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Ethnopharmacological survey and in vitro evaluation of wound-healing plants used in South-western Nigeria

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ABSTRACT

Ethnopharmacological relevance: Traditional healers in Nigeria employ a range of plant preparations as wound healing agents. Despite the use of local plants in wound healing, there is only scant literature on the wound healing properties of these plants to support the continued therapeutic application of these herbal remedies.
Aim of the study: To document plants commonly used to treat wounds in South-western Nigeria and to test the scientific basis of such claims using relevant in vitro tests.
Materials and methods: Structured questionnaires were used to determine which plant preparations are in common use, via interviews with Yoruba traditional healers. Aqueous and ethanolic extracts of the nine most common plants cited by the healers were collected, identified and tested using relevant in vitro wound healing assays. Minimum inhibitory concentrations (MIC) were determined against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. Antioxidant activity was measured by DPPH assay and fibroblast proliferation determined by neutral red assay.
Results: A total of 20 traditional healers from South-western Nigeria were involved in the study. Thirty-six plant species were recorded with their local names and parts used in the traditional wound healing preparations. Ethanolic extracts of nine species most frequently cited by the healers exhibited strong antioxidant activities (3.8–31.3 μg/ml) comparable to ascorbic acid (7.3 μg/ml). Crude extracts of the selected plants also inhibited the growth of bacteria with MIC values 0.3–7.6 mg/ml. Ethanol extracts of *Bridelia ferruginea* Benth. (1–30 μg/ml) and *Parkia biglobosa* Jacq. (15–30 μg/ml) influenced the proliferation of dermal fibroblasts significantly ($p < 0.05$). Extracts from the remaining seven plants either had no effect on fibroblast proliferation or were cytotoxic.
Conclusion: Traditional use of many wound-healing plants from Nigeria can be rationalised by activity determined in relevant in vitro investigations of ethanol and aqueous extracts. These results support the traditional selection of these plants in South-western Nigeria for wound healing.
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1. Introduction

In most of the developing world plants or herbal products play an important role in the treatment of wounds (Phillipson, 2001; Mensah et al., 2006). The choice of herbal products for the treatment of wounds varies between regions and cultures (Sofowora, 1993). In South-western Nigeria, traditional healers provide crude extracts from a range of plants to treat skin afflictions including wounds such as sores, bites, burns and lacerations. This form of traditional herbal medicine makes a significant contribution to the healthcare provision for rural communities (Okeke et al., 2006). Despite the Nigerian Government providing funding for development and integration of traditional medicine into Nigeria's primary healthcare system, the scientific basis for the use of many of these plant extracts for wound healing remains poorly understood. The present study investigates selected wound-healing plants commonly employed by traditional healers in Oyo State using three key in vitro assays of antibacterial activity, antioxidant activity and fibroblast growth stimulation.

2. Materials and methods

2.1. Study area and survey

An ethnopharmacological survey of traditional healers was carried out in Oyo State in South-western Nigeria. Data collection was based on the strategy of mutual trust suggested by Sofowora (1993) and Heinrich (2000). Information was compiled through

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general conversations and using standard questionnaires (which is a modification of the format recommended by Joly et al. (1987) and Sofowora (1993). The language used for interview was Yoruba since most traditional herbal practitioners were illiterate. The questionnaire was written in English language, but was translated to Yoruba language as the question was posed and answer written down in English except for the local names of plants.

Prior to the interviews, the healers were given information about participation in the project. The conversations were built on mutual trust with the common goal to improve the health situation in the country and increase the knowledge on Nigerian medicinal plants. More than thirty traditional healers from various suburbs and villages in South-western Nigeria were approached, with twenty volunteering to complete the survey. The information collected included local names of the plants used for wound healing, methods of preparation and details of administration (Table 1). The plants used by the healers are listed alphabetically by Latin names, along with the respective families and vernacular (Yoruba) names. The number of times each plant was cited was recorded. Nine of these plants are most frequently cited for treatment of wounds including burns and ulcers (Fig. 1). Specimens of the sample were collected and these were useful in the translation of the Yoruba names to botanical names.

2.2. Plant materials

Of the 36 species recorded in the survey (Table 1), only the nine with most frequent occurrence (Fig. 1) were collected. Relevant plant parts were collected from Oyo State in South-western Nigeria during the rainy season of August to September 2006. This corresponded to the time when most of the plant species were available and growing well. The plant materials were identified by a qualified botanist (Mr. T.K. Odewo) at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria, and voucher specimens were deposited in the herbarium at FRIN. All plants collected were air-dried in a moisture-regulated room (temperature 25–28 °C).

2.3. Preparation of plant extracts

The leaves or stems of the nine most frequently cited plants were collected for screening. The protocol used for extraction in this work reflects the preparation used by traditional healers and so simple aqueous and alcoholic extracts were prepared. The plant materials were ground into fine powder and then extracted (100 g each in 500 ml of solvent) by maceration for three days in distilled water or ethanol at room temperature. Extracts were filtered through a Whatman No. 1 filter paper and the filtrates collected. The aqueous filtrates were freeze-dried and the organic filtrates reduced using a rotary evaporator at 37 °C. The yield corresponding to each plant material is shown in Table 2. The reduced extracts were stored at 4 °C until required for analysis.

2.4. In vitro assays relevant to wound healing

2.4.1. Antibacterial assay

The extracts were tested against the selected bacteria strains for their inhibitory activity, using a common broth microdilution method (Eloff, 1998) in 96-well plates. The anti-bacterial activity of the extracts was tested against four reference bacterial strains: *Escherichia coli* UEL57, *Pseudomonas aeruginosa* NCTC10701, *Staphylococcus aureus* NCTC 7447 and *Bacillus subtilis* NCTC3610. One hundred microliter of Mueller Hinton broth were distributed from the first to the 12th test wells. One hundred microliter of ethanolic or water extract (20 mg/ml) in 1% DMSO were added to the first test well of each microtiter line, and then 100 μl of scalar dilution were transferred from the second to the ninth well. The 10th well was considered as growth control, since no extract solutions were added. Then, 100 μl of a microbial suspension (0.5 McFarland standard), obtained from an overnight growth at 37 °C, were added to each well and incubated overnight at 37 °C. The final concentration of the extracts adopted to evaluate the antibacterial activity ranged from 10 mg/ml (first well) to 0.019 mg/ml (11th well).

A blank control was taken using 1% DMSO alone (100 μl) added to the 96-well plate and the MIC was evaluated as described above. The determination of the MICs for the positive control chloramphenicol against all the reference strains was simultaneously carried out. After the incubation period, 40 μl of 2 mg/ml of *p*-iodonitrotetrazolium violet (Sigma) (INT) dissolved in water was added to each well. The extracts were then incubated for a further 30 min and bacterial growth was indicated by the red colour of the INT formazan produced. The MIC was determined as the lowest sample concentration at which no red colour (i.e. no bacterial growth) appeared.

2.4.2. Cell proliferation and viability assay of crude extracts

The method described by Mensah et al. (2001) was used. Human foreskin fibroblast (FS5) cells were seeded at a density of 1×10^3 cells per well in 96-well plates maintained at 37 °C in a humidified incubator of 5% CO_2: 95% air atmosphere. The medium was replaced after 48 h with 100 μl of DMEM containing 0.5% FCS. The ethanolic extract residues from the plants were initially dissolved in 1 ml of DMSO and filtered to give the sterile stock solution (20 mg/ml) and further diluted to give a final concentration of 1–60 μg/ml in the wells. The final volume of the medium was 200 μl per well. Two well plate columns were maintained on DMEM/0.5% FCS and DMEM/10% FCS as maintenance (negative) and positive growth stimulation controls, respectively. The cells were incubated for 48 h and cell growth determined using neutral red uptake assay. After incubation the cells were washed with phosphate buffered saline (PBS), 100 μl freshly prepared neutral red solution was added to each well and the cells were incubated at 37 °C for 4 h. The neutral red was then removed by washing with 1% HCHO/1% $CaCl_2$ and neutral red in the lysosomes was eluted with 100 μl of 1% acetic acid/50% ethanol over 30 min in an orbital shaker and the optical density measured at 540 nm using a 96-well plate reader. Three independent experiments were conducted and the results obtained are expressed as mean ± standard error of the mean of the absorbance. The data from the experiments were compared with the control (0.5% FCS) by one-way analysis of variance and Dunnet's test. Differences at $p < 0.05$ were considered to be significant.

2.4.3. Free radical scavenging activity of selected plants

The modified methods of Brand-Williams et al. (1995) and Chen et al. (1999) were used in this study. Scavenging activity was determined by dissolving the extracts to obtain a final concentration of 500–1.9 μg/ml in methanol. For a typical reaction, 200 μl of 6.5×10^{-5} M DPPH solution in methanol was mixed with 20 μl of extracts, in 96-well plates. The diluted solutions were allowed to stand in the dark for 30 min and thereafter the optical density was recorded at 517 nm using 96-well plate reader. For the control, 200 μl of DPPH• solution in methanol was mixed with 20 μl of methanol and the optical density of the solution was recorded after 30 min. The assay was carried out in triplicate and 3 independent experiments were conducted. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (%IP) of DPPH•.

$$\text{Percentage inhibition (\%IP)} = \frac{A_0 - A_1}{A_0} \times 100$$

Table 1
Medicinal plants used in the treatment of wounds in South-western Nigeria as identified by the ethnopharmacological survey.

Latin names	Family	Local name(s) (Yoruba)	Part used	Mode of application	No of times cited
Aframomum melegueta (Roskoe) K. Schum.	Zingiberaceae	Ata-ire	Fruits	The fruit is ground and mixed with palm oil and used as paste to treat wound	4
Acalypha wilkesiana Müll.Arg.	Euphorbiaceae	Aworoso	Leaves	The poultice of the leaves are applied to wounds, burns, rashes and itches	6
Ageratum conyzoides L.	Asteraceae	Imin-esu	Leaves	The leaves are ground and applied to wounds and sores	6
Annona senegalensis Pers.	Annonaceae	Abo	Leaves	A decoction of the leaves is used to clean wounds	2
Anogeissus leiocarpa (DC.) Guill. & Perr.	Combretaceae	Ayin	Stem-bark	The stem-bark is ground, powdered and applied to wound and sores	2
Azadirachta indica A. Juss	Meliaceae	Afoforo oyimbo	Leaves	The leaves are used as a paste for wound dressing	3
Bridelia ferruginea Benth.	Euphorbiaceae	Ira	Leaves	The poultice of the leaves are applied to wounds, open cuts, and sores	10
Capsicum frutescence L.	Solanaceae	Atarodo	Leaves	Ground leaves are mixed with palm oil and are applied to cuts and wounds	1
Carica papaya L.	Caricaceae	Ibepe	Leaves	The roasted leaf pulp is placed on sores or wounds for healing	1
Chromolaena odorata (L.) King & H. Rob	Asteraceae	Awolowo Akintola Taku	Leaves	An infusion is used as an external wash for wounds	2
Citrullus lanatus (Thunb.) Matsum. & Nakai	Cucurbitaceae	Osan	Leaves	The softened warm leaf is applied to sores and wounds	2
Cocos nucifera L.	Arecaceae	Igi agbon	Fruits	The fruit is ground and applied to the wound	1
Crassocephalum crepidioides (Benth.) S. Moore	Asteraceae	Ebolo	Leaves	Chopped leaves are placed on the sore	1
Datura stramonium L.	Solanaceae	Apikan	Leaves and seeds	The crushed leaves and seeds are mixed with palm oil and applied to wounds and burns.	4
Dichrostachys glomerata Chiov.	Fabaceae	Kara	Leaves	An infusion of the leaves is used as wash for wounds	3
Dioscorea hirtiflora Benth.and Hook.	Dioscoreaceae	Isanyinahun	Leaves	The fresh leaves are made as paste and applied to the wound	2
Diospyros canaliculata De Wild	Ebenaceae	Orile ijebu	Leaves	The leaves are used as paste for wound dressing	3
Euphorbia heterophylla L.	Euphorbiaceae	Oro	Leaves	Hot ash in a cloth is dipped in the oil from the leaves and applied to the wound	1
Euphorbia poissonii L.	Euphorbiaceae	Oro-adete	Leaves and sap	The fresh leaves or leaf sap are applied as a wound closure	2
Ficus asperifolia Miq.	Moraceae	Eripin	Leaves	The leaves are used as paste for wound dressing	3
Flabellaria paniculata Cav.	Malpighiaecea	Lagbolagbo	Leaves	A decoction of the leaf is used for cleaning of wounds	3
Hibiscus sabdariffa L.	Malvaceae	Amukan-zobo	Fruits	The dried fruit is finely powdered and applied to sores and wounds	1
Jatropha curcas L.	Euphorbiaceae	Botuje, Lapalapa	Leaves	The juice from the leaves is applied to wounds	1
Lawsonia inermis L.	Lythraceae	Lali	Leaves	An infusion of the leaves is used as wash for wounds	10
Lycopersicon esculentum L.	Solanaceae	Igi Tomato	Leaves	Fresh leaves are used for wound dressing	1
Morinda lucida Benth	Rubiaceae	Oruwo	Leaves	The leaves are used as paste for wound dressing	2
Nymphaea lotus L.	Nymphaeaceae	Ira	Leaves	The poultice of the leaves are applied to wounds and burns	2
Ocimum gratissimum L.	Lamiaceae	Effirin	Leaves	An infusion of the leaves is used as a wash for wounds	10
Olax subscorpioides Oliv.	Olacaceae	Ifon	Leaves	The fresh leaves are applied as paste on wounds	2
Parkia biglobosa (Jacq.) R.Br. ex G.Don	Leguminosae	Igi-Igba	Bark	The ground bark is used as a paste used for wound dressing	7
Piliostigma thonningii (Schumach.) Milne-Redh.	Leguminosae	Abafe	Leaves	The apical part of young leaves are macerated in water for topical application	1
Ricinus communis L.	Euphorbiaceae	Ewé ogohoun, ilara	Leaves	The fresh leaves are made as paste and applied to the wound	2
Sida acuta Burm.f	Malvaceae	Isekete	Leaves	A decoction of the leaves is used to clean wound	6
Tridax procumbens L.	Asteraceae	Kodele yiri	Leaves	The fresh leaves are applied as a wound closure	5
Vernonia amygdalina L.	Asteraceae	Ewuro	Leaves	A decoction is used as a wash and applied on wounds	8
Xylopia aethiopica (Dunal) A. Rich.	Annonaceae	Eeru	Leaves	The fresh leaves are applied as paste on wounds	4

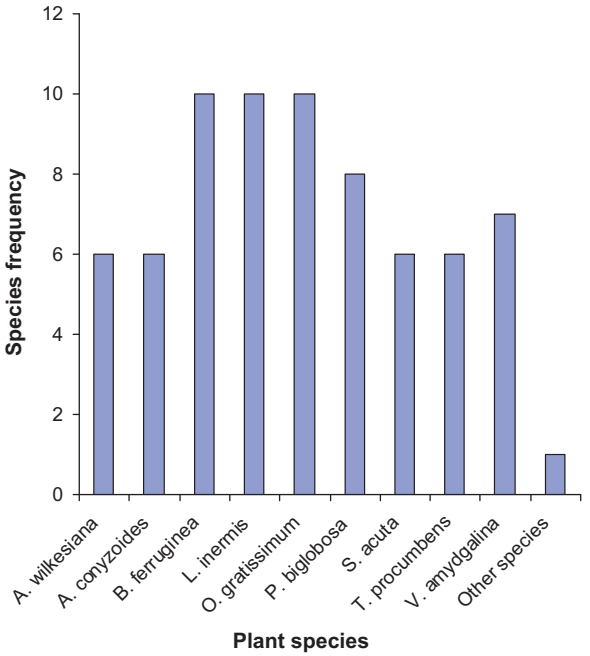

Fig. 1. Species citation frequency in the ethnopharmacological survey of plants commonly used for wound-healing in South-western Nigeria.

where A_0 is the absorbance of the control and A_1 is the absorbance of the test sample. The concentration required to inhibit DPPH radical formation by 50% was calculated as the IC_{50} of each sample.

3. Results and discussion

3.1. Survey on wound healing plants

In the present study 36 species belonging to 22 plant families have been identified as wound-healing remedies used by healers in Oyo State Nigeria (Table 1). The survey has shown the range and extent of medicinal plants employed in the traditional treatment of wounds in South-western Nigeria. The most common plant families are Euphorbiaceae (six cites) and Asteraceae (five cites). Solanaceae has three cites and those families with two cites include Annonaceae, Leguminosae and Malvaceae. Leaves are the most frequent plant parts used, constituting about 86% of the preparations followed by stem bark, sap, seeds and fruits (approximately 2% each). The plants are used as first aid, in the cleaning of wounds, as well as for wound dressing. The most common treatment seems to either be using a decoction as a wash followed by application of a plant paste or applying a plant dressing directly on the wound. The treatments are usually repeated every day until the wounds are healed. Wound dressing most often consists of whole or fresh leaves used in form of paste. The present inventory of wound healing plants used by the Yorubas in South-western Nigeria opens new avenues to scrutinize such a rich natural plant resource for further pharmacological screening towards the development of potential phytotherapy and to validate the traditional knowledge as an adjunct to Nigeria's primary healthcare system. Therefore, the plants most frequently cited by the healers were subjected to in vitro pharmacological investigations relevant to wound-healing. The ultimate goal is providing efficient and non-toxic medicines to the rural populations of Nigeria, where medicinal plants play a vital role in the primary health care. The demonstration of pharmacological activity by plant extracts that correspond to traditional formulations may support the traditional use of these medicinal plants.

Table 2
The percentage yield of crude extracts.

Plant species	Plant part used	% Yield of plant extract residues	
		Ethanol	Water
Ageratum conyzoides	Leaves	12.5	9.5
Acalypha wilkesiana	Leaves	9.0	8.2
Bridelia ferruginea	Leaves	7.1	6.3
Lawsonia inermis	Leaves	10.1	11.2
Ocimum gratissimum	Leaves	8.6	6.5
Parkia biglobosa	Stem bark	9.5	8.0
Sida acuta	Leaves	7.0	6.0
Tridax procumbens	Leaves	5.6	4.6
Vernonia amygdalina	Leaves	7.2	6.6

3.2. In vitro wound healing assays

3.2.1. Antibacterial assay

Wounds provide an environment for the growth of microorganisms (Somboonwong et al., 2003). An infected wound is less likely to heal, thus removal and prevention of further infection is a key to rapid and effective would healing. Many authors have proposed is in vitro and in vivo evidence to support the use of plant materials as topical anti-microbial agents to enhance wound healing (Chah et al., 2006; Muthusamy et al., 2006; Perumal et al., 2006). The results of our antibacterial screening of nine plants against four bacteria species are summarised in Table 3 (MIC values). *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* were selected for this study because they are common to infected wounds (Mertz and Ovington, 1993; Bowler et al., 2001). Amongst the test organisms used, *Staphylococcus aureus* was found to be most sensitive, followed by *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Initially water extracts of the plants were used in this study in order to reflect the traditional healers' formulations. These were not as active as ethanolic extracts against bacteria (Table 3). This is in accordance with work by other researchers who have reported water extracts showing low activities especially towards bacteria, as compared to organic extracts (Shale et al., 1999; Lall and Meyer, 2000). An initial observation is that the healer's extraction techniques may be inefficient and variable, however given that there are some activities in the aqueous extracts, there may still be some efficacy if used in very high doses.

In this study all the plant extracts were able to inhibit the growth of at least two of the tested standard strains (Table 3). The highest activity was shown against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. The findings of our study agreed with, or showed greater antimicrobial activity, to that of earlier research for *Lawsonia inermis* L. (Malekzadeh, 1968), *Bridelia ferruginea* Benth. (Talla et al., 2002) and *Vernonia amygdalina* L. (Iwalokun and Bamiro, 2003). In other cases however the results of this study showed less activity. Such variation may be due to the different assays used by different researchers, the different extraction methods employed, the sources of plant materials and the different strains of bacteria tested. In the field such variation in plant formulations must have a significant impact on the efficacy of the treatment used by traditional healers.

One of the most active plants *Parkia biglobosa* (Jacq.) showed similar activity for both aqueous and ethanolic extracts. The bark extracts showed anti-bacterial activity against *Staphylococcus aureus* with MIC = 0.31 ± 0.01 mg/ml for both the aqueous extract and the ethanolic extract. Previous screening for antibacterial agents from the leaves and seeds of this plant showed that gram positive bacteria were less susceptible to the leaf extracts than the gram negative bacteria (Ajaiyeoba, 2002). It has previously been suggested that the outer membrane surrounding the cell wall

Table 3
Minimum inhibitory concentrations (MICs) of the aqueous and ethanolic extracts of the selected plants.

Plant species	Extract	MIC (mg/ml)			
		Staphylococcus aureus	*Escherichia coli*	*Bacillus subtilis*	*Pseudomonas aeruginosa*
Ageratum conyzoides	Aqueous	0.95 ± 0.02	1.86 ± 0.07	0.95 ± 0.02	1.25 ± 0.05
	Ethanolic	0.47 ± 0.01	1.25 ± 0.05	0.31 ± 0.01	0.63 ± 0.01
Acalypha wilkesiana	Aqueous	0.94 ± 0.02	0.95 ± 0.01	0.95 ± 0.01	1.56 ± 0.05
	Ethanolic	0.31 ± 0.01	0.47 ± 0.01	0.47 ± 0.02	0.95 ± 0.02
Bridelia ferruginea	Aqueous	0.95 ± 0.02	1.25 ± 0.05	0.95 ± 0.03	1.25 ± 0.06
	Ethanolic	0.47 ± 0.03	0.95 ± 0.03	0.47 ± 0.01	0.63 ± 0.02
Lawsonia inermis	Aqueous	0.31 ± 0.02	0.95 ± 0.01	0.31 ± 0.01	0.63 ± 0.01
	Ethanolic	0.47 ± 0.03	0.78 ± 0.02	0.31 ± 0.02	0.78 ± 0.02
Ocimum gratissimum	Aqueous	2.50 ± 0.09	5.00 ± 0.25	3.75 ± 0.10	5.00 ± 0.20
	Ethanolic	1.25 ± 0.05	3.75 ± 0.10	3.75 ± 0.09	2.50 ± 0.12
Parkia biglobosa	Aqueous	0.31 ± 0.01	0.63 ± 0.02	0.31 ± 0.01	0.95 ± 0.04
	Ethanolic	0.31 ± 0.01	0.63 ± 0.02	0.47 ± 0.02	0.63 ± 0.02
Sida acuta	Aqueous	5.00 ± 0.24	5.00 ± 0.12	2.50 ± 0.14	5.00 ± 0.15
	Ethanolic	3.75 ± 0.20	5.00 ± 0.25	2.50 ± 0.10	5.00 ± 0.25
Tridax procumbens	Aqueous	2.50 ± 0.30	5.00 ± 0.20	3.75 ± 0.03	7.50 ± 0.50
	Ethanolic	1.86 ± 0.10	3.75 ± 0.10	2.50 ± 0.15	5.00 ± 0.25
Vernonia amygdalina	Aqueous	0.63 ± 0.02	1.25 ± 0.02	0.47 ± 0.01	1.25 ± 0.01
	Ethanolic	0.47 ± 0.01	0.94 ± 0.02	0.47 ± 0.02	0.63 ± 0.01
Chloramphenicol		0.001 ± 0.00	0.001 ± 0.00	0.08 ± 0.01	0.09 ± 0.01

Values represent means ± SEM of three independent experiments.

of Gram-negative bacteria may restrict diffusion of hydrophobic compounds through its lipopolysaccharide covering (Burt, 2004; Davidson et al., 2005).

In our study, both ethanol and aqueous extracts of the leaves of *Bridelia ferruginea* Benth. inhibited the growth of all the tested bacteria; the greatest activity was from the ethanolic extracts. This result confirms previous studies by others on antibacterial activity for this plant. Talla et al. (2002) demonstrated that the methanol, ethylacetate and hexane extracts of *Bridelia ferruginea* Benth. leaves exhibited significant antimicrobial activity against *Pseudomonas frutescens*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *S. faecalis*. Akinpelu and Olorunmola (2000) reported that 60% methanolic extract of *Bridelia ferruginea* fruit exhibited antimicrobial activity against seven bacterial isolates. Muanza et al. (1995) also reported that methanol extract of the leaves showed a marked antibacterial activity against the microorganisms *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Streptococcus mutans*. The use of decoctions of *Bridelia ferruginea* Benth. is widespread in Africa and such extracts are reported to contain antimicrobial constituents and activity (Muanza et al., 1995; Akinpelu and Olorunmola, 2000).

Vernonia amygdalina L. was active against most of the strains tested in this study. Few reports exist on the antimicrobial property of compounds isolated from this plant. Previous reports indicate antibacterial activity of extracts of this plant and the two constituent sesquiterpene lactones, vernolide and vernodalol (Akinpelu, 1999; Iwalokun and Bamiro, 2003; Erasto et al., 2006). The crude extracts of *Ocimum gratissimum* L. inhibited the growth of all strains of tested bacteria with an MIC range of 5.00 ± 0.20 to 1.25 ± 0.05 mg/ml. The data obtained here varied from what was reported by other researchers who reported antibacterial activity of *Ocimum gratissimum* L. against Gram positive and Gram negative bacteria (Ngassoum et al., 2003; Ijeh et al., 2005). The difference in extraction procedure, plant part and geographical origin may contribute to different results. The ethanol but not the water extracts of *Acalypha wilkesiana* Müll. Arg. inhibited the growth of both the Gram-positive and Gram-negative bacteria. Similar observations were reported by Alade and Irobi (1993) on water and methanol extracts of the plant, who found that most of the antibacterial activity observed was in the methanol extracts. Results obtained here on antibacterial activity of *Lawsonia inermis* L. leaves (Table 3), are similar to those obtained by Malekzadeh (1968) who reported that aqueous extracts showed antimicrobial activity against *Escherichia coli*, and *Staphylococcus aureus*. In addition, Awadh et al. (2001) reported that the ethanolic and ethyl acetate extract of *Lawsonia inermis* L. were active against *Staphylococcus aureus*, *Escherichia coli,* and *Pseudomonas aeruginosa.* Our results were comparable to Awadh's study with aqueous extracts (Table 3) producing similar activity to the ethanol extracts by

Table 4
Influence of ethanolic extracts at 1–60 μg/ml for 48 h on human dermal skin fibroblasts using neutral red uptake (NRU)-assay. Data and SEM-values are from 3 independent experiments with $n = 6$ replicates. Negative control: untreated cells; positive control: 10%FCS. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the untreated control group (ANOVA).

Plant species	Percentage proliferation					
	1 μg/ml	5 μg/ml	15 μg/ml	30 μg/ml	60 μg/ml	Catalase 250 IU/ml
Ageratum conyzoides	101.4 ± 9	106.8 ± 8	109.5 ± 9	94.6 ± 6	97.3 ± 5	168.9** ± 12
Acalypha wilkesiana	102.7 ± 12	101.8 ± 13	101.8 ± 10	111.1 ± 12	107.4 ± 9	185.4** ± 14
Bridelia ferruginea	123.3* ± 8	133.3** ± 9	123.3* ± 7	125.0* ± 8	70.0 ± 6	181.7** ± 12
Lawsonia inermis	100.3 ± 9	95.6 ± 7	91.2 ± 8	88.2 ± 6	80.9 ± 5	161.0** ± 12
Ocimum gratissimum	103.3 ± 9	101.6 ± 9	104.9 ± 10	105.7 ± 10	93.4 ± 9	174.0** ± 13
Parkia biglobosa	111.9 ± 9	110.4 ± 8	114.9* ± 11	131.3** ± 12	104.4 ± 11	186.6** ± 15
Sida acuta	98.27 ± 8	100.9 ± 9	94.8 ± 9	87.2 ± 7	92.2 ± 8	170.1** ± 14
Tridax procumbens	102.7 ± 9	103.1 ± 10	100.3 ± 9	105.4 ± 8	105.5 ± 11	187.2** ± 15
Vernonia amygdalina	104.2 ± 9	101.3 ± 9	100.5 ± 10	98.6 ± 9	98.5 ± 8	147.1** ± 14

Malekzadeh (1968) cited above. The slight variation in the values of MICs (Table 3) obtained from this study and that of Awadh et al. (2001) could be explained partly because of different concentrations of plant extracts used and method of plant extraction employed.

By comparison to the plants described above, the antibacterial activity *Ageratum conyzoides* L. has well been studied (Pari et al., 2000). The essential oil showed weak activity against *Staphylococcus aureus*, *Staphylococcus epidermidis* and was inactive against *Escherichia coli* (Pari et al., 2000). Our data demonstrates that the leaf extracts also showed some activity against all the tested bacteria. In contrast to the generally broad-spectrum activity from the plant extracts highlighted above, the extracts from the leaves of *Sida acuta* Burm. F and *Tridax procumbens* L. were found to exhibit low antibacterial properties mainly against Gram positive species. This is in accordance with previous work reporting that Gram-negative bacteria are resistant to most plant extracts (Lall and Meyer, 2000; Cos et al., 2006). The antibacterial activity of *Sida acuta* Burm. F components have been reported by Damintoti et al., 2006. Cryptolepine was shown to contribute, at least in part, to the antibacterial effects by causing cell lysis and morphological changes of *Staphylococcus aureus* (Sawer et al., 2005).

3.2.2. Influence of extracts on human fibroblast skin cells in vitro

Skin fibroblast proliferation is important in tissue repair as fibroblasts are involved in migration, proliferations, contractions and collagen production (Woodley et al., 1985; Mimura et al., 2004). The ability to stimulate fibroblast cell growth is a useful model for testing wound healing activity in vitro (Graham et al., 1984; Mensah et al., 2001). *Parkia biglobosa* (Jacq.) and *Bridelia ferruginea* Benth. stimulated the growth of fibroblasts at 15–30 and 1–30 μg/ml, respectively (Table 4). The findings obtained indicate that these two plants may promote wound healing by the stimulation of fibroblasts, although it is difficult to relate these concentrations to those achievable by the healers in practice. Flavonoids, tannins, saponins and terpenoids have been found in these plants (Cimanga et al., 2001; Tringali et al., 2000). Previous reports have associated these classes of compounds with wound healing activities by stimulating the growth of fibroblasts (Kim et al., 1998; Stevenson et al., 2002). Conversely, *Vernonia amygdalina* L., *Tridax procumbens* L., *Acalypha wilkesiana* Müll. Arg. and *Sida acuta* Burm. F (Table 4), at various concentrations tested did not affect human skin fibroblasts proliferation. Ethnopharmacological uses of these plants however suggest that they might have wound healing properties. Therefore, the wound healing properties of these plants may be achieved through other properties such as anti-inflammatory, antioxidant or antibacterial action and not directly by fibroblast growth stimulation.

The data obtained in this study indicates stimulation of fibroblast growth with the lower concentrations (1–15 μg/ml) of extracts of some of the selected plants. However, at higher concentrations (30–60 μg/ml) for some of the plants there is evidence of cytotoxicity or inhibition since the absorbance was less than the control (Table 4). Most traditional healers apply these extracts with little understanding of the concentration being applied. The indirect evidence of cytotoxicity of some of these extracts at high concentration implies that caution must be taken when using infusion of these plant species in treating wounds.

3.2.3. Free radical scavenging activity of selected plants

Infected wounds attract high levels of phagocytic cells which release reactive oxygen species in an attempt to fight infection, however these molecules can damage the host cells and delay the healing process (Altomare et al., 1995). As wound healing can be positively influenced by antioxidant agents, the radical scavenging activity of the ethanolic test extracts was determined by DPPH assay and IC_{50} values were calculated (Table 5). The extracts which showed strong DPPH radical scavenging activity (IC_{50} < 16.0 μg/ml) were those of *Lawsonia inermis* L, *Bridelia ferruginea* L., *Acalypha wilkesiana* Müll. Arg. and *Parkia biglobosa* (Jacq.). The observed antioxidant effects offered by some of the extracts compare well with antioxidant effects demonstrated by plant species which find their use in the treatment of wounds in Vietnam (Hien et al., 1997), Oman (Marwah et al., 2007), Ghana (Mensah et al., 2006) and in similar studies involving the wound-healing properties of *Buddleja globosa* (Mensah et al., 2001). Thousands of compounds occur in the medicinal herbs which are believed to be responsible for antioxidant activities (Xiao et al., 2000). Over 4000 flavonoids and hundreds of coumarins and lignans have been reported as naturally occurring antioxidant compounds (Iwashina, 2000 and Xiao et al., 2000). The antioxidant activity observed in this study could be one possible mechanism which contributes to the selected plants potential for enhanced wound healing.

Table 5
DPPH scavenging activity for the selected plants estimated by means of IC_{50}. Ascorbic acid was used as a positive control. IC_{50} values (mean ± SD, $n = 3$).

Plant (ethanolic extract)	IC_{50} (μg/ml)
Ageratum conyzoides	31.25 ± 0.26
Acalypha wilkesiana	15.25 ± 0.25
Bridelia ferruginea	12.50 ± 0.31
Lawsonia inermis	3.80 ± 0.02
Ocimum gratissimum	18.0 ± 0.25
Parkia biglobosa	15.65 ± 0.20
Sida acuta	500.0 ± 2.95
Tridax procumbens	62.50 ± 0.31
Vernonia amygdalina	31.25 ± 0.20
Ascorbic acid	7.26 ± 0.12

4. Conclusion

The different phases of the wound healing process overlap and ideally a plant-based remedy should affect at least two different processes before it can be said to have some scientific support for its traditional use (Houghton et al., 2005). Most of the plant extracts reported here displayed both antioxidant and antibacterial activities thereby suggesting all have some potential for wound healing. By contrast only two plants were effective at stimulating fibroblast growth with *Bridelia ferruginea* L. showing activity at low concentrations. From the above results there is strong indication that the traditional use of plant materials for wound healing by South-western Nigerian healers in many cases can be rationalized by in vitro investigations. In conclusion, these findings support the use of the nine plants selected by citation frequency. However further studies to isolate the pharmacologically active compounds contributing to the wound healing properties of these plants are needed.

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