound tissues showed different degrees of fibrosis. Wounds treated with 10% w/w *M. arboreus* and 5% w/w aqueous creams of both aqueous and ethanol leaf extracts of *A. cordifolia* showed higher degrees of fibrosis with faster wound healing than that of the untreated. There was increased proliferation

C of **Discussion** f A. The phytochemical screening test showed the presence of tannins, alkaloids, glycosides, saponins, flavonoids and terpenoids in the plant

alkaloids, glycosides, saponins, flavonoids and terpenoids in the plant extracts. The strong antioxidant effect of plant extracts with  $IC_{50}$  values below 3.0 µg/mL may be primarily due to the presence of polyphenols such as phenols, flavonoids and tannins [21,22]. Phytochemical constituents present in the extracts may play a major role in wound healing. Flavonoids and tannins are known to promote wound healing process due to their astringent and antimicrobial activities which are responsible for the increased wound contraction and faster reepithelization. They prevent oxidative damage in wound site that could arise from production of free radicals during the inflammatory phase of the healing process and also inhibit the growth of possible microbial contaminants that may cause infection in wounds [1].

of fibroblasts and collagen formation in the treated wound tissues. The

untreated wounds showed only thin layer of fibrosis of the epidermis

Methanol leaf extract of *M. arboreus* exhibited antimicrobial activity at higher concentration compared with the extracts of *A. cordifolia*. The extracts of *M. arboreus* and *A. cordifolia* had MIC range of 2.5 to 8.0 mg/mL against the Gram-positive bacteria and 3.0 to 10.0 mg/mL against the Gram-negative bacteria. The antimicrobial activity exhibited by *A. cordifolia* confirms the earlier reports by Ebi [23] and Mavar-Manga et al. [11]. The comparatively low MIC of *M. arboreus* against the test organisms with the micro-dilution method may be due to the inability of the active principles in the extracts to diffuse into the agar medium [15]. The MIC values show the plant extracts

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Figure 4: Free radical scavenging activity of *M. arboreus, A. cordifolia* and α-tocopherol. MMAL=methanol leaf extract of *M. arboreus*; AqACL=aqueous leaf extract of *A. cordifolia*; EACL=ethanol leaf extract of *A. cordifolia*.

Mean wound diameter (mm) ± SEM								
Day	Untreated	Cream only	S/S (1% w/w)	MMAL (10% w/w)	AqACL (5% w/w)	EACL (5% w/w)		
1	27.00 ± 1.09	30.20 ± 2.22	25.80 ± 0.96	22.80 ± 0.58 *	22.60 ± 0.67 *	25.40 ± 0.40		
3	$20.54 \pm 0.45$	24.60 ± 1.32	19.60 ± 0.87	21.40 ± 0.60	19.40 ± 1.12	22.80 ± 1.06		
5	19.94 ± 0.36	23.60 ± 1.83	17.32 ± 0.84	21.20 ± 1.24	17.80 ± 0.86	20.20 ± 0.80		
7	18.28 ± 0.75	19.00 ± 1.22	14.00 ± 0.79 *	17.20 ± 0.96	14.40 ± 0.67	17.20 ± 0.80		
9	15.26 ± 0.82	11.80 ± 0.37	9.88 ± 0.91**	9.20 ± 0.58 ***	9.60 ± 0.60 **	11.60 ± 0.92		
11	10.84 ± 0.41	8.20 ± 0.20	7.08 ± 0.90	6.80 ± 0.80 *	6.60 ± 0.97 *	8.40 ± 0.74		

MMAL=methanol leaf extract of *M. arboreus*; AqACL=aqueous leaf of *A. cordifolia*; EACL=ethanol leaf extract of *A. cordifolia*; S/S= silver sulphadiazine; N=5 rats per group; \*p<0.05, \*\*p<0.01, \*\*\*p<0.01, \*\*



have significant activity against the bacteria and *C. albicans*. According Fabry et al. [23], extracts with MIC values below 8.0 mg/mL show effective antimicrobial activity.

Common pathogens that cause infections in wounds are *Streptococcus sp, Staphylococcus sp and Pseudomonas sp* [5,6]. From results, it was observed that all the extracts exhibited some level of antimicrobial activity against *S. aureus* and *P. aureginosa* based on

their low MICs. And topical application of antimicrobials is an efficient therapeutic method of destroying microbial population because of the availability of the drug at the wound sites.

The antimicrobial activity of the extracts may be attributed to the presence of terpenoids and other secondary metabolites. Terpenoids are known to have this activity due to the possible effect on the nonmevalonate pathway. This pathway is very essential in Gram-negative bacteria, fungi, protozoans and other microorganisms for the synthesis of cell membrane components and as a secondary source of carbon [24].

With respect to the wound healing rate, wound size decreased significantly with time in the animals treated with aqueous leaf extract of A. cordifolia at day 1 (p<0.05), 9 (p<0.01) and day 11 (p<0.05) compared with the untreated groups. The methanol leaf extract of *M. arboreus* also significantly increase the wound contraction at day 1 (p<0.05), day 9 (p<0.001) and day 11 (p<0.05) with 69.95% wound closure observed on day 11 compared to the untreated. There was no significant (p>0.05) influence of the ethanol extract of A. cordifolia compared to untreated wounds during the treatment period (Table 4). The increased wound contraction observed in this group can be better explained using the histological findings. This was an increase granulation tissues, proliferation of fibroblasts and fibrosis in treated wound tissues compared with the untreated. There was also increase in fibrosis because of the high levels of fibroblasts and this will therefore lead to increases in collagen formation. Thus, the increase wound contraction in the treated group may be due to the enhanced proliferation of fibroblasts and successful elimination of microbial agents [25]. These findings may support the claims by herbalists and traditional healers that wound treated with these plant extracts heal better and faster.

#### Conclusion

The methanol leaf extract of *M. arboreus* as well as the aqueous and ethanol and aqueous leaf extracts of *A. cordifolia* exhibited antimicrobial and antioxidant activities. There was an increment in the wound contraction, fibroblast proliferation and fibrosis of wounds treated with methanol leaf extract of *M. arboreus* and aqueous leaf extract of *A. cordifolia* compared with the untreated.

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