Abstract

An ethnopharmacological survey was carried out among 78 traditional healers in Bosomtwi-Atwima-Kwanwoma area, Ashanti region, Ghana with the aim of collecting and documenting indigenous knowledge on medicinal plants used in the treatment of wounds. Additionally data were collected how traditional healers recognize wounds, how they classify and treat wounds with medicinal plants, how the respective plants are collected, identified, which parts of the plants are used, how they are obtained, prepared and applied to wounds.

Evaluation of the biodata of the healers (62 % male, 38% female) revealed that professional traditional healers are mainly older than 40 years (86 %), 14 % older than 80 years and only 14 % are younger than 40 years. Level of education of healers does not influence the potential qualification to practice as a traditional healer (only 1 % with university degree, 40 % no education, 50 % middle school/junior secondary school, 8 % high school).

Most of the healers defined wound as any opening that results from damage to the skin or superficial tissues. Healers classified wounds as acute, chronic or old wounds, injuries, abrasions, bites, boils, carbuncles, burns, hemorrhoids, skin rashes, parasitic wounds, fractures and internal ulcers. Leaves and aerial parts (86 %) are the main plant parts used in the herbal remedies and are mostly prepared in the form of poultice. The identified plants are mostly collected from forests (71 %) and organoleptic characters (odour, colour and shape) are mainly used by healers to identify the plants.

The survey revealed 104 plant species from 47 families been used as wound healing agents. Based on the frequency of use of plant and published work related to wound healing, 11 plants were selected for *in vitro* screening on skin cells. Aqueous extracts of *Phyllanthus muellerianus* (Kuntze.) Exell., *Pycnanthus angolensis* (Welw.) Warb. and *Combretum smeathmanni* G. Don. exerted positive influence on viability and proliferation of HaCaT keratinocytes and human dermal fibroblasts (pNHDF). *P. muellerianus* was selected for further bioassay-guided fractionation due to the *in vitro* activity of the aqueous extract on skin cells and the fact that secondary compounds of this plant are mainly unknown. Also, *P. muellerianus* was among one of the most frequently used plants in the treatment of wounds by healers.

Bioassay-guided fractionation of a methanolic extract of *P. muellerianus* lead to the isolation and characterization of 13 compounds reported for the first time for *P. muellerianus*: the ellagitannins geraniin (**1**), corilagin (**2**) and furosin (**3**), the flavonoids kaempferol-3-O-*β*-D-

glucoside (astragalin) (**4**), quercetin-3-O-*β*-D-glucoside (isoquercitrin) (**5**), quercetin-3-O-rutinoside (rutin) (**8**), further on brevifolin carboxylic acid (**6**), caffeoyl malic acid (phaselic acid) (**7**), chlorogenic acid (**9**), methyl gallate (**10**), gallic acid (**11**), caffeic acid (**12**) and 3,5-dicaffeoylquinic acid (**13**). Geraniin (3 to 6 %) was shown to be the dominant component of the extracts.

A suitable HPLC method for quality control analysis of geraniin content in *P. muellerianus* extract was developed and validated based on ICH-compliant protocol.

Geraniin and furosin increased under *in vitro* conditions the cellular energy status of human skin cells (MTT test on pNHDF and HaCaT-keratinocytes), triggering the cells towards higher proliferation rates, with fibroblasts being more sensitive than keratinocytes. Highest stimulation of pNHDF by geraniin was found at 5 μM, and of keratinocytes at 50 to 100 μM. Furosin stimulated pNHDF at about 50 μM, keratinocytes at about 150 to 200 μM. Necrotic cytotoxicity of geraniin, as measured by LDH release, was observed at 20 μM for pNHDF and above 105 μM for keratinocytes. Toxicity of furosin less than that of geraniin was observed at more than 400 μM.

Geraniin at 105 μM significantly stimulated the terminal differentiation of primary human epidermal keratinocytes while furosin had a minor influence on the expression of involucrin and cytokeratins CK1 and CK10. Geraniin and furosin stimulated the biosynthesis of collagen from pNHDF at 50 μM and 5 to10 μM respectively.