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An ethnopharmacological survey and *in vitro* confirmation of ethnopharmacological use of medicinal plants used for wound healing in Bosomtwi-Atwima-Kwanwoma area, Ghana

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ABSTRACT

Aims of the study: Wounds represent a major health burden and drain on healthcare resources in the world including Ghana and Africa. The majority of the people of Ghana and Africa still patronize traditional medicine for their health needs including various forms of wounds. The aim of this study is the identification of medicinal plants, type of wounds, dosage forms and collection methods used traditionally in treating wounds in the Bosomtwi-Atwima-Kwanwoma district, Ghana. *In vitro* screening of selected extracts from these plants on cell physiology of human dermal fibroblasts and keratinocytes was to be performed.

Materials and methods: Validated questionnaires were administered to 78 traditional healers in 54 communities of the district. Interviews and structured conversations were used to administer the questionnaires. Selected herbal material dominantly used by the healers was collected, identified and aqueous and ethanolic extracts were investigated *in vitro* on influence on cell physiology of keratinocytes and dermal fibroblasts (MTT-, BrdU-, LDH-assay). Antioxidant activities of ethanolic extracts were determined by free radical scavenging activity. Antiadhesive activity against *Helicobacter pylori* on human stomach cells was investigated for extracts reported to be used for stomach ulcer treatment.

Results: The ethnopharmacological survey revealed 104 plants species belonging to 47 families. The detailed use of these plants is documented. Aqueous extracts of *Phyllanthus muellerianus, Pycnanthus angolensis* and *Combretum smeathmanni* influenced the mitochondrial activity and proliferation of dermal fibroblasts and keratinocytes significantly. Ethanolic extracts of selected plants exhibited strong antioxidant activities comparable to α-tocopherol. For *Spathodea campanulata*, *Hoslundia opposita* and *Pycnanthus angolensis*, which were reported by the healers to be used also for wound healing in case of stomach ulcers, strong antiadhesive activity against *Helicobacter pylori* was demonstrated, while the extracts did not exhibit any direct cytotoxicity against the bacterium.

Conclusions: Traditional use of many wound-healing plants from Ghana can be well rationalized by the *in vitro* investigation of aqueous extracts. E.g. extracts of *Phyllanthus muellerianus, Pycnanthus angolensis* and *Combretum smeathmanni* exhibited significant influence on the cell viability and proliferation of keratinocytes and dermal fibroblasts.

1. Introduction

Wounds have a tremendous impact on the healthcare economy. Chronic wounds represent a major health burden and drain on healthcare resources in the developed countries (Harding et al., 2002; JanBen, 2006). It is estimated that 70 to 80% of patients in Africa, and also in Ghana are treated by traditional healers and herbal practitioners (Diallo et al., 1996; Nyika, 2007). People in Africa rely on traditional medicine for their health needs, including management of wounds because of the high cost of orthodox medicines, inadequate health facilities and healthcare professionals, coupled with a lack of training of health workers on skin disorders and diseases (Mahé et al., 2006). Traditional medicines and medicinal plants used for management of skin disorders and as wound healing agents (Inngjerdingen et al., 2004; Ram et al., 2004; Njoroge and Bussmann, 2007) are easily available and afford-

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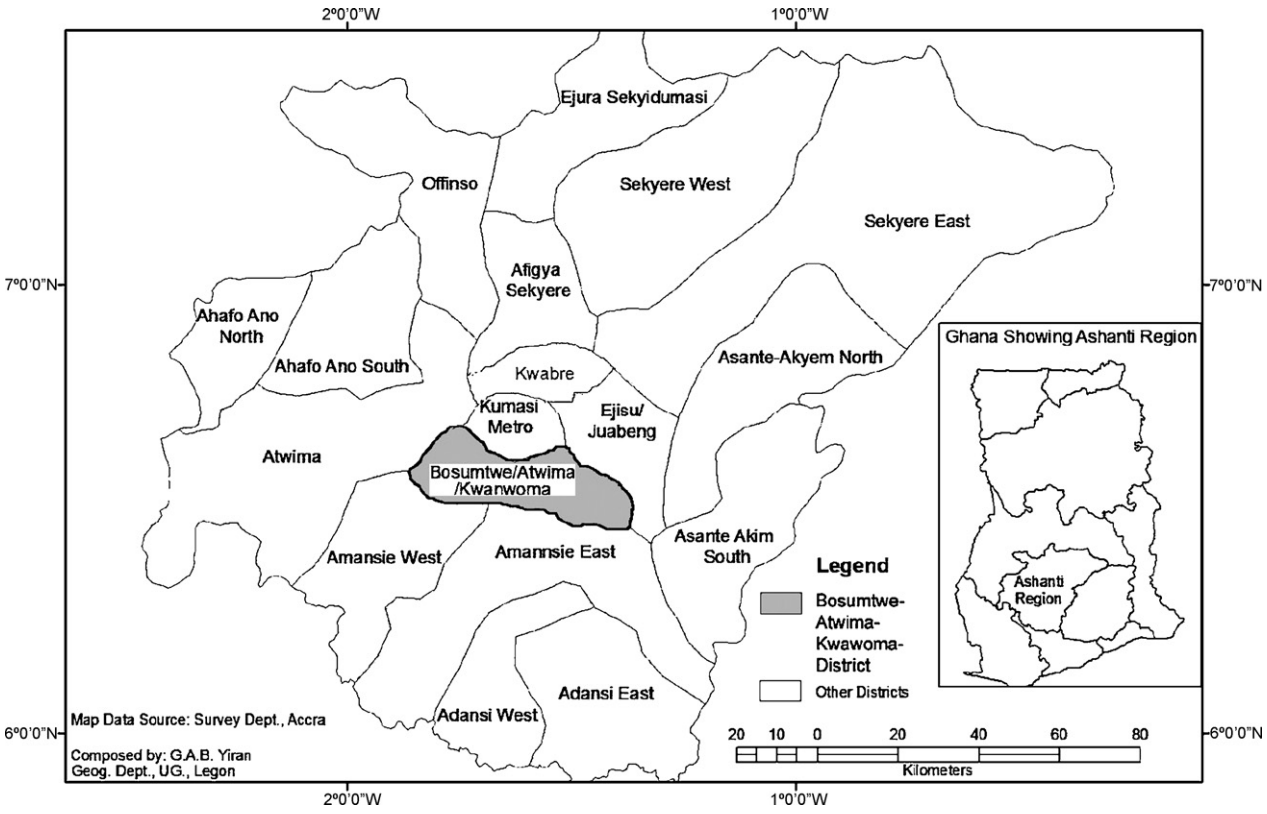

Fig. 1. Geographical description of study area. Map of Ashanti region showing the location of Bosomtwi-Atwima-Kwanwoma District.

able, sometimes free of charge. Most of these medicinal plants have been used for a long time and are assessed to be safer than isolated active compounds (Fabricant and Farnsworth, 2001). Ethnopharmacological investigations on medicinal plants used traditionally for wound-healing have shown that many active extracts and compounds can be identified which stimulate wound healing by induction of skin cell proliferation or differentiation (Deters et al., 2004, 2005a,b; Houghton et al., 2005; Zippel et al., 2009), with additional benefits by potential antioxidant and antimicrobial activities (Mensah et al., 2001; Konning et al., 2004; Jimoh et al., 2007).

Because of the huge reservoir of traditional knowledge on wound-healing plants in Ghana the following study was performed to obtain validated, quantified and reliable data on the use of wound-healing plants in an exactly defined part of the country. The aims of the study were to find out how traditional healers in the Bosomtwi-Atwima-Kwanwoma area of Ghana recognize wounds and how they classify and treat wounds with medicinal plants. It was to be investigated how the respective plants were collected and identified, which part of plants are used, how they are obtained, prepared and applied to wounds and what importance these plants have to the healers beside other non-plant based methods within the management of wounds. Additionally it was to be investigated if under *in vitro* condition with common methods of cell biology, effects associated with skin cell activity and wound healing use can be identified.

2. Material and methods

2.1. Study area and survey

Study area: Bosomtwe-Atwima-Kwanwoma District (Fig. 1), one of the 21 districts in the Ashanti Region of Ghana, 0.15–2.25°W, 5.50–7.46°N. The ethnopharmacological survey was carried out from June to September 2007 in close cooperation with Ghana Federation of Traditional Medicine Healers Association (GHAFTRAM), an umbrella organization including all traditional healers such as herbal practitioners, fetish priests, divine healers, psychic practitioners and traditional medicine practitioners in Ghana. The executives and members of the district branch of GHAFTRAM were contacted prior to the study and informed about the objectives of the investigations. They assisted and cooperated with the survey team. Also non-members of the district branch of GHATRAM participated in the survey. All participants were informed about the survey and personal visits were made to their facilities, centers and homes. In respect to the local tradition, gifts in form of money or local alcoholic drinks were bestowed upon the healers prior to the interviews and administration of questionnaires. Interviews and conversations were used to administer the questionnaires.

Questionnaires were designed in English, translated into the local dialect (Asante-Twi) and administered to 78 traditional healers and herbal practitioners. Interviews were conducted together with a curator/botanist. Voucher specimens of all plants have been deposited in the Ghana Herbarium, Department of Botany, University of Ghana, with defined ID-numbers.

2.2. Plant material and chemicals

Leaves of *Parquetina nigrencens* (Afzel). Bullock., *Phyllanthus muellerianus* (Kuntze.) Exell, *Ficus exasperata* Vahl., *Pupalia lappacea* (L.) Juss., *Hoslundia opposita* Vahl., *Combretum smeathmanni* G. Don., stem bark of *Pycnanthus angolensis* (Welw.) Warb., *Alstonia boonei* Wild. and roots from *Anchomanes difformis* (Bl.) Engl. were collected from Bosomtwi-Atwima-Kwanwoma area and identified by Dr. A. Asase, Department of Botany, University of Ghana. If not stated otherwise all chemicals were purchased by Sigma (Deisenhofen, Germany).

2.3. Preparation of plant extracts

Aqueous extracts: 10 g of the powdered plant material in 100 mL water were heated at 90 °C/15 min and centrifuged (6.000 × *g*, 10 min). The supernatant was concentrated at a temperature not exceeding 40 °C and lyophilized.

Ethanolic extracts: 10 g of the powdered plant material in 100 mL ethanol/water (60:40 v/v), were heated at 70 °C/30 min and centrifuged (6.000 × *g*, 10 min). The supernatant was concentrated and lyophilized.

Tannin contents were determined according Glasl (1983).

2.4. Methods of cell biology

HaCaT keratinocytes were kindly provided by Prof. Fusenig, Heidelberg, Germany. Primary dermal fibroblasts were obtained from surgical resectates of various Caucasian subjects. Approvals of the studies were made by the local ethical committee of University of Münster (2006-117-f-S). Isolation of dermal fibroblasts, cultivation of cells, and determination of the influence of the test extracts on cell physiology, MTT-, BrdU-, LDH-test were performed according Deters et al. (2008) and Zippel et al. (2009).

Antiadhesion assay against Helicobacter pylori was performed according methods described by Niehues and Hensel (2009).

2.5. Free radical scavenging activity of ethanolic extracts

Assay was performed according to Chizzola et al. (2008) using 1,1-diphenyl-2-picryl-hydrazyl (DPPH). 0.1 mM solution of DPPH in methanol was prepared and 10 μL of this solution were added to 100 μL of the ethanolic test extracts at 0.5, 1, 10, 25, 50, 100, 150 μg/mL. The plates were shaken for 30 s and after 30 min absorbance was measured at 517 nm. Inhibition of radical scavenging was calculated as (%) = $[(A_0 - A_1)/A_0 \times 100]$, with A_0 being the absorbance of the control and A_1 the absorbance of the test sample at 517 nm.

2.6. SPSS evaluation of data

Quantitative evaluation of survey data was performed with SPSS 16.0 (PASW Statistics Inc.).

3. Results and discussion

3.1. Survey on wound healing plants

An ethnopharmacological survey was carried out in the Bosomtwi-Atwima-Kwanwoma district, Ashanti region, Ghana (Fig. 1). Professional healers were mainly older than 40 years (86%), 14% older than 80 years. The respective age distribution is shown in

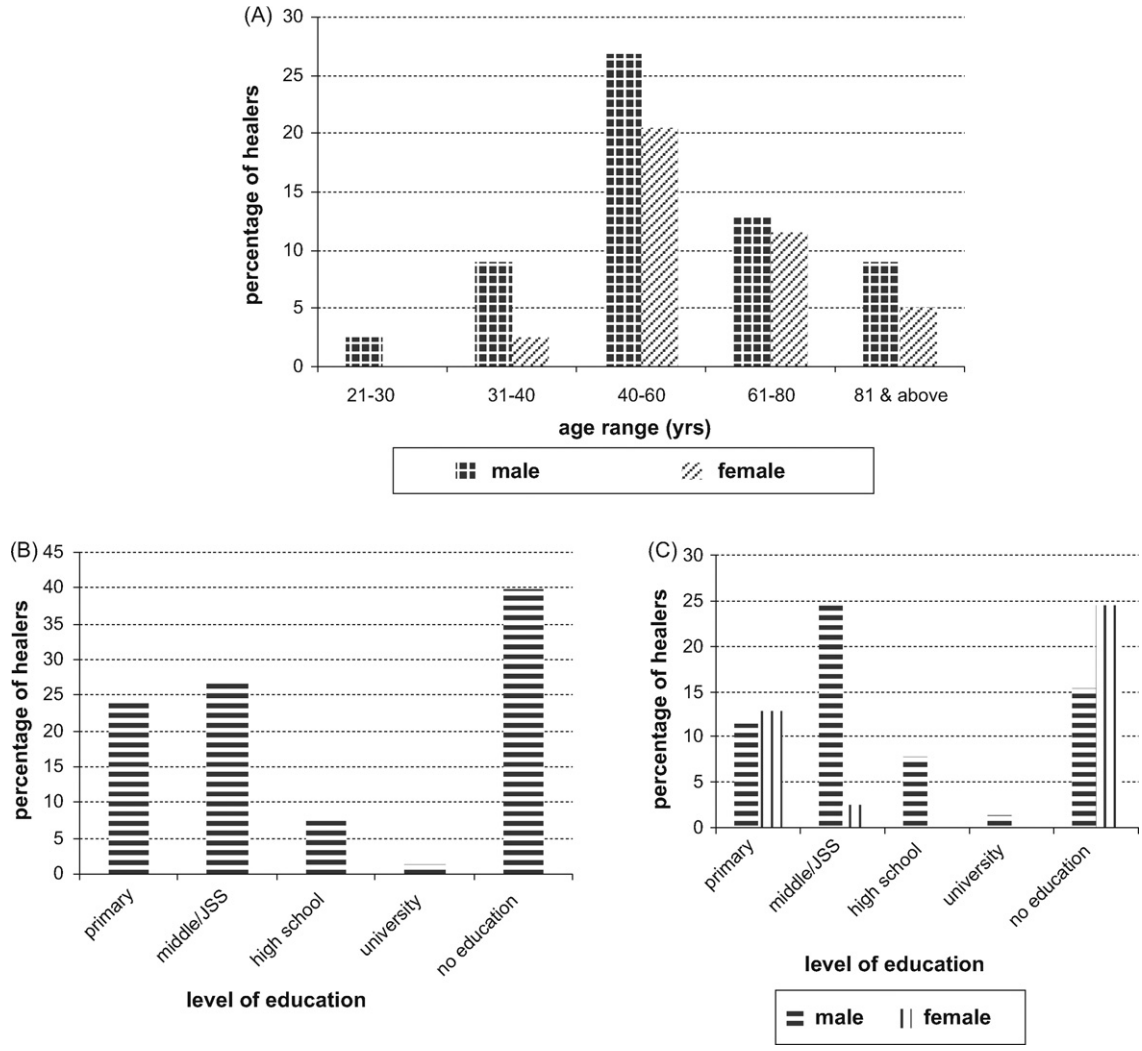

Fig. 2. Biodata analysis of healers: (A) age distribution and sex distribution. (B) Level of education. (C) Distribution of level of education and sex of healers.

Table 1
Period of practice of herbal medicine practitioners in Bosomtwi-Atwima-Kwanwoma district Ghana.

Time of practice	Frequency	(%)	Cumulative percent
0–5 years	8	10.3	10.3
5–10 years	9	11.5	21.8
11–20 years	10	12.8	34.6
21–40 years	32	41.0	75,6
>41 years	19	24.4	100.0

Fig. 2A. About two thirds of the healers are men, one third women. The respective sex and age distribution is shown in Fig. 2A.

Level of education of healers does not increase the potential qualification to practice as a traditional healer. Only 1% of them has an university degree, while the majority (40%) has no education; 50% have at most middle school/Junior secondary school (elementary) education and small proportion (8%) has high school education (Fig. 2B). Except for primary level education, male healers have higher education than female healers (Fig. 2C). Younger healers have comparably higher levels of education than the very old practitioners. The only practitioner who has university education falls within the 21 to 30 years age group.

The level of education could determine the level of readiness of healers to apply scientifically proven methods of preparation of their medicines and treatment of the ailment. The practical work of healers depends mostly on their intuition and experience achieved during practical work over the years.

Herbal practitioners constitute the majority (58%) of the healers. Fetish priests and traditional healers constitute 36 and 6%, respectively. It is important to note however, that there is no clear distinction of practice between the three kinds of healers. Each could play the role of the other, and this is common amongst traditional medical practice.

A significant proportion of the healers have been in practice for long periods of time (Table 1). Moreover, the profession requires long experience to be able to identify plants for effective management and treatment of diseases. As expected, Asantes constitute the majority of the practitioners (79%), a reflection of the fact that Asantes are the indigenous tribe of the district.

Most of the healers defined wound as a result of damage to the skin or superficial tissues caused by sharp objects, burns, microbial infections, fractures, metabolic diseases (diabetic wounds), bites and stings. It was interesting to note that also stomach sores or peptic ulcers were considered as internal wounds which are also subjected to the treatment with medicinal plants.

Healers classified wounds as cuts, injuries, abrasions, bites, acute, chronic, old wounds, boils, carbuncles, burns, hemorrhoids, skin rashes, parasitic wounds, fractures and internal ulcers. Also parasitic infections in the skin area were classified as wounds. Often non-healing wounds were considered to have spiritual reasons.

Concerning the herbal medicines 104 plant species from 89 genera, belonging to 47 families were recorded as wound healing agents (Table 2). Most recorded species (9 species/8.7%) were from the family Euphorbiaceae, followed by Apocynaceae (6 species/5.8%), Moraceae and Solanaceae (each with 5 species/4.8%). In order to investigate whether these species are already known plants for wound-healing cross-referencing was made to standard literature (Neuwinger, 1998; Burkill, 2000) (Table 2). From the 104 plants found in our survey only 26 are referenced for wound-healing by Burkill, 2000, 9 by Neuwinger and 9 by both references. This clearly shows that the survey performed within this study revealed 60 plants not described until now for wound-healing

These plants are applied mostly as poultice (for which the fresh leaves are grounded), decoction (for both superficial and internal wounds), as powdered material, washings, enemas (hemorrhoids) and as gargle for mouth sores. Leaves and aerial parts (86%), stem bark (9%), roots (4%), seeds and fruits (1%) of these plants are used either in the fresh or dried forms for the respective preparations. The healers mentioned formulation problems such as storage and microbial contamination affecting their herbal preparations.

The identified plants are mainly collected from forests (71%), farms/gardens (18%), special farms for medicinal plants (8%) and to lesser extent from markets (3%). Bushfires, deforestation, farming and environmental pollution are having a severe impact on the availability of some of these plants.

For identification of the plants most healers (92%) use organoleptic characteristics, a typical location or habitat of the plants and long experience with the plants as the various methods used in identifying these plants. Some of the healers (6%) use or collect a particular plant(s) based on spiritual intuition for a specific type of wound.

The study shows that 28% of the healers used burnt ashes of shell of black snail (*Chrysemys scripta*), salt (12%), shea butter (10%), soil/sand (8%), clay (4%), animal faeces (3%) and fats from python (1%) as non-plant means of treatment of various wounds.

Survey data were analyzed quantitatively by SPSS 16.0 to evaluate potential correlations between different parameters and variables such as type of wound, plants used, sex of healers, level of education and location of healer within the district. It was deduced that the stem bark of *Alstonia boonei* is used for the treatment of chronic wounds by healers ($p < 0.001$). There is a direct relationship between the use of leaves of *Chromolaena odorata*, *Justicia flava* and *Hoslundia opposita* for the treatment of new wounds such as cuts, bruises and abrasions ($p < 0.03$; $p < 0.04$, $p < 0.05$, respectively). The wound healing effects of *Chromolaena odorata* has been demonstrated by Phan et al. (1998, 2001) and *Hoslundia opposita* has been shown to possess antimicrobial properties (Gundidza et al., 1992), both properties are essential in wound healing (Mensah et al., 2006).

Also, there is an increased use of *Alstonia boonei* for treatment of chronic wounds in Akosomo and Pramso areas (both $p < 0.04$) within the survey district, indicating that only in this limited case only a local use is made, while all other herbal remedies are used over the entire survey district. This indicates a good communication between the healers and a broad exchange and spreading of knowledge in the area.

The relationship between increasing level of education of healers and the use of leaves of *Ficus exasperata* for treatment of new wounds was significant ($p < 0.04$) in the district. Increasing level of education of healers has a direct influence on the use of *Alchornea cordifolia* for the treatment of stomach ulcer/sores ($p < 0.01$) Also significant correlation between the higher level of education of healer and the use of leaves of *Ficus exasperata* for treatment of boils ($p < 0.05$) is found. This may indicate a high level of knowledge of medicinal plants among educated herbal/traditional medicine practitioners in the area.

There is also a higher tendency of male healers using *Pupalia lappacea* for treatment of boils ($p < 0.032$). Healers within the age range of 41–60 years with higher level of education treat more clients with stomach ulcer/sores ($p < 0.02$). Healers within the age group of 61–80 years tend to treat boils ($p < 0.03$). This is not surprising since most people in rural and even urban areas who suffer from frequent infections tend to consult old and experienced healers who had managed difficult and severe infections with medicinal plants.

3.2. Selection of plants for in vitro investigations

Regarding the frequency of use medicinal plants recorded during the survey were clustered into 3 groups: group I (very frequently used plants, named by healers more than 10 times), group II (frequent plants, recorded between 7 to 9 times), and group III with

Table 2
Medicinal plants used for the management of various wounds in Bosomtwi-Atwima-Kwanwonwa district, Ghana, according ethnopharmacological survey. [1]Wound healing activity cross-referenced in published literature by Neuwinger (1998), or [2]Burkill (2000).

Plant family/scientific name/local name (Asante-Twi)	Type of wounds	Formulations	Part of plant used
Acanthaceae			
Justicia flava Vahl.	New, old, deep, chronic wounds, boils, burns, stomach ulcer	Poultice, decoction	Fresh leaves
Afema	Also described in [2]		
Amaranthaceae			
Althernanthera pungens (L.) Link.	New wounds, boils	Decoction	Leaves
Abirimmuro			
Pupalia lappacea (L.) A Juss.	New wounds, boils	Poultice	Leaves
Aposompo	Also described in [2]		
Anacardiaceae			
Lannea welwitschii (Hiern) Engl.	Chronic wounds, stomach ulcer/sores	Decoction	Leaves, stem bark
Kumnini			
Mangifera indica L.	Chronic and new wounds	Poultice	Leaves, stem bark
Mango	Also described in [2]		
Annonaceae			
Annona squamosa L.	Burns	Poultice	Leaves
Apre			
Pachypodanthium staudtii Engl. et Diels	Measles, skin rashes	Cream with shear butter	Leaves
Duawusa	Also described in [1]		
Uvaria mocoli De Wild & T. Durand.	Shingles	Poultice	Leaves
Apraduro			
Xylopia aethiopica A.Rich.	Chronic wounds	Powder, poultice	Fruits
Hwentia	Also described in [1]		
Apocynaceae			
Alstonia boonei De Wild.	Chronic, new wounds, boils	Poultice	Stem bark, root
Nyamedua	Also described in [2]		
Funtumia elastica (Preuss) Stapf.	Stomach ulcer/sores	Decoction	Stem bark
Funtum			
Hunteria ghanensis J. B. Hall & Leeuwenb.	Stomach ulcer/sores	Enema	Seeds
Akuama			
Rauwolfia vomitoria Afzel.	Haemorrhoids	Root	Decoction
Kakapenpen	Also described in [1]		
Strophanthus hispidus DC.	Chronic wounds, stomach ulcer	Leaves, root	Poultice, decoction
Omaatwa	Also described in [1]		
Voacanga africana Stapf ex Scott-Eliot	New, old wounds	Decoction	Stem bark
Paaku			
Araceae			
Anchomanes difformis Engl.	Snake bites, burns	Decoction poultice	Leaves, stem bark
Opɛ	Also described in [1]		
Colocasia esculentus (L.) Schott	Stings/bites, chronic, deep wounds	Poultice, decoction	Leaves, stem bark
Kooko	Also described in [2]		
Arecaceae			
Elaeis guineensis Jacq.	Chronic wounds	Powder	Leaves
Abe			
Asclepiadaceae			
Parquetina nigrescens (Afzel). Bullock.	Boils, carbuncles, snake bites, new, old wounds	Poultice	Leaves, root
Abakamo	Also described in [1]		
Secamone afzelii (Roem. & Shult.) K.Schum	Chronic wounds, skin tumor, bites	Poultice	Aerial parts
Kwantemaa			
Asteraceae			
Ageratum conyzoides L.	New, old wounds, burns	Poultice	Leaves
Guakro	Also described in [2]		
Blumea aurita DC.	New, old wounds	Poultice	Leaves
Plaaduru	Also described in [2]		
Chromolaena odorata (L.) R.M King & H. Rob.	New, old wounds	Poultice	Leaves
Mfofo (Acheampong)			
Eclipta alba Hassk.	Deep wounds	Poultice	Leaves
Ntum	Also described in [2]		
Melanthera scandens Schu, Nach. & Thonn.	Stomach ulcer/sores	Decoction	Leaves
Mfofo	Also described in [2]		
Vernonia amygdalina Delile.	Stomach sores/ulcer	Decoction (enema)	Leaves
Odwono			
Bignoniaceae			
Kigelia africana (Lam.) Benth.	Stomach ulcer/sores	Decoction	Stem bark
Nufuhene	Also described in [1,2]		
Nerbouldia laevis Seem.	Stomach ulcer	Decoction, enema	Leaves, stem bark
Sesemasa/Sasanemasa	Also described in [2]		
Spathodea campanulata P. Beauv.	Skin rashes, haemorrhoids, stomach ulcer	Decoction	Leaves, stem bark
Kuokuonesuo	Also described in [1,2]		

Table 2 (*Continued*)

Plant family/scientific name/local name (Asante-Twi)	Type of wounds	Formulations	Part of plant used
Bombacaceae			
Bombax buonopozense P. Beauv. Akata	Stomach ulcer, burns	Decoction	Leaves
Ceiba pentandra (L.) Gaetn. Onyina	Stomach ulcer/sores Also described in [2]	Decoction	Stem bark
Capparidaceae			
Euadenia trifoliolata Oliv. Densinkro	Chronic wounds	Poultice	Leaves
Caricaceae			
Carica papaya L. Bofere	New, old, stomach ulcer/sores Also described in [2]	Poultice, decoction	Leaves
Caesalpiniaceae			
Cassia alata L. Osempe	Chronic wounds, shingles, burns	Poultice	Leaves
Cassia occidentalis L. Mmofraborodee	Stomach ulcer/sores	Decoction	Leaves
Erythrophleum ivorensis A. Chev. PɔtrɔdomPɔtrɔdom	Old wounds	Decoction	Leaves
Cecropiaceae			
Myrianthus arboreus P. Beauv. Nyankama	Stomach ulcer/sores	Decoction	Leaves
Chrysobalanceae			
Acioa dinklagei Engl. Atwere	Old and new wounds	Poultice	Leaves
Combretaceae			
Combretum smeathmannii G. Don. Hwiremoo	New, old wound, boils, burns Also described in [2]	Poultice	Leaves
Terminalia ivorensis A. Chev. Emire	Haemorrhoids, stomach ulcer, burns	Decoction	Stem bark
Crassulaceae			
Kalanchoe integra Kuntze. Egorɔ	Boils	Poultice	Leaves
Cucurbitaceae			
Momordia charantia L. Nyanya	Mouth sores, chronic wounds, stomach ulcer Also described in [1,2]	Poultice, decoction	Leaves, stem bark
Dioscoreaceae			
Dioscorea cayensis Lam. Bayere	Stomach ulcer/sores	Decoction	Leaves
Euphorbiaceae			
Acalypha ciliata Forssk. Mofoa	Chronic wounds, stomach ulcer Also described in [2]	Decoction	Leaves
Alchornea cordifolia Muell.Arg. Gyama	Deep wounds, fractures, haemorrhoids, stomach ulcer Also described in [2]	Decoction poultice	Leaves, stem bark
Euphorbia hirta L. Kakaweadwe	Deep wounds, carbuncles Also described in [1,2]	Poultice	Leaves
Jatropha curcas L. Nkrangyedua	Chronic and old wounds, boils, stomach sores Also described in [1,2]	Poultice, decoction	Leaves
Mallotus oppositifolius Muel. Arg. Nyanyafurowa (Pimpim)	New, old, chronic wounds, fractures, burns Also described in [2]	Poultice	Leaves
Manihot esculentus L. Bankye	New wounds, stomach ulcer/sores Also described in [2]	Poultice, decoction	Leaves
Phyllanthus muellerianus (Kuntze.) Exell. Awobɛ	New, old, deep and chronic wounds Also described in [2]	Poultice	Leaves
Phyllanthus urinaria L. Bowomaguwokyi	Chronic wounds	Poultice	Leaves
Ricinodendron heudelotii (Baill.) Pierre ex Heckel Nwama	Stomach ulcer/sores	Decoction	Stem bark
Fabaceae			
Acacia pennata Willd. Nwere	Burns, new and old wounds	Poultice	Leaves, stem bark
Albizia ferruginea Benth. AwiemfoɔsamenaAwiemfoɔsamena	Measles	Poultice	Leaves
Albizia zygia J.F. Macbr. Ɔkoro	Chronic wounds, swellings, carbuncles	Powder	Seeds, bark
Baphia nitida Lodd. Odwono	New, old and deep wounds, stomach ulcer	Poultice	Leaves, root
Milletia zechiana Harm. Frafraha	New, old wounds	Decoction	Root, stem bark

Table 2 (*Continued*)

Plant family/scientific name/local name (Asante-Twi)	Type of wounds	Formulations	Part of plant used
Lamiaceae			
Hoslundia opposita Vahl. Nunum nini	Chronic and deep wounds, stomach ulcer	Poultice, decoction	Leaves, root
Ocimum gratissimum L. Nunum	Burns, boils, stomach ulcer	Poultice, decoction	Leaves
Lecythidaceae			
Petersianthus macrocarpus (P. Beauv.) Liben. Asia	Boils	Poultice, decoction	Leaves
Loranthaceae			
Tapinanthus bangwenis Engl.& K. Krause Nkranpan	New and chronic wounds	Poultice	Leaves, stem bark
Malvaceae			
Abelmoschus esculentus Moench. Nkuruma	Chronic wounds	Powder	Seeds
Gossypium hirsutum L. Asaawa	Boils, skin rashes	Poultice	Leaves
Sida acuta Burm. f. Tweta	New and old wounds Also described in [2]	Poultice	Leaves
Meliaceae			
Khaya ivorensis A. Chev. Dubini	Boils, haemorrhoids, swellings, fractures	Poultice	Stem bark, leaves
Khaya senegalensis A. Juss. Kuntunkuri	Snake bites, chronic wounds	Powder, decoction	Stem bark
Mimosaceae			
Caepsalpinia bunduc (L.) Roxb. Abubuo	Haemorrhoids Also described in [1]	Decoction	Stem bark
Moraceae			
Ficus capensis Thunb. Doma	New and old wounds	Poultice	Leaves
Ficus elastica Roxb. Amanyedua	Boils, new and old wounds	Poultice	Leaves, stem bark
Ficus exasperata Vahl Nyankyerenee	New, old, deep wounds, boils, burns Also described in [1]	Poultice	Leaves, stem bark
Ficus leprieurii Miq. Amasusuwa	Boils	Decoction	Stem bark
Ficus sur Forskal Doma	Stomach ulcer Also described in [1]	Decoction	Stem bark
Musaceae			
Musa paradisiaca L. BƆƆdee	New wounds Also described in [2]	Poultice	Stem, leaves
Myristicaceae			
Pycnanthus angolensis (Welw.) Warb. Otie	Haemorrhoids, stomach ulcer, chronic wounds Also described in [2]	Decoction	Stem bark, leaves
Myrtaceae			
Psidium guajava L. Gua (Guava)	Chronic wounds	Decoction	Leaves
Passifloraceae			
Adenia cissampeloides (Planch.ex Benth.) Harms. Homakyem	Stings/bites	Decoction	Whole plant
Piperaceae			
Piper guineense Schumach. & Thonn. Sorowisa	Stomach ulcer/sores	Enema	Dried fruits
Piper umbellatum L. Mumuaha	Burns Also described in [1,2]	Poultice	Leaves
Phytolaccaceae			
Hilteria latifolia (Lam.) H. Wall. Anafranaku	Chronic and deep wounds	Poultice	Leaves
Poaceae			
Brachyachne obtusiflora (Benth.) C.E. Hubb. Aberekyere abodwesɛ	Boils	Poultice	Roots, leaves
Cymbopogon citratus (DC) Stapf. Akutukankan	Boils, swellings	Poultice	Leaves
Eleusine indica (L.) Gaetn Nsensan	New and old wounds Also described in [2]	Poultice	Aerial parts
Zea mays L. Aburo	Boils, carbuncles	Poultice, powder	Fruits, leaves

Table 2 (*Continued*)

Plant family/scientific name/local name (Asante-Twi)	Type of wounds	Formulations	Part of plant used
Portulaceae			
Portulaca oleracea L.	Boils	Poultice	Leaves
Adwera	Also described in [2]		
Rubiaceae			
Morinda lucida Benth.	Haemorrhoids, stomach ulcer	Decoction	Stem bark
Konkroma			
Psydrax subcordata (DC.) Bridson	Haemorrhoids, stomach ulcer	Decoction	Stem bark
Ntatiadupon			
Rutaceae			
Zanthoxylum gilletii (De Wild.) Waterman	Stomach ulcer/sores	Decoction	Stem bark
Okuo	Also described in [2]		
Zanthoxylum leprieurii (Guil. & Perr.) Engl.	Stomach ulcer/sores	Decoction	Stem bark
Oyaa	Also described in [2]		
Sapindaceae			
Blighia sapida Kon.	Snake bites, stings	Poultice	Stem bark
Akyee			
Paullinia pinnata L.	New, old and chronic wounds, haemorrhoids, stomach ulcer	Poultice, decoction	Roots, leaves
Toa-ntini	Also described in [1,2]		
Solanaceae			
Datura metel L.	Chronic, old and new wounds, stomach ulcer, haemorrhoids	Decoction, poultice	Leaves
Pepediewuo			
Lycopersicum esculentum Mill.	Chronic wounds, burns	Poultice	Fruits
Tomato			
Nicotiana tabacum L.	Stomach ulcer/sores	Decoction	Leaves
Ataa/taa	Also described in [1,2]		
Physalis angulata L.	Chronic wounds	Poultice	Leaves
TotↃtotↃ			
Schwenchia americana L.	Snake bites, chronic and new wounds	Powder, poultice	Leaves
Agyennyensu			
Solanum torvum Sw.	New wounds, boils	Poultice	Leaves
Kwaonsusua	Also described in [1,2]		
Sterculiaceae			
Cola nitida (Vent.) Schott & Endl.	Boils	Poultice	Leaves
Bese (Kola)			
Theobroma cacao L.	Deep wounds	Poultice	Leaves
Kokoo			
Triplochiton scleroxylon K. Schum.	Burns, stomach ulcer, haemorrhoids	Decoction, powder	Stem bark
Wawa			
Tiliaceae			
Duboscia viridiflora Mildbr.	New wounds	Poultice, powder	Stem bark, leaves
Akokoragyehin			
Urticaceae			
Laportea ovalifolia (Schumach.) Chew.	Boils, swellings	Poultice	Leaves
Akyekyenswonsa			
Vitaceae			
Ampelocissus multistriata (Bak.) Planch.	New and old wounds	Poultice	Leaves
Anunum			
Zingiberaceae			
Aframomum melegueta K. Schum.	Mouth sores, boils, skin rashes, fractures	Decoction (gargle), powder	Dried seeds
Famwisa	Also described in [2]		
Zingiber officinale Roscoe.	Chronic wounds, boils	Poultice	Rhizome
Akakaduro			

Table 3
Plants used for wound healing from group I (very frequent use, recorded more than 10 times by the 78 healers) and group II (frequent use, recorded 7 to 9 times by the 78 healers). Species in bold were selected for further detailed *in vitro* investigations.

Group I		Group II	
Plant species	Frequency	Plant species	Frequency
Hoslundia opposita	16	*Aframomum melegueta*	9
Alchornea cordifolia	13	***Phyllanthus muellerianus***	9
Ficus exasperata	15	*Mallotus oppositifolius*	8
Chromolaena odorata	11	*Paulina pinnata*	8
Justicia flava	11	***Pycnanthus angolensis***	8
Pupallia lappacea	11	***Spathodea campanulata***	8
Colocasia esculentus	10	***Alstonia boonei***	7
		Anchomames difformis	7
		Combretum smeathmanii	7
		Musa paradisica	7
		Parquetina nigrescens	7

plants recorded less than 7 times. Table 3 comprises all species found for group I (7 plants/7.3% of all species recorded, 87 hits/24% from total 363 hits during the survey) and group II (11 plants/11.5% of all species recorded, 84 hits/23% from total 363 hits during the survey). This means that 18 medicinal plants comprise 57% of all records from the healers.

The "top-18" plants were subjected to an intense review concerning details of use given by the healers. 11 plant species were selected (Table 3) for a further *in vitro* investigation for influence on cell physiology of human skin cells. One of the most cited species, *Chromolaena odorata* L., was excluded from this screening because skin activity is well documented and rationalized (Phan et al., 1998, 2001). Also *Colocasia esculentus* L. was excluded because it remained unclear from the survey which part of the plant should be used.

In three cases we found that the traditional use is related also to the treatment of stomach ulcer and therefore it was decided to investigate the three herbal remedies (*Hoslundia opposita, Pycnanthus angolensis, Spathodea campanulata*) into a screening against *Helicobacter pylori*.

3.3. Influence of extracts on skin cells under in vitro conditions

Aqueous and EtOH/H_2O extracts were prepared from 11 selected plants from group I and II (Table 4). The lyophilized extracts were investigated at 10 and 100 μg/mL concerning a potential influence on mitochondrial activity on keratinocytes (HaCaT cell line). Aqueous extracts from *Combretum smeathmanii, Phyllanthus muellerianus* and *Pycnanthus angolensis* turned out to induce the mitochondrial activity significantly.

Aqueous extracts from these three species were subjected to a more detailed investigation on human keratinocytes and primary human dermal fibroblasts (pNHF) using MTT assay and BrdU proliferation ELISA (Table 5). The stimulating effects of *Phyllanthus muellerianus* on keratinocytes were dose dependent from 10 to 100 μg/mL and the increased cellular energy status, as measured by MTT test, was accompanied by an increased cellular proliferation. Dermal fibroblasts were more sensitive against the *Phyllanthus muellerianus* extract and were stimulated within MTT and BrdU test between 5 and 20 μg/mL, while higher concentrations had inhibitory effects. Test on necrotic activity of the extract on fibroblasts was conducted at 1, 5, 10 and 20 μg/mL by quantification of LDH-release: No significant LDH-increase was obvious, indicating the absence of necrotic activity in this dose range on human fibroblasts.

Aqueous extracts from *Pycnanthus angolensis* stimulated keratinocytes at 50 to 100 μg/mL in MTT and BrdU assay. MTT activity in dermal fibroblasts was also increased, while the cell proliferation decreased.

Combretum smeathanni extract increased keratinocytes and fibroblasts mitochondrial activity at 10 μg/mL significantly, while higher concentrations turned out to have an inhibitory effect.

3.4. Influence of extracts on adhesion of Helicobacter pylori on human stomach cells

Aqueous extracts from *Spathodea campanulata*, *Hoslundia opposita* and *Pycnanthus angolensis* in concentrations from 0.5 to 2.5 mg/mL were investigated in an agar diffusion test against *Helicobacter pylori*, using amoxicillin as positive control. The extracts did not effect the bacterial growth in any of the test concentrations. Within a further assay the influence of the test extracts on the adhesion of *Helicobacter pylori* to AGS human stomach cells was investigated. In principle the bacteria were pretreated with the test extracts for 2 h. Unbound extract components were washed out. The bacteria were fluorescent-labeled and coincubated with a monolayer of AGS cells. Non-adhering bacteria were washed out; cells were trypsinized and measured by flow cytometry. An untreated negative control with maximal bacterial adhesion served as 100% control, positive control with strongly reduced adhesion was an extract from fruits of *Abelmoschus esculentus* (Lengsfeld et al., 2004a,b). The respective adhesion values are given in Table 6, indicating that all three extract are strong antiadhesive agents. This proves a well-documented rational for the traditional use of the plants for treatment of stomach ulcer.

3.5. Free radical scavenging activity of selected plants

Because wound healing can positively influenced by antioxidant agents, the radical scavenging activity of the ethanolic test extracts was determined by DPPH assay (Chizzola et al., 2008) and IC_{50} values were calculated (Table 7). Strong antioxidatives potential were found for most of the extracts. Especially for *Phyllanthus muelleri-*

Table 4
Influence of aqueous extracts and ethanolic extracts at 10 and 100 μg/mL for 72 h on mitochondrial activity of HaCaT keratinocytes (MTT-test). Data with standard deviation SD are from 3 independent experiments with each $n = 6$ replicates. Negative control: untreated cells; positive control: FCS 1%. $^*p < 0.05$, $^{**}p < 0.01$ compared to the untreated control group (ANOVA).

Plant and extracts	10 μg/mL	100 μg/mL	Plant and extracts	10 μg/mL	100 μg/mL
Anchomames difformis			***Astonia boonei***		
H_2O extract	97 ± 7%	94 ± 8%	H_2O extract	101 ± ±5%	103 ± 9%
EtOH/H_2O extract	95 ± 7%	98 ± 7%	EtOH/H_2O extract	101 ± 8%	103 ± 8%
Combretum smeathanii			***Ficus exasperata***		
H_2O extract	112 ± 7%*	100 ± 5%	H_2O extract	97 ± 6%	98 ± 6%
EtOH/H_2O extract	100 ± 6%	98 ± 8%	EtOH/H_2O extract	97 ± 6%	102 ± 8%
Hoslundia opposita			***Justicia flava***		
H_2O extract	100 ± 8%	102 ± 8%	H_2O extract	95 ± 9%	97 ± 9%
EtOH/H_2O extract	96 ± 8%	97 ± 9%	EtOH/H_2O extract	95 ± 7%	89 ± 5%
Parquetina nigrescens			***Phyllanthus muellerianus***		
H_2O extract	84 ± 9%*	84 ± 6%*	H_2O extract	107 ± 6%*	116 ± 7%*
EtOH/H_2O extract	91 ± 4%*	94 ± 7%*	EtOH/H_2O extract	105 ± 5%	99 ± 7%
Pycnanthus angolensis			***Pupalia lappacea***		
H_2O extract	104 ± 6%	115 ± 7%*	H_2O extract	102 ± 8%	100 ± 5%
EtOH/H_2O extract	99 ± 9%	104 ± 8%	EtOH/H_2O extract	99 ± 9%	98 ± 7%
Spathodea campanulata			***Positive control***		
H_2O extract	102 ± 6%	113 ± 8%*	*123 ± 6%**		
EtOH/H_2O extract	98 ± 8%	103 ± 8%			

Table 5
Influence of aqueous extracts at 10, 50 and 100 μg/mL for 72 h on mitochondrial activity (MTT test) and mitogenic proliferation (BrdU incorporation ELISA) of HaCaT keratinocytes and primary skin fibroblasts. Data and SD-values are from 3 independent experiments with $n = 6$ replicates. Negative control: untreated cells; positive control: FCS 1%. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the untreated control group (ANOVA).

Extract/(μg/mL)	Keratinocytes						Primary fibroblasts											
	MTT-assay (%)			BrdU-ELISA (%)			MTT-assay (%)						BrdU-ELISA (%)					
	10	50	100	10	50	100	1	5	10	20	50	100	1	5	10	20	50	100
Phyllanthus muellerianus	107 ± 6	112* ± 7	116* ± 7	106 ± 7	123* ± 7	126* ± 8	103 ± 6	109 ± 5	114* ± 5	119* ± 5	74 ± 10	68 ± 9	102 ± 12	100 ± 8	116 ± 15	121* ± 13	62** ± 8	54** ± 9
Pycnanthus angolensis	102 ± 6	108 ± 5	115* ± 7	105 ± 7	122* ± 11	115 ± 8			106 ± 9		122 ± 7	131* ± 15			116 ± 5	90 +/10		78* ± 13
Combretum smeathmanni	112* ± 7	105 ± 5	96 ± 8	106 ± 10	131** ± 12	169 ± 10			116* ± 9		108 ± 8	82 ± 8			88 ± 9		76 ± 11	64** ± 12
Positive control		123* ± 6			118* ± 9			121* ± 6			115* ± 8			115* ± 10			119* ± 7	

Table 6
Relative adhesion of FITC-labeled *Helicobacter pylori* to AGS cells, preincubated for 2 h with aqueous test extracts at 5 and 10 mg/mL. Data are related to the untreated control (=100%), positive control okra fresh extract. Data are mean ± S.D. ($n = 3$); $^{*}p < 0.05$; $^{**}p < 0.01$. n.d.: not determined.

Plant/extract	Relative adhesion (%)	
	5 mg/mL	10 mg/mL
Spathodea campanulata		
H_2O extract	46 ± 22*	49 ± 14**
EtOH extract	59 ± 5**	44 ± 11**
Hoslundia opposita		
H_2O extract	134 ± 14	48 ± 12*
EtOH extract	n.d.	15 ± 8**
Pycnanthus angolensis		
H_2O extract	79 ± 15	34 ± 10**
EtOH extract	60 ± 11*	44 ± 10**
Positive control (*Abelmoschus esculentus* fruit extract)	9 ± 6**	

Table 7
Free radical scavenging activities of ethanolic extracts of 10 selected plants used traditionally for wound healing within the district. DPPH assay plants; positive control: α-tocopherol.

Extracts	IC_{50} (μg/mL)	Extracts	IC_{50} (μg/mL)
Anchomas difformis	68.0	*Astonia boonei*	51.8
Combretum smeathmanii	7.2	*Ficus exasperata*	26.2
Hoslundia opposita	9.8	*Justicia flava*	81.3
Parquentina nigrescens	10.0	*Phyllanthus muellerianus*	7.6
Pycnanthus angolensis	7.8	*Pupallia lappacea*	0.8
Positive control (α-tocopherol)	0.6		

anus and *Combretum smeathmanii* the effects may be correlated to the high polyphenol content in form of tannins (14 resp. 10%),

4. Conclusion

From the above results there is strong indication that the traditional use of plant materials for wound healing as it is used by Ghanaians healers is assessed to be a valuable phytotherapeutic tool, which in many cases can be rationalized by *in vitro* investigations. The ethnopharmacological and traditional selection of plants in this African area seems to be highly effective, supporting people with effective medicines for wound healing. On the other side there is a strong need to characterize the plant material concerning quality aspects, but also for phytochemical aspects to isolate the compound(s) responsible for activity. Further *in vivo* studies should be conducted on the extracts to ascertain the pharmacological activities.

Aqueous extracts of *Phyllanthus muellerianus, Pycnanthus angolensis* and *Combretum smeathmanni* exhibited significant influence on the cell physiology of human keratinocytes and dermal fibroblasts *in vitro* beside strong antioxidative effects, correlated to the high tannin content. The use of plant species traditionally used against stomach ulcer seems to be due to an indirect antiadhesive effect, indicating a good and reliable base for the development of a rational phytotherapy based on the traditional herbal medicine of Ghana.

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References

Burkill, H.M., 2000. The Useful Plants of West Tropical Africa, third ed. Royal Botanic Gardens, Kew.

Chizzola, R., Michitsch, H., Franz, C., 2008. Antioxidative properties of *Thymus vulgaris* leaves: comparison of different extracts and essential oil chemotypes. Journal of Agricultural Food Chemistry 27, 6897–6904.

Deters, A., Schröder, K.R., Hensel, A., 2005a. Kiwi fruit (*Actinida chinnisis* L.) polysaccharides exert stimulating effects on cell proliferation via enhanced growth factors receptors, energy production and collagen synthesis of human keratinocytes, fibroblasts and skin equivalents. Journal of Cell Physiology 202, 717–722.

Deters, A., Brunold, C., Hensel, A., 2004. Polysaccharides from *Hibiscus sabdariffa* flowers stimulate proliferation and differentiation of human keratinocyte. Planta Medica 70, 370–373.

Deters, A., Petereit, F., Schmidgall, J., Hensel, A., 2008. N-Acetyl-D-glucosamine oligosaccharides induce mucin secretion from colonic tissue and induce differentiation of human keratinocytes. Journal of Pharmacy and Pharmacology 60, 197–204.

Deters, A.M., Schröder, K.R., Smiatek, T., Hensel, A., 2005b. Ispaghula (*Plantago ovata*) seed husk polysaccharides promote proliferation of human epithelial cells (skin keratinocytes and fibroblasts) via enhanced growth factor receptors and energy production. Planta Medica 71, 33–39.

Diallo, D., Paulsen, B.S., Hveem, B., 1996. Production of traditional medicine: preparations accepted as medicine in Mali. In: Hostettmann, K., Chinyanganya, F., Millard, M., Wolfender, J.J. (Eds.), Chemistry, Biological and Pharmacological Properties of African Medicinal Plants. University of Zimbabwe Publications, Harare, pp. 235–241.

Fabricant, D.S., Farnsworth, N.R., 2001. The value of plants used in traditional medicines for drug discovery. Environment Health Perspectives (Supplement) 109, 69–75.

Glasl, H., 1983. Zur Photometrie in der Drogenstandardisierung. Deutsche Apothekerzeitung 123, 1979–1987.

Gundidza, G.M., Deans, S.G., Svoboda, K.P., Mavis, S., 1992. Antimicrobial activity of essential oil from *Hoslundia opposita*. Central African Journal of Medicine and Medical Sciences 38, 290–293.

Harding, K.G., Morris, H.L., Patel, G.K., 2002. Science, medicine, and the future: healing chronic wounds. British Medical Journal 324, 160–163.

Houghton, P.J., Hylands, P.J., Mensah, A.Y., Hensel, A., Deters, A.M., 2005. *In vitro* tests and ethnopharmacological investigations: wound healing as an example. Journal of Ethnopharmacology 100, 100–107.

Inngjerdingen, K., Nergard, C.S., Diallo, D., Mounkoro, P.P., Paulsen, B.S., 2004. An ethnopharmacogical survey of plants used for wound healing in Dogonland, Mali, West Africa. Journal of Ethnopharmacology 92, 233–244.

JanBen, H., 2006. Integrationsversorgung und Begleitstudie, Pflegekongress Bremen, Berechung fur die stationäre Versorgung von Ulcus Cruris Patienten, Data base. Betriebskrankenkassen Verlag Deutschland, Frankfurt, Germany.

Jimoh, F.O., Sofidiya, M.O., Afolayan, A.J., 2007. Antioxidant properties of the methanol extracts from leaves of *Paullinia pinnata*. Journal of Medicinal Food 10, 707–711.

Konning, G.H., Agyare, C., Ennison, B., 2004. Antimicrobial activity of some medicinal plants from Ghana. Fitoterapia 75, 65–67.

Lengsfeld, C., Titgemeyer, F., Faller, G., Hensel, A., 2004a. Glycosylated compounds from okra inhibit adhesion of *Helicobacter pylori* to human gastric mucosa. Journal of Agricultural Food Chemistry 52, 1495–1503.

Lengsfeld, C., Deters, A., Hensel, A., 2004b. High molecular weight polysaccharides from black currant seed inhibit adhesion of *Helicobacter pylori* to human gastric mucosa. Planta Medica 70, 620–626.

Mahé, A., Faye, O., N'Diaye, H.T., Konare, H., Keita, S., Traore, A.K., Hay, R., 2006. Definition of an algorithm for the management of common skin disease at primary care level in sub-Suharan Africa. Transactions of the Royal Society of Tropical Medicine and Hygiene 99, 39–47.

Mensah, A.Y., Houghton, P.J., Dickson, R.A., 2006. In vitro evaluation of effects of two Ghanaian plants relevant to wound healing. Phytotherapy Research 20, 941–944.

Mensah, A.Y., Sampson, J., Houghton, P.J., Hylands, P.J., Westbrook, J., Dunn, M., Hughes, M.A., Cherry, G.W., 2001. Effects of *Buddleja globosa* leaf and its constituents relevant to wound healing. Journal of Ethnopharmacology 77, 219–226.

Neuwinger, H.D., 1998. Afrikanische Arzneipflanzen und Jagdgifte, 2nd ed. Wissenschaftliche Verlagsgesellschaft, Stuttgart.

Niehues, M., Hensel, A., 2009. *In vitro* interaction of l-Dopa with bacterial adhesins of *Helicobacter pylori*: an explanation for clinicial differences in bioavailability? Journal of Pharmacy and Pharmacology, doi:10.1211/jpp/61.10.00xx, in press.

Njoroge, G.N., Bussmann, R.W., 2007. Ethnotherapeutic management of skin diseases among Kikuyus of Central Kenya. Journal of Ethnopharmacology 111, 303–307.

Nyika, A., 2007. Ethical and regulatory issues surrounding African traditional medicine in the context of HIV/AIDS. Developing World Bioethics 7, 25–234.

Phan, T.T., Hughes, M.A., Cherry, G.W., 1998. Enhanced proliferation of fibroblast and endothelial cells treated with an extract of leaves of *Chromolaena odorata* (Eupolin) an herbal remedy for treating wounds. Plastical Reconstruction Surgery 101, 65–76.

Phan, T.T., Hughes, M.A., Cherry, G.W., 2001. Effect of an aqueous extract from the leaves of *Chromolaena odorata* (Eupolin) on the proliferation of human keratinocytes and on the migration in an *in vitro* model of reepithelialization. Wound Repair Regeneration 9, 301–313.

Ram, A.J., Bhakshu, L.M., Raju, R.R.V., 2004. In vitro antimicrobial activity of certain medicinal plants from Eastern Ghats, India, used for skin diseases. Journal of Ethnopharmacology 90, 353–357.

Zippel, J., Deters, A., Pappai, D., Hensel, A., 2009. A high molecular arabinogalactan from *Ribes nigrum* L.: influence on cell physiology of human skin fibroblasts and keratinocytes and internalization into cells via endosomal transport. Carbohydrate Research 344, 1001–1008.