In Vitro Evaluation of Effects of Two Ghanaian Plants Relevant to Wound Healing

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Commelina diffusa and Spathodea campanulata are used as wound-healing agents in Ashanti traditional medicine in Ghana. The methanol extracts of Commelina diffusa herb and Spathodea campanulata bark showed some level of antimicrobial activity with C. diffusa exhibiting selective antifungal activity against Trichophyton species. The extracts reduced the peroxidation of bovine brain extract with an IC50 value of 1.39 mg/mL and 0.24 mg/mL, respectively. In addition the extracts also exhibited significant antioxidant activity by protecting MRC-5 cells from hydrogen peroxide induced oxidant injury at concentrations between 1 μg/mL and 10 μg/mL. The extracts showed no inhibition of NF-xB at 100 μg/mL. The antioxidant activities and antimicrobial activities suggest that the use of the plants in wound healing may be based on antioxidant and antiseptic effects of its constituents. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords: Commelina diffusa; Spathodea campanulata; ringworm fungi; bacteria; wound healing; lipid peroxidation.

INTRODUCTION

Recent years have witnessed an increase in interest in plants used in traditional medicine for healing wounds (Bodeker and Hughes, 1996; Houghton et al., 2005). Commelina diffusa Burn. F. (Commelinaceae) and Spathodea campanulata Beav. (Bignoniaceae) are two such species used in Ghana, which were identified as being used by several healers of the Ashanti ethnic group following a survey carried out amongst them. C. diffusa, also known as ‘spreading dayflower’, is a versatile perennial tropical herb with numerous traditional uses (Akobundu and Agyakwa, 1987; Burkill, 1985). Its tenacious nature is adequately exemplified by its local Ghanaian name which when translated literally means ‘God will die before I die’. In Ghana the leafy aerial parts are used to treat boils and sores (Ayensu, 1979; Burkill, 1985). S. campanula is widely distributed in Africa and the bark is used traditionally as an antimalarial, hypoglycaemic agent and also for the treatment of ulcers (Irvine, 1961; Makinde et al., 1988; Niyonzima et al., 1993). Phytochemical investigation of S. campanulata bark has shown the presence of triterpenes and sterols (Ngouela et al., 1991). There is, however, no documented information on the phytochemistry of C. diffusa. In Ghana, decoctions of both plants are made with water and the concentrated aqueous extracts obtained are applied to wounds to aid their healing.

Wound healing is a complex process involving three main overlapping stages; inflammation, cell proliferation and contraction of the collagen lattice formed (Bodeker and Hughes, 1996). Inflammation is a fundamental physiological process common to all wounds and begins immediately injury occurs. It involves the constriction and activation of the coagulation process producing a platelet plug to limit blood loss. In addition to this the local immune system is activated and soluble biological active mediators are released which attract leukocytes and monocytes to enter the wound and attack contaminating foreign objects. The neutrophils form the first line of defence against invading microorganisms. They phagocytose microorganisms then kill the ingested microbes by the production of oxygen-free metabolites such as hydroxyl radicals and superoxide ions. Paradoxically, normal tissues may be damaged by some of these reactive oxygen species in excess amount, thus prolonging the inflammatory phase of wound healing. The inflammatory phase may also be prolonged when the wound is colonized by bacteria (Cherry et al., 1994; Morrison and Kindlen, 1997; Laupattarakasem et al., 2003).

Some aspects of the inflammatory process exploited for screening for antiinflammatory extracts in vitro include evaluating the protection offered by extracts against oxidant injury to cells and monitoring the inhibition of peroxidation of lipid membranes (Aruoma et al., 1989; Mensah et al., 2001). Inhibition of NF-xB activation is used as an indicator of possible antiinflammatory action (Karin et al., 2004).

The present study was undertaken to determine whether there was any scientific justification for the...
MATERIALS AND METHODS

The leafy young stems and leaves of Commelina diffusa Burn. F. (Commelinaceae) and the stem bark of Spathodea campanulata Beav. (Bignoniaceae) were collected in April from the campus of Kwame Nkrumah University of Science and Technology, Kumasi, Ghana (KNUST) and authenticated by the curator of the herbarium at the Department of Pharmacognosy, Voucher specimens of the Commelina diffusa and Spathodea campanulata (CD1 and SC1) are deposited at the Faculty of Pharmacy, KNUST.

The plant materials were dried in sunlight and powdered. 200 g of each powdered material was continuously extracted with MeOH in a Soxhlet apparatus for 8 h to obtain 3.5 g of greenish syrupy extract of the Commelina diffusa and 2.4 g of a dark brown extract of the Spathodea campanulata, respectively.

In this investigation the inhibition of NF-kB was used as an indicator of possible anti-inflammatory action (Karin et al., 2004). Inhibition of NF-κB activation was measured utilizing luciferase in HeLa cells under the control of an IL-$6$ promoter (a gene target for activated NF-κB). The cells were exposed to the extracts (100 μg/mL) in 1 mL of growth media in replicates of three and incubated at 37 °C for 1 h. At this point phorbol myristic acetate (PMA) (50 ng/mL) was added to stimulate NF-κB activation. PMA stimulated and unstimulated cells acted as positive and negative controls, respectively. The cells were then incubated for a further 7 h before cell harvesting and lumimetric measurements in an automated 96-well format. Details of the experimental protocol can be found in Brenmer et al. (2004).

Inflammation also involves the formation of excess reactive oxygen species (ROS) so antioxidant properties in a plant extract can be anti-inflammatory and also protect the new cells formed during the proliferation stage from oxidative damage. Two tests for antioxidant activity was performed, one using liposomes and the other for protection of cultured cells. The liposome assay measured oxidative damage in terms of malondialdehyde (MDA) through its reaction product with thiobarbituric acid (Burits and Bucar, 2000). Serial dilutions (10 mg/mL to 0.32 mg/mL) based on the previous protocol of each extract were tested, assayed according to the method described by Aruoma et al. (1989) and Guvenc et al. (2003) and propyl gallate was used as the positive control. The percentage inhibition ($I$) of lipid peroxidation was calculated by the equation

$$I(\%) = 100 \times \left(\frac{A_{0} - A_{1}}{A_{0}}\right)$$

where $A_{0}$ is the absorbance of the control reaction (full reaction, containing no test compound) and $A_{1}$ is the absorbance in the presence of the inhibitor.

The other test for antioxidant protection was performed using a confluent MRC-5 cell line (Sigma, UK) challenged with H$_2$O$_2$ according to a method described previously (Murrell et al., 1990; Mensah et al., 2001). Catalase (250 IU/mL) was used as the positive antioxidant control and the normal control consisted of the cells alone (exposed only to the HBSS). Different doses of the extracts were tested and 1 μg/mL, 5 μg/mL and 10 μg/mL doses of the extract were selected and used since they gave reproducible and consistent results. The neutral red assay was used to assess the protection offered by the doses (Zhang et al., 1990). The cells were also visually examined to check for any visible sign of damage. Four separate experiments were carried out and each experiment was done with seven replicates. The data from both experiments were analysed by one-way analysis of variance and Dunnet’s test using the Microsoft Excel package. Differences at the 95% level were considered to be significant.

As noted above, antimicrobial effects in the extract would aid wound-healing so possible activity of the extracts in this respect was also tested. The organisms used were: Staphylococcus aureus (ATCC 2593); Bacillus subtilis (ATCC 6633); Escherichia coli (ATCC 25922); Pseudomonas aeruginosa (NCTC 5055); Candida albicans (ATCC 10231); Saccharomyces cerevisiae (NCTC 080178); Trichophyton interdigitale (NCPF 654) Trichophyton tonsurans (NCPF 656) and Microsporum gypseum (NCPF261). The bacteria were maintained on nutrient agar at 37 °C and the yeast and moulds on Sabouraud’s dextrose agar maintained at 30 °C and 26 °C, respectively. The agar serial dilution method was used to determine the minimum inhibitory concentration (MIC) for each extract with concentrations of the extracts from 125 to 1000 μg/mL (Vanden Berghe and Vlietinck, 1991; Mensah et al., 2000). Chloramphenicol and clotrimazole were used as positive controls for the bacteria and fungi, respectively, whilst the culture media used for the assay were used as negative control and the assay was performed in triplicate.

RESULTS AND DISCUSSION

In the tests for antioxidant effect using liposomes, the methanol extract of C. diffusa gave an IC$_{50}$ of 1.39 mg/mL and that of the S. campanulata was 0.24 mg/mL, propyl gallate having an IC$_{50}$ of 1 x 10$^{-4}$ M (21.2 mg/mL).

When the extracts were tested for protection of cultured MRC-5 cells, the methanol extract of C. diffusa at concentrations of 1.0, 5.0 and 10 μg/mL gave statistically significant protection ($p < 0.05$) against hydrogen peroxide-induced damage (Fig. 1), although the lowest
concentration of 1 µg/mL gave the greatest protection, possibly due to some cytotoxicity of the extracts at higher concentrations. Similar results were also obtained with the extract of S. campanulata (Fig. 2) and again an inverse dose-response trend was noted. Observation using a microscope showed changes in the shape of the cells treated with peroxide, which was not seen in the cells treated with the lowest dose extracts. This supported the hypothesis that the extracts gave protection against oxidative damage. However, cells treated with the 5 µg/mL and 10 µg/mL S. campanulata extracts showed characteristic signs of damage such as a rounded appearance and detachment from the wells, hence the higher doses of the S. campanulata are probably cytotoxic. Previous reports have shown that the antioxidant effect noted with cell lines was not due to direct interaction of the extract and hydrogen peroxide, but possibly due to an alteration of the cell membrane thus limiting the damage induced by the hydrogen peroxide (Tran et al., 1997; Mensah et al., 2001).

The results for the anti-NF-κB assay showed that neither fraction possessed inhibitory activity in this IL-6/Luc dependent cell based assay. The measurement of luciferase activity showed those cells exposed to 100 µg/mL of each extract had readings equivalent to the positive control values.

The antimicrobial experiments (Table 1) showed that the extracts had no activity against S. aureus, P. aeruginosa and the yeasts at the maximum test concentration of 1000 µg/mL, but they gave slight inhibition of the growth of B. subtilis with MIC of 500 µg/mL and 750 µg/mL, respectively. S. campanulata extract also showed some activity against E. coli (MIC 750 µg/mL).

Table 1. Minimum inhibitory concentrations (MIC µg/mL) of aqueous extracts of C. diffusa and S. campanulata

<table>
<thead>
<tr>
<th>Test organism</th>
<th>S. campanulata</th>
<th>C. diffusa</th>
</tr>
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<tbody>
<tr>
<td>S. aureus (ATCC 2593)</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>B. subtilis (ATCC 6633)</td>
<td>500</td>
<td>750</td>
</tr>
<tr>
<td>E. coli (ATCC 25922)</td>
<td>&gt;1000</td>
<td>750</td>
</tr>
<tr>
<td>P. aeruginosa (NCTC 5055)</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>C. albicans (ATCC 10231)</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>S. cerevisiae (NCTC 080178)</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>M. gypseum (NCPF 261)</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>T. interdigitale (NCPF 261)</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>T. tonsurans (NCPF 856)</td>
<td>250</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

The C. diffusa extract exhibited moderate antifungal activity against the Trichophyton species with MIC values of 500 µg/mL and 250 µg/mL against T. interdigitale and T. tonsurans, respectively, but no such activity was observed for the S. campanulata extract. Thus there is a weak level of antimicrobial activity in the crude extracts of the assayed plants, although the C. diffusa shows marked activity against the Trichophyton species, so may be of use in preventing fungal infection of wounds.

The antioxidant activities in particular show that there is some scientific justification for the traditional uses of the plants as wound healing agents in Ghana. The antimicrobial activity is unlikely to be very effective against infection of wounds at the concentration used, and there was no indication of NFκB inhibition as an indicator of antiinflammatory effects. Other tests for properties such as fibroblast stimulation or other mechanisms associated with antiinflammatory action will be of interest to investigate other activities related to wound-healing properties.

Acknowledgements

Financial support was provided by the WelCome Trust Fund as an International Research Development in Tropical Medicine Award. Research at ULSOP has been supported by the European Union, FP 6.

REFERENCES


