

Medicinal plants used for treatment of wounds and skin infections: assessment of wound healing and antimicrobial properties of *Mallotus oppositifolius* and *Momordica charantia*

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Abstract

Mallotus oppositifolius and *Momordica charantia* are two medicinal plants used in folk medicine for the treatment of skin infections and disorders, wounds, peptic ulcer, fever, piles and parasitic infections. The aim of the study was to determine the antimicrobial, antioxidant and wound healing properties of the methanol leaf extracts of *M. oppositifolius* and *M. charantia*. The two plants extracts were screened for their phytochemical composition and their antimicrobial activity was also determined against three gram-positive, two gram-negative bacteria and a fungus. The antioxidant or free radical scavenging activity of the methanol leaf extracts was also determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH). The wound healing activity of the extracts was determined by the excision wound model in rats. The results indicated that both extracts contain tannins, alkaloids, flavonoids and glycosides and *M. charantia* contains saponins. The two extracts exhibited activity against all the test organisms. MICs of the methanol leaf extract of *M. oppositifolius* was between 0.01 to 3.75 mg/mL and that of *M. charantia* was between 1.88 to 7.5 mg/mL. The IC₅₀ of methanol leaf extracts of *M. oppositifolius* and *M. charantia* were 16.11 and 7.09 µg/mL respectively. The wound contraction rate was significant ($p < 0.05$) for both extracts of *M. oppositifolius* and *M. charantia* on the 11th day compared to the untreated group. The extracts exhibited high antimicrobial and antioxidant activities and increased fibrosis and collagenation in the wound tissues and the rate of wound closure compared with the untreated wounds at the late phase of the wound healing process.

Keywords: Antimicrobial, antioxidant, *Mallotus oppositifolius*, *Momordica charantia*, wound healing.

Introduction

Wound is defined as the breaking of the cellular and anatomical continuity of a tissue. It may come about through physical, chemical, thermal, microbial or immunological attack to the tissue [1]. The processes leading to wound healing consist of several cellular and biochemical events including haemostasis, inflammation, proliferation and regeneration and these eventually lead to reinstatement of the structural and functional integrity with regain of the strength of the injured tissue [2]. Following the breaking of the tissue, microorganisms may invade the wound leading to infection which may result in delayed healing or non-healing wounds. Non-healing wound is associated with significant morbidity and mortality in many populations especially aging population [3]. Therefore, if an agent with activity against the microorganisms commonly implicated in wounds is employed it may facilitate the healing process and reduce the healing period. In inflammatory conditions chemical mediators such as macrophages and neutrophils are released in an attempt to control foreign debris and microorganisms. This leads to the generation of

free radicals which the body's natural anti-oxidants should mop up. But if there is excess this can lead to tissue damage which can also prolong wound healing. Antioxidants therefore play an essential part in protecting the human body against damage caused by reactive oxygen species [4]. The endogenous antioxidant enzymes such as superoxide dismutase, catalase, glutathione NADPH oxidase and glutathione peroxidase help to neutralize the harmful effects of these oxygen free radicals [5]. Hence, if an antioxidant is used it can help reduce the excess free radicals which will ultimately lead to reduced healing period. Several medicinal plants are used for the treatment of various forms of wounds for several centuries [6]. Some of these plants such as *Justicia flava*, *Phyllanthus muellerianus* etc have been evaluated and found to increase the wound healing process [7, 8].

Mallotus oppositifolius (Geiseler) Muel Arg. belongs to the family Euphorbiaceae. In Ghana among the Asante-Twi speaking community it is called 'Nyanyafurowa'. In Africa the plant is distributed in Gabon, Nigeria, Senegal, Ethiopia, Angola and Mozambique. The leaves are used for the treatment of skin and



eye infections, wounds, pains, parasitic diseases and swellings [6, 9, 10]. The leaves and stem bark have been found to possess some medicinal properties such as analgesia, anti-inflammatory, antibacterial, anthelmintic and haemostatic agents [11, 12]. The ethanol root extract has been shown to exhibit antibacterial activity [9]. Hydro-alcoholic extract of the leaves has been found to exert central nervous effects including antidepressant, anticonvulsant and analgesic effects [13].

Momordica charantia L. also known as 'bitter melon' or 'bitter melon' belongs to the family Cucurbitaceae. In Ghana, among the Asante-Twi speaking people it is known as 'Nyanya'. *M. charantia* is found in Asia, Amazonia, Caribbean regions, Africa, South America and sub-tropical regions as well as the temperate regions [14]. It is used in folklore medicine for management of skin infections, wounds, peptic ulcer, fever, piles and parasitic infections [6, 10]. Essential oils from seeds of *M. charantia* has been found to possess antidiabetic, carminative, anthelmintic, antimalarial, antimicrobial and antiviral properties [15]. The methanolic fruit extract of *M. charantia* has been found to possess healing and preventive effect on gastric ulcer in rats [16]. The aim of this study was to determine the extent and potential of methanol leaf extracts of *M. oppositifolius* and *M. charantia* to influence wound healing process and also to determine the antimicrobial and antioxidant properties of the extracts.

Materials and methods

Plant materials and chemicals

Fresh leaves of *Mallotus oppositifolius* and *Momordica charantia* were collected from Krofom, Atwima-Kwanwoma District of Ashanti Region in Ghana in July, 2012 and identified by Dr. Alex Asaase, Department of Botany, University of Ghana, Legon, Ghana and voucher specimen each the of plant material has been kept at the Ghana Herbarium, University of Ghana, Ghana.

All chemicals and reagents used were purchased from Sigma-Aldrich, Disendorf, Germany unless otherwise stated.

The plant materials (fresh leaves of *M. oppositifolius* and *M. charantia*) were air dried (28 to 30°C), pulverized and extracted with Ultra-Turrax® T25 (IKA, Wilmington, North Carolina, USA) at 4000 rpm using methanol-water (7:3) for 3 min under ice-cooling, centrifuged and filtered. The filtrate was concentrated under vacuum at 40°C and lyophilized.

Phytochemical screening

Preliminary phytochemical tests were conducted on the methanol leaf extracts of *M. oppositifolius* and *M. charantia* to determine the presence of tannins, alkaloids, saponins, glycosides and flavonoids as described by Evans [17] and total amount of tannins in each extract determined [18].

HPLC finger-printing of methanol extracts

The HPLC analysis of methanol leaf extracts of *M. charantia* and *M. oppositifolius* were performed on a Thermo Finnigan HPLC system using Hypersil Gold C₁₈, reversed-phase column (150 x 4.6 mm). The concentration of extracts used for the analysis was 10 mg/mL. The optimum HPLC conditions for the analysis were: injection volume: 10 µL, 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.

Antimicrobial activity

Determination of antibacterial and antifungal activity of extracts by agar well diffusion method

The antimicrobial activities of the extracts of *M. oppositifolius* and *M. charantia* as well as the reference drugs (ciprofloxacin and ketoconazole) were determined according to the modified method described by Wiegand and his colleagues [18]. Nutrient agar and sabouraud agar (Oxoid Limited, United Kingdom) were used for the determination of antibacterial and antifungal activities respectively. One hundred microliters (10⁶ cfu/mL) of the test organisms (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Bacillus subtilis* NCTC 10073, *Staphylococcus aureus* ATCC 25923 and clinical strains of *Streptococcus pyogenes* were used to seed 20 mL nutrient agar; and a similar amount of *Candida albicans* was used to seed 20 mL sabouraud agar plates. In each of these plates, five (5) equidistant wells with diameter of 10 mm were made using a sterile cork borer. The wells were filled with different concentrations of the extracts and reference drugs dissolved in dimethyl sulfoxide (DMSO) and allowed to diffuse at room temperature (28-30) for 1 h. The mean zones of growth inhibition were measured after incubation at 37°C for 24 h for the bacteria and 72 h at 30°C for the fungus. DMSO was found to exhibit no activity against the test organisms.

Determination of minimum inhibitory concentration (MIC)

The MICs of the extracts of *M. oppositifolius* and *M. charantia* against the test bacteria were determined using the modified micro-dilution technique as described by Agyare *et al.* [19] and Eloff [20]. Concentrations of 1.2, 2.5, 4.5, 7.5, 10, 25 mg/mL for each of the methanol leaf extract of were prepared and volumes of the respective concentrations of both extracts equivalent to specific concentrations were diluted by 50% with 100 µL of double strength nutrient broth and 20 µL of test organisms of *S. pyogenes*, *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* and *C. albicans*. Volumes of 20, 50, 61.25, 67.5, 69.58, 70.63 and 70.23 µL of sterile water were added to all in a 96-well micro-titer plate such that they make a required volume of 200 µL for each respective well. The plate was covered and incubated at 37°C for 24 h. Ten microliters of 2.5



mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well with blue colouration as an indicator for growth of microorganism and yellow for absence of microbial growth.

Determination of free radical scavenging activity of extracts

The free radical scavenging activity of the extracts was investigated using the method described by Chizzola *et al.* [21]. Various concentrations of 12.5, 25.0, 50.0 and 100.0 µg/ml in methanol of methanol leaf extract of *M. oppositifolius* and 3 mL of 20 µg/mL of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in methanol added to 1 mL each concentration. The absorbance of mixtures was recorded at 517 nm after they have been shaken and allowed to stand for 30 min in the dark. The above procedure was repeated for the methanol leaf extract of *M. charantia* at concentrations of 3.91, 7.81, 15.63, 31.25, 62.5 and 125.0 µg/mL. The percentage inhibition of free radical scavenging activity determined.

Excision wound model

Thirty five healthy Sprague-Dawley male rats weighing between 120 to 210 g were housed in metal cages and maintained on normal commercial diet for rats (GAFCO, Tema, Ghana). 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 by the Department of Pharmacology Ethics Committee, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

The animals were anaesthetized with 50 mg/kg ketamine body weight by intra-muscular injection prior to the creation of the wounds. The wounds were created along the markings using toothed forceps, surgical blades and pointed scissors and clean with 70% ethanol. The animals were divided into five groups of seven animals each. The first group was left untreated and cleaned with normal saline (0.9% w/v NaCl) and the second group was topically treated with 1% w/w silver sulphadiazine cream (SS) (Ayrton Drugs, Ghana). The third group was treated with cream without extract (vehicle alone). The fourth and fifth groups were treated with 10% w/w aqueous creams of methanol leaf extracts of *M. oppositifolius* and *M. charantia* respectively. Wound treatment started was the next day after wound excision till the 10th day post treatment. In the course of treatment, scaled photographs of the wound areas were taken (by means of high-resolution Olympus digital camera, Cameron Sino, Hong Kong) alongside a millimeter

scale. Wound measurement was done every other day, starting from the first day of wound treatment till the 10th day.

Histological examination

Wound tissue specimens from control, test and standard groups were taken on day 10 post-treatment. After usual processing, 6 mm thick sections were cut and 10% v/v of neutral formalin solution was used to fix the granulation tissues for 24 h and then dehydrated with a sequence of ethanol-xylene series of solutions. The wound tissues were embedded with paraffin at 40-60°C. Microtome sections were taken at 4 µm thicknesses. The processed sections were stained with haematoxylin eosin and observed under microscope using slightly modified method of Varshney *et al.* [20].

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 ± 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

Preliminary phytochemical screening

The yield of the extracts for *M. oppositifolius* and *M. charantia* were 9.97 and 7.19% w/w, related to the dried material, respectively. The methanol leaf extracts of both *M. oppositifolius* and *M. charantia* were found to contain alkaloids, tannins, flavonoids and glycosides. In addition, *M. charantia* extract contained saponins. The tannin content of *M. oppositifolius* and *M. charantia* extracts were 1.2 and 0.95% w/w related to polygallo (HPLC 99.5%) respectively.

HPLC finger-printing of extracts

The HPLC finger-printing of the leaf extracts of *M. oppositifolius* and *M. charantia* (Figures 1 and 2, respectively) were determined to identify the main peaks in the extracts for the purpose of identification of the two extracts.

Antimicrobial activity

The methanol leaf extracts of *M. oppositifolius* and *M. charantia* were found to be active against the test organisms with varying mean zones of inhibition (Table 1). The minimum inhibitory concentration ranges for *M. charantia* were from 1.88 to 7.5 mg/mL whilst those of *M. oppositifolius* were from 0.01 to 3.75 mg/mL (Table 2).



Table 1: Antimicrobial activity of methanol leaf extracts of *M. charantia* and *M. oppositifolius* against test organisms

Mean zones of growth inhibition ± SEM						
Test organisms						
Extract (w/v %)	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. Pyogenes</i>	<i>C. albicans</i>
<i>M. charantia</i>						
2.5	14.3 ± 0.6	17.0 ± 1.0	18.0 ± 0.0	18.3 ± 0.6	15.0 ± 0.0	16.3 ± 0.6
5.0	18.3 ± 1.1	19.7 ± 0.6	21.3 ± 1.1	20.7 ± 1.1	21.0 ± 1.2	20.0 ± 0.6
7.5	20.3 ± 0.9	20.1 ± 1.0	24.3 ± 0.6	22.7 ± 1.0	24.3 ± 0.6	20.0 ± 1.0
10.5	21.3 ± 0.8	23.0 ± 0.0	27.7 ± 1.4	24.3 ± 0.6	22.0 ± 0.0	22.7 ± 0.6
<i>M. oppositifolius</i>						
2.5	-	-	-	-	-	15.0 ± 0.0
5.0	-	-	*	-	-	17.0 ± 1.0
7.5	12.0 ± 0	-	11.3 ± 0.6	12.0 ± 1.0	15.0 ± 0	20.0 ± 1.0
10.0	13.3 ± 0.6	12.3 ± 0.4	13.7 ± 0.6	14.0 ± 1.6	16.0 ± 1.0	19.3 ± 0.6
CPZ	25.3 ± 0.6	34.0 ± 1.0	27.7 ± 0.6	30.3 ± 1.2	25.0 ± 1.0	ND
KTZ	ND	ND	ND	ND	ND	24.7 ± 0.9

Diameter of well = 10 mm, CPZ: Ciprofloxacin, KTZ: Ketoconazole, ND: Not Determined, SEM= Standard Error Mean, N = 3

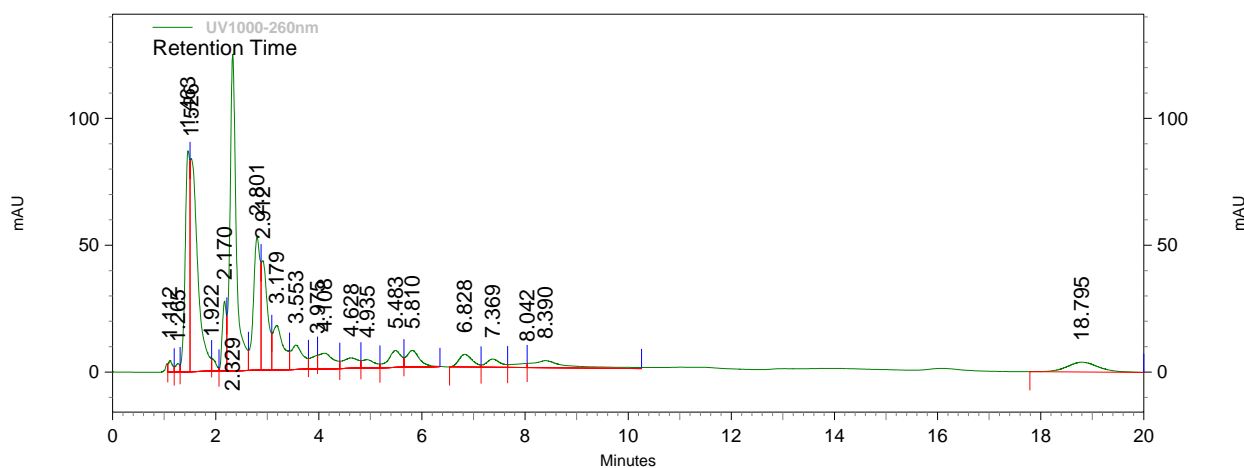


Figure 1: HPLC chromatogram (finger-printing) of methanol leaf extract of *M. charantia* at 260 nm



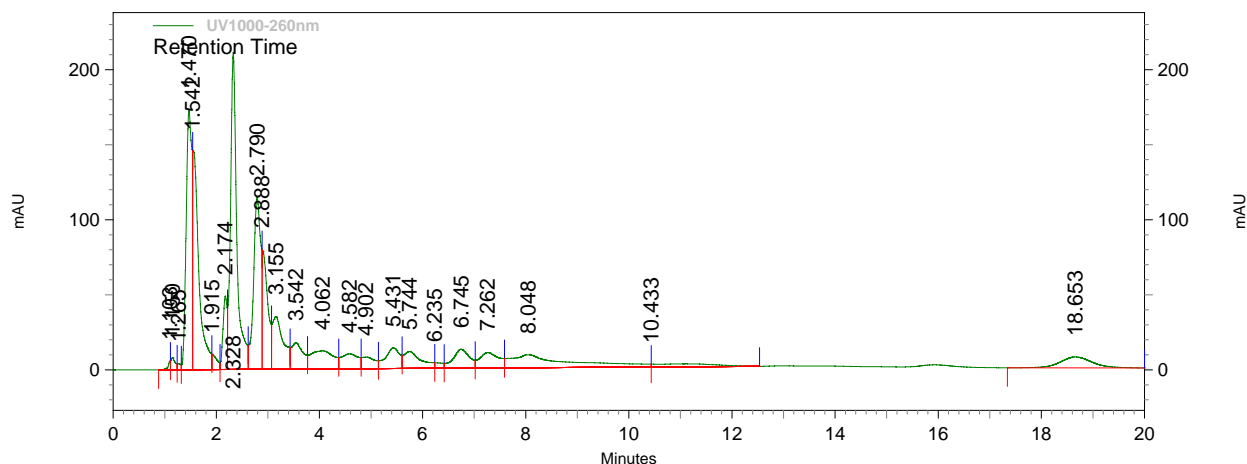


Figure 2: HPLC chromatogram (finger-printing) of methanol leaf extract of *M. oppositifolius* at λ 260 nm

Table 2: Minimum inhibitory concentration (MIC) of methanol leaf extracts of *M. charantia* and *M. oppositifolius* against test organisms

Organisms	<i>M. charantia</i> (mg/mL)	<i>M. oppositifolius</i> (mg/mL)
<i>S. aureus</i>	3.75	0.23
<i>C. albicans</i>	3.75	0.01
<i>P. aeruginosa</i>	1.88	0.94
<i>E. coli</i>	7.50	7.50
<i>S. pyogenes</i>	3.75	3.75
<i>B. subtilis</i>	1.88	0.94

Table 3: Free radical scavenging activity of methanol leaf extracts and reference antioxidant

Extract/Agent	IC ₅₀ (μ g/mL)
<i>M. oppositifolius</i>	16.11
<i>M. charantia</i>	7.09
-Tocopherol	0.43

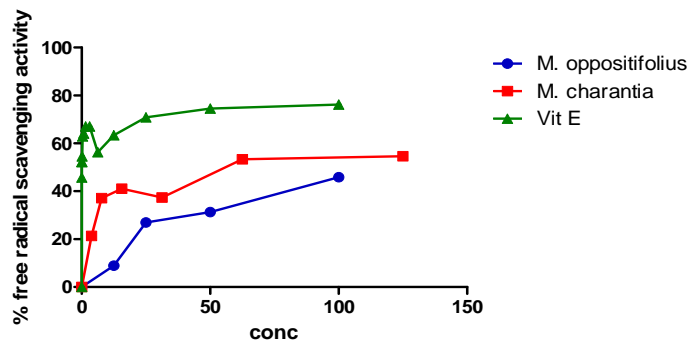


Figure 3: Free radical scavenging activity of *M. charantia*, *M. oppositifolius* and reference antioxidant -tocopherol

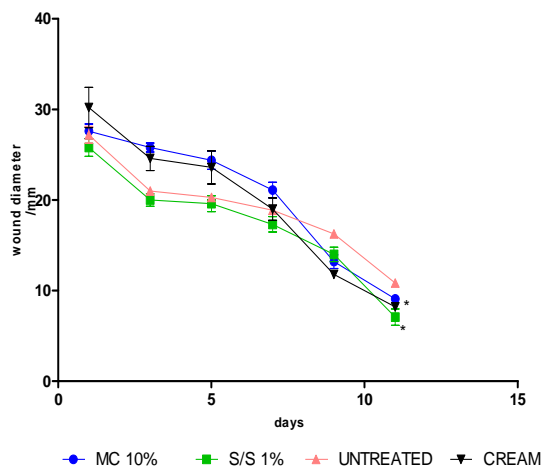


Figure 4: Influence of 10% w/w methanol leaf extract of *M. charantia* on wound closure against 1% w/w silver sulphadiazine, aqueous cream only and the untreated wounds using Two-way Anova Bonferroni post test, * $p < 0.05$.



Table 4: Wound diameter for selected time points. Values are mean wound area (mm) \pm SEM for untreated wounds and wounds treated with 1%w/w silver sulphadiazine, 10.0 % w/w of methanol leaf extracts of *M. charantia* and *M. oppositifolius* creams and cream only. *N*=5 rats per group. Data analyzed by one-way ANOVA followed by Dunnett's multiple comparison test.

Day	WOUND DIAMETER (mm)				
	Untreated	Cream only	Silver Sulphadiazine	<i>M. oppositifolius</i>	<i>M. charantia</i>
1	27.2 \pm 0.917	30.2 \pm 2.223	25.8 \pm 0.970	26.2 \pm 1.158	27.6 \pm 0.812
3	2.001 \pm 0.567	24.6 \pm 1.327	20 \pm 0.691	23.8 \pm 0.490	25.8 \pm 0.490
5	20.28 \pm 0.196	23.6 \pm 1.83	19.6 \pm 0.871	22.9 \pm 0.748	24.4 \pm 0.992
7	18.88 \pm 0.203	19 \pm 1.225	17.32 \pm 0.850	18.1 \pm 0.430	21.1 \pm 0.872
9	16.26 \pm 0.232	11.8 \pm 0.374	14 \pm 0.799	12.2 \pm 0.583	13.2 \pm 0.752
11	10.84 \pm 0.412	8.2 \pm 0.2	7.08 \pm 0.904	8.2 \pm 0.889*	9.1 \pm 0.332*

SEM- Standard Error Mean, *p < 0.05

Table 5: Percentage increase in wound contraction for selected time points. Values are mean percentage increase \pm SEM for untreated wounds and wounds treated with 1%w/w silver sulphadiazine, 10.0 % w/w of methanol leaf extracts of *M. charantia* and *M. Oppositifolius* creams and cream only. *N*=5 rats per group. Data analyzed by one-way ANOVA followed by Dunnett's multiple comparison test.

Day	Untreated	Cream only	Silver Sulphadiazine	<i>M. oppositifolius</i>	<i>M. charantia</i>
1	8.258 \pm 3.702	19.186 \pm 2.317	18.414 \pm 3.916	10.138 \pm 4.300	8.928 \pm 2.761
3	18.672 \pm 1.076	18.355 \pm 2.769	22.273 \pm 5.015	18.434 \pm 1.556	14.617 \pm 3.669
5	25.590 \pm 1.985	23.551 \pm 3.021	26.841 \pm 3.998*	21.545 \pm 2.234	19.378 \pm 3.979
7	32.195 \pm 3.552	38.056 \pm 2.866	44.268 \pm 3.608*	37.994 \pm 1.084	30.067 \pm 4.438
9	50.945 \pm 2.735	60.751 \pm 3.803	62.834 \pm 0.965*	58.217 \pm 1.777	56.251 \pm 3.279
11	66.510 \pm 3.246	73.037 \pm 1.467	74.123 \pm 1.154	71.842 \pm 3.092	69.842 \pm 1.801

SEM: Standard Error Mean, *p < 0.05



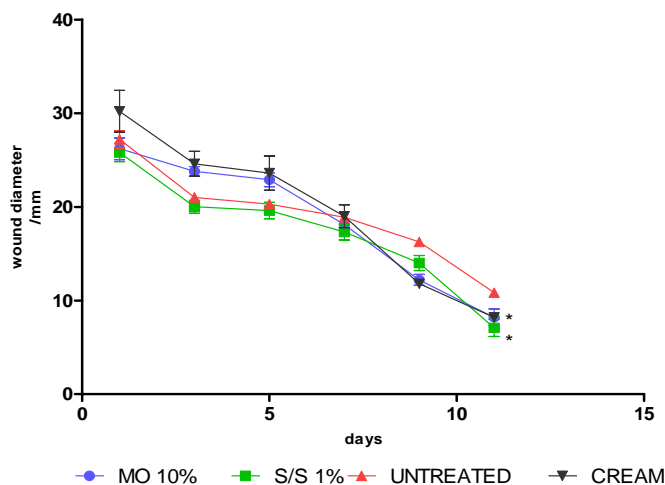


Figure 5: Influence of 10% w/w methanol leaf extract of *M. oppositifolius* on wound closure against 1% w/w silver sulphadiazine, aqueous cream only and the untreated wounds using two-way Anova Bonferroni post test, * $p < 0.05$.

Free radical scavenging activity

Both plant extracts possess some level of antioxidant property with *M. charantia* having the lowest IC_{50} of 7.1 $\mu\text{g/mL}$ and *M. oppositifolius* having the lowest free scavenging activity (Table 3 and Figure 3).

Rate of wound closure

Both extracts increased the rate of wound closure compared to the untreated but 1% w/w silver sulphadiazine (positive control) were slightly better than the extracts (Tables 4 and 5).

Histological examination

Images of the specimen taken on day 10 of wound healing showed that, both 10% w/w methanol leaf extracts of *M. oppositifolius* and *M. charantia* formulated with aqueous cream bases exhibited more fibrosis, collagen formation and less ulcer-based granulation tissues (Figure 6A & 6B). The scar tissue from the silver sulphadiazine had a dense connective tissue (Figure 6D). For the cream only there was fibrosis, more pronounced collagenisation but the presence of ulcer-based granulation tissue (Figure 6C). Lastly, the untreated tissue exhibited more ulcer-based granulation tissues with more foreign-body giant cells and lots of inflammatory cells although there was more fibrosis and collagenisation (Figure 6E).

Figure 6: Monographs of histological sections of the rat wound tissues stained with hematoxylin and eosin and viewed at magnification 40x Low power. (A): Tissues from wounds treated with the extract of 10% w/w of *M. oppositifolius*; (B): Tissues treated with the extract of 10% w/w of *M. charantia*; (C): Tissues from wounds treated with cream only; (D): Tissues from wounds treated with 1% w/w silver sulphadiazine ointment; (E): Tissues from untreated wounds.



Discussion

Management of wounds is very vital in that improper wound healing may contribute to undesirable consequences such as chronic wounds, non-healing wounds and delayed wound healing [2, 23]. Several factors may contribute to wound healing. These may include age, the presence of a disease, presence of pathogens, antimicrobial activity of some medicaments, oxidants as well as antioxidants, idiopathic factors, presence of contaminants and many others that may directly or indirectly affect wound closure and contraction and therefore wound healing [24].

Preliminary phytochemical screening of the methanol leaf extracts of *M. oppositifolius* and *M. charantia*, showed that each of them contains tannins, alkaloids, flavonoids and glycosides; and in addition to the presence of saponins in *M. charantia* extract. Tannins are known to have antimicrobial, astringent and protein coagulatory properties that aid in wound healing and together with the contribution of the other secondary metabolites which gives positive indication of the two plants having wound healing properties [25].

The MICs of the two extracts against test organism were between 0.01 and 7.5 mg/mL (Table 2). These micro-organisms were selected because they are among the most commonly found organisms on the skin and therefore in wounds [26]. The MIC is the lowest concentration of the extract at which there is no microbial growth and it gives an indication of the relative potency of medicinal substances with antimicrobial activity [27]. Since the MICs *M. oppositifolius* leaf extract were lower than that of *M. charantia* for four of the organisms, the former may have a better relative potency against all the organisms than *M. charantia*, except for *S. pyogenes* and *E. coli*. The antimicrobial activity of the extracts may be attributed to the tannins and other phytochemicals with astringent property.

For the activity with the agar diffusion method, both leaf extracts inhibited the growth of the test organisms at concentrations ranging from 2.5 to 10 % w/v. However for *M. oppositifolius* the mean zones of inhibition was relatively small compared to *M. charantia* (Table 1). This is at variance with the MIC which seems to suggest that *M. oppositifolius* has a greater antimicrobial activity than *M. charantia*. This may be due to the inability of the bioactive constituents in the former extract to diffuse well in the agar medium at those lower concentrations [7] and the presence of relatively higher amount of polyphenols in the *M. oppositifolius* leaf extract.

Oxidative stress known to be caused by excessive oxidants is very important in wound healing because it causes further damage to wound tissues [4, 28] and therefore reduces or delays wound healing process. The IC₅₀ is an indicator of how much of both extracts required in mopping up 50% of the oxidants [27]. It was determined to be 16.11 µg/mL for methanol leaf extract of *M. oppositifolius*, 7.09 µg/mL for *M. charantia* extract (Table 3). From

these results it could be observed that the two test samples have relatively higher values compared with the reference standard. This shows that the two extracts have potent antioxidant activity which may be due to the presence of the polyphenols in the extracts.

Extracts of *M. oppositifolius* and *M. charantia* showed no significant reduction in the rate of wound closure until day 11 when they both showed a significant ($p < 0.05$) reduction compared with the untreated. The histological studies of wound tissues (Figure 6) revealed that the untreated wounds exhibited more ulcer-based granulomatous tissues due to foreign-body giant cells and lots of inflammatory cells giving an indication of either delayed healing or non-healing wound. In an attempt to correct this anomaly, there was increased fibrosis and collagenisation. The tissues from cream only treated wounds were just slightly better than the untreated because there were no foreign-body giant cells but some level of ulcer based granulation tissues due to inflammatory cells which is also a negative indicator of wound healing. Collagenisation and fibrosis are indicators of wound healing but their presence may be deceptive depending on the prevailing conditions. These results suggest that the methanol leaf extracts of *M. oppositifolius* and *M. charantia* had some influence on the wound healing in rats compared to the untreated wound at late phase of the wound healing process. Hence the wound healing property may be due their antimicrobial and antioxidant and other biological properties. There is a need to isolate and characterize the bioactive agents or compounds responsible for the above biological activities from these two plant extracts.

Conclusion

The methanol leaf extracts of *M. oppositifolius* and *M. charantia* were active against the test microbes with respective MICs of 0.01 to 3.75 and 1.88 to 7.5 mg/mL. The extracts also exhibited good antioxidant property. The 10.0 % w/w methanol leaf extracts had influence on the wound healing process in treated wounds compared with the untreated wounds in the rats.

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Conflict of interest

The authors declare that there are no conflicts of interest.



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