WOUND HEALING POTENTIAL OF METHANOL EXTRACT OF SPATHODEA CAMPANULATA STEM BARK FORMULATED INTO A TOPICAL PREPARATION

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Abstract

This study evaluated the wound healing potential of *Spathodea campanulata* stem bark in Sprague Dawley rats using the excision wound model. The methanol extract contained glycosides, flavonoids and tannins, and was relatively stable when stored at the room temperature for six (6) months. Solvent-free, semi-solid extract of *S. campanulata* was incorporated into an aqueous cream and applied (10 % w/w and 20 % w/w) on excision wounds of thirty two (32) rats. Cicatrin® cream was used as a standard wound healing agent. Prior to the remedial cream application, done later on twice daily, sixteen (16) rats had their wounds infected with *Staphylococcus aureus,* while in the remaining sixteen the wounds were kept clean. The surface area of the excision wounds was monitored planimetrically every four (4) days until a complete wound closure or healing took place. Excision wounds treated with 20 % w/w Spathodea cream and Cicatrin® cream showed a rapid and comparable decrease ($p > 0.05$) in wound size. In uninfected wounds, both 20 % w/w Spathodea cream and Cicatrin® cream application resulted in ~ 95 %-wound closure seen on Day 20, and a complete closure seen on Day 24. In infected wounds, both 20 % w/w Spathodea cream and Cicatrin® cream administration led to ~ 91 %-wound closure on Day 24 and a complete wound contraction on Day 28. The results of this study justify the folkloric use of *S. campanulata* stem bark to the effect of wound treatment.

Key words: *Spathodea campanulata*, wound healing, excision wound model, Spathodea cream, Cicatrin® cream, wound contraction

Introduction

A wound is described as a break in continuity of tissue arising on the grounds of violence or trauma, and acts as a portal of entry of microbial infective agents into inner tissues. Wound healing as a complex biochemical process, involves the interrelated processes of inflammation, cell proliferation and collagen lattice contraction (Bodeker and Hughes, 1998). Wound healing seeks to replace damaged tissue with a living one, or to regenerate the tissues at wounded or inflamed sites. Wound healing process can be affected by microbial infections and the presence of free oxygen radicals (Houghton et al., 2005). Other factors that may inhibit wound healing include necrotic tissue, diabetes mellitus, lymphatic blockage, certain drugs, and vitamin and mineral deficiencies (Puratchikody et al., 2006; Odimegwu et al., 2008).

Spathodea campanulata P. Beauv. (Bignoniaceae) is a plant species used in folkloric medicine in Ghana and several African countries to the effect of wound healing. Among the Ashanti ethnic group in Ghana, *S. campanulata* stem bark is applied on wounds by traditional healers in form of a paste (Mensah et al., 2003; Houghton et al., 2005). The plant is widely distributed in Africa, while its stem bark has hypoglycemic, anti-complement, anti-HIV and anti-malarial properties (Amusan et al., 1996; Niyonzima et al., 1999). Antimicrobial and antioxidant properties of *S. campanulata* significantly contribute to its wound healing potential. We have recently reported that extracts and topical products prepared from *S. campanulata* stem bark exhibit a broad-spectrum antibacterial activity, its methanol extract thereby demonstrating the most superior antibacterial activity of them all (Ofori-Kwakye et al., 2009). Several chemical constituents, to which the wound healing properties of *S. campanulata* stem bark are ascribed, have been isolated (Ngouela et al., 1988; 1990; Mbosso et al., 2008).

The present study was undertaken in order to evaluate the wound healing potential of *S. campanulata* stem bark claimed by traditional healers. Semi-solid, solvent-free methanol extract of *S. campanulata* stem bark was formulated into a topical preparation, applied on excision wounds of experimental rats, and thence evaluated for its wound healing potential.

Materials and Methods

Harvesting and preparation of the plant material

The stem bark of *S. campanulata* (voucher number FP/FP/SC201303/KOK) was harvested, authenticated, prepared and stored as previously described (Kwapong, 2007; Ofori-Kwakye et al., 2009).

Chemicals and standard drug

Nutrient agar, emulsifying ointment BP, chlorocresol, sodium hydroxide, hydrochloric acid, methanol, ethanol, acetone, petroleum ether, chloroform and dimethylsulphoxide (DMSO) were obtained from the Chemical store of the Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana. Cicatrin® antibiotic cream (GlaxoSmithKline, UK), each gram thereby containing 3,300 units of neomycin sulphate, 250 units of bacitracin zinc, 2 mg of L-cysteine, 10 mg of glycine, and 1 mg of dl-threonine, was used as a standard wound healing agent.

Test microorganism

Gram - positive *Staphylococcus aureus* NCTC 10788 bacterium was obtained from the stock of the Pharmaceutical Microbiology laboratory, Department of Pharmaceutics, Faculty of Pharmacy & Pharmaceutical Sciences, KNUST, Ghana. The bacterial strain was grown and maintained on a nutrient agar at 37 ºC, while a diluted preparation containing 5 x 10^7 *S. aureus* colony-forming units (cfu)/ml was used for *in vivo* study.

Animals

Thirty-two (32) Sprague Dawley rats of both sexes (16 of each gender), weighing 225 - 290 g and obtained from the stall of the Department of Pharmacology, KNUST, Kumasi, Ghana, were used for *in vivo* study. In order to stabilize prior to the experiment, the animals had been housed under standard environmental conditions, i.e. at the temperature of 31 ± 1^o C with 12:12 light/dark alteration for 7 days, fed with a standard pellet diet, and given tap water *ad libitum*. The study was conducted in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals (National Academy Press, 1996) and with approval by the Departmental Ethics Committee.

Plant extraction

About 100 g of the coarsely milled stem bark of *S. campanulata* was continuously methanol-extracted using a Soxhlet extraction apparatus for 24 hrs. The extract was filtered and concentrated under reduced pressure and the controlled temperature (50 - 55 ºC), so as to achieve dryness and ultimately obtain a solvent-free semi-solid extract.

The effect of storage time on extract's physicochemical properties

Physical appearance, solubility, pH, absorbance and phyto-chemical content of both freshly prepared and six (6) month-stored *S. campanulata* methanol extract were determined. The solubility of concentrated semi-solid extract was determined by virtue of vigorous shaking of 0.1 g of extract in 3 ml of a range of polar and non-polar solvents. Methanol extract's pH-value was tested using a standardized pH-meter. Absorbance of the extracts in reference was determined spectrophotometrically (Cecil CE spectrophotometer, England) at 268 nm wavelength (Kwapong, 2007). Methanol extract was screened for glycosides, flavonoids, tannins, saponins, coumarins, terpenoids, steroids and alkaloids using the methodology proposed by Harbourne (1998).

Formulation of *S. campanulata* topical cream

Aqueous cream BP was prepared using the fusion method (British Pharmacopoeia, 1993). Twenty grams (20 g) of semi-solid *S. campanulata* methanol extract were incorporated into the aqueous cream by virtue of pestle trituration in a ceramic mortar so as to obtain 100 g of topical preparation (Spathodea cream) containing 20 % w/w of *S. campanulata* extract. Another batch of Spathodea cream was prepared so as to contain 10 % w/w of *S. campanulata* extract. The remedial creams were stored in a refrigerator until used.

Excision wounds

Wounds were inflicted on thirty-two (32) Sprague Dawley rats in line with the excision wound model. The Sprague Dawley rats were divided into two equal groups (A and B), each group consisting of sixteen (16) rats. Animals assigned to each group were further divided into four subgroups, each subgroup contained four rats. In each group, wound healing effects of *S. campanulata* cream on infected (Group A) and non-infected (Group B) wounds were evaluated. The rats were anaesthetized with

diethyl ether and had their back skin hairs (dorsal region towards the tail section) shaved with sterilized razor blades. The depilated skin was cleaned using 70 %- ethanol; in further course, a circle having 20 mm in diameter was marked. A circular incision was made in the marked skin surface area; the skin was thence carefully dissected so as to end up with a wound roughly occupying a 314 mm^2-surface (Esimone et al., 2005; Perumal-Samy et al., 2006; Odimegwu et al., 2008). The excision wound area was measured immediately, i.e. traced out using a transparent tracing paper and then counted for a number of squares. The excision wounds inflicted to Group A animals were infected with *S. aureus* by virtue of in-swabbing of 24 h-bacterial broth culture (5 x 10^7 cfu/ml) using a sterile cotton swab (Perumal-Samy et al., 2006). Group B animals were left uninfected. Prior to treatment, the excision wounds of all experimental animals were left untreated for 24 hrs post wounding.

Wound treatment and wound healing measurement

Throughout the study period, the excision wounds were cleaned with normal saline every morning prior to medication. In each animal group (infected and uninfected), the wounds inflicted to animals constituting each of the four subgroups were treated with Cicatrin® cream (positive control), 20 % w/w *S. campanulata* cream, 10 % w/w *S. campanulata* cream, and normal saline (negative control), respectively. The remedial creams (or normal saline in case of negative controls) were applied topically twice daily and the wounds were left undressed, i.e. opened to the environment. Change in the wound area was monitored periodically every 4th day. Wound contractions were monitored planimetrically by tracing the wound margin on a graph paper (a transparent paper equipped with a millimeter scale) and calculated as percentage reduction in wounded area size (Mukherjee and Suresh, 2000; Esimone et al., 2005; Perumal-Samy et al., 2006). The Sprague Dawley rats were observed for two (2) weeks post complete wound closure and healing for any observable signs of adverse reactions to Spathodea cream.

Statistical analysis

Tracings of excision wounds were counted; the latter was supplemented by the surface area estimation. The wound closure surface area was also estimated using Scion image software (Scion Corporation, Frederick, Maryland – USA). Three readings were taken for each excision wound and the results were subsequently averaged. The statistical analysis made use of 2-way ANOVA processed using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). The results are presented as Mean ± Standard Deviation (SD) and P value, with the threshold value set at 0.05. Values of $P < 0.05$ were considered statistically significant.

Results

Table 1 depicts the solubility of freshly prepared and 6 month-stored *S. campanulata* stem bark extracts in different solvents. The extract was soluble in water, ethanol (70 %), methanol, DMSO, 1 M sodium hydroxide, and 1M sodium chloride; poorly soluble in chloroform, petroleum ether and ethanol (96 %); and insoluble in acetone. Extract storage did not affect its solubility in the solvents tested. Table 2 shows the influence of storage time on some physicochemical extract properties. The extract was shown to be acidic and the pH remained stable throughout storage. The absorbance of the extract, however, slightly changed during storage. Glycosides, flavonoids and tannins were present in fresh and stored extracts, whilst saponins, coumarins, terpenoids, steroids and alkaloids were not detected.

Table 3 shows the effect of *S. campanulata* cream on the contraction of uninfected excision wounds. Wound contraction or closure attained by virtue of 20 % w/w *S. campanulata* cream application was comparable ($p > 0.05$) to that attained by Cicatrin® cream application. Wound contraction seen with 20 % Spathodea and Cicatrin® cream on Day 20 was 95.50 ± 3.24 %, and 94.75 ± 1.95 %, respectively, while the ultimate wound contraction or closure was in both cases attained on Day 24. Wound contraction induced by 20 % w/w Spathodea cream was not statistically different ($p > 0.05$) from that seen with 10 % w/w Spathodea cream. In 10 % w/w Spathodea cream, a complete wound contraction was achieved on Day 28, as compared to Day 32 in the negative (normal saline-treated) controls. The group of rats whose excision wounds were left uninfected, no clinical signs of infection during and after the study had been revealed.

Table 4 shows the influence of *S. campanulata* cream on the contraction of excision wounds infected with *S. aureus*. During the study period, the excision wounds in reference appeared pale-red with patches of pus indicating the presence of a clinical infection.. Wound contraction attained by 20 % w/w Spathodea cream was comparable ($p > 0.05$) to that seen with Cicatrin® cream. On Day 24, wound contraction achieved with 20 % w/w Spathodea cream was 90.58 ± 9.55 %, as compared to 90.92 ± 3.53 % seen with Cicatrin® cream. Complete wound contraction or closure occurred on Day 28 both in Cicatrin® and 20 % w/w Spathodea creams. The results obtained by virtue of 10 % w/w Spathodea cream and normal saline administration in rats infected with *S. aureus,* were not statistically reliable as indicated by major standard deviations (Table 4).

Discussion

The solubility of *S. campanulata* stem bark methanol extract was determined in acidic, alkaline, polar and non-polar solvents. The extract was soluble in polar solvents and poorly soluble in non-polar solvents, indicating that the extract contains more polar than non-polar components. The stability of the extract was evaluated upon a 6 month-storage at the room

temperature; on the occasion, pH-value, absorbance and phytochemical content were determined. Upon a 6 month-storage at the room temperature, the extract's pH value, revealed to be acidic in the first place, remained relatively stable. In our *in vitro* studies, *S. campanulata* stem bark extract was found to contain glycosides, flavonoids, and tannins.

Table 1: The influence of storage time on the solubility of concentrated semi-solid extract of *S. campanulata* stem bark in different solvents

Solvent	Fresh SC extract	6month-stored SC extract
Water	Soluble	Soluble
1M NaOH	Soluble	Soluble
1M HCl	Soluble	Soluble
Chloroform	Poorly soluble	Slightly soluble
Petroleum ether	Poorly soluble	Slightly soluble
Ethanol (96 %)	Poorly soluble	Slightly soluble
Ethanol (70 %)	Soluble	Soluble
Methanol	Soluble	Soluble
Acetone	Insoluble	Insoluble
DMSO	Soluble	Soluble

SC = *S. campanulata,* DMSO = dimethylsulphoxide

Table 2: The influence of storage time on some physical and phytochemical properties of *S. campanulata* stem bark methanol extract

Parameter	Fresh SC extract	6 month-stored SC extract
Appearance	Coffee brown	Coffee brown
pH	4.58 ± 0.01	4.57 ± 0.05
*Absorbance	0.57 ± 0.01	0.52 ± 0.12
Glycosides	Present	Present
Flavonoids	Present	Present
Tannins	Present	Present

*λ_{max} = 268 nm, nominal concentration of *S. campanulata* extract used ~ 0.016 % w/v; SC = *S. campanulata*

Note: Both fresh and 6 month-stored SC extracts were tested for the presence of other phytochemicals, namely saponins, coumarins, terpenoids, steroids, and alkaloids, but all of the aforementioned tested negative.

Table 3: The effect of *S. campanulata* cream on the contraction of uninfected excision wounds inflicted to Sprague Dawley rats (mean ± SD, n = 4).

Day	Wound contraction (%)			
	Cicatrin® cream	20 % w/w *Spathodea campanulata* cream	10% w/w *Spathodea campanulata* cream	Normal saline
4	20.17 ± 4.81	32.75 ± 15.52	21.58 ± 8.19	20.00 ± 3.66
8	44.33 ± 17.57	62.83 ± 3.45	62.75 ± 7.71	36.00 ± 6.90
12	80.67 ± 3.50	87.17 ± 2.78	83.58 ± 6.84	44.00 ± 31.45**
16	86.50 ± 4.67	91.33 ± 4.50	85.83 ± 1.40	68.33 ± 45.60
20	94.75 ± 1.95	95.50 ± 3.24	88.75 ± 3.51	69.58 ± 46.44
24	100.00 ± 1.05	100.00 ± 0.80	93.50 ± 3.18	71.83 ± 47.89*
28	Healed	Healed	Healed	72.58 ± 48.39
32	Healed	Healed	Healed	Healed

*P < 0.05, **P < 0.01 when compared to Cicatrin® cream (positive control) and 20 % w/w *Spathodea campanulata* cream, Normal saline = negative control, Healed = 100 % wound contraction or closure

This confirms earlier reports on *S. campanulata* stem bark methanol extract content (Ngouela et al., 1990; Tsuchyia et al., 1996). No qualitative effects of extract storage on phytochemicals present therein had been revealed; however, possible quantitative effects could not be established. The tested extract did not contain phytochemicals such as saponins, coumarins, terpenoids, steroids, and alkaloids. Upon a 6 month-storage at the room temperature, the change in extract's absorbance properties ($p > 0.05$) was only slight. This means that storage induces only slight or no changes in extract's chemical composition. On the whole, 6 month-storage of *S. campanulata* extract at the room temperature had no significant effect on its physicochemical properties. The stability of the extract would minimize any physical or chemical breakdown which may affect its activity when stored for a longer period of time.

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissues to their normal state as closely as possible. In the current study, *in vivo* wound healing potential of *S. campanulata* stem bark-based cream was established in Sprague Dawley rats of both sexes using the excision wound model.

Table 4: The effect of *S. campanulata* cream on the contraction of excision wounds of Sprague Dawley rats infected with *S. aureus* (mean ± SD, n = 4)

Day	Wound contraction (%)			
	Cicatrin® cream	20 % w/w *Spathodea campanulata* cream	10 % w/w *Spathodea campanulata* cream	Normal saline
4	31.50 ± 10.15	15.17 ± 4.84	15.25 ± 11.74	12.83 ± 5.23
8	44.08 ± 2.27	34.92 ± 11.67	17.83 ± 20.76	35.50 ± 14.56
12	66.83 ± 3.72	56.67 ± 22.10	18.92 ± 28.31**	43.25 ± 21.74
16	74.75 ± 2.89	70.17 ± 15.74	31.92 ± 28.75*	53.17 ± 15.15
20	87.08 ± 1.10	91.42 ± 7.33	[b]44.83 ± 28.68*	61.17 ± 19.16
24	90.92 ± 3.53	90.58 ± 9.55	66.00 ± 10.53	73.25 ± 17.77
28	100.00 ± 1.95	100.00 ± 2.55	[a]59.75 ± 40.06*	84.33 ± 10.84
32	Healed	Healed	61.50 ± 42.93	62.08 ± 41.74
36	Healed	Healed	69.83 ± 47.01	64.50 ± 44.11
40	Healed	Healed	73.00 ± 48.81	64.67 ± 44.39
44	Healed	Healed	71.42 ± 47.83	71.08 ± 47.59
48	Healed	Healed	[b]50.00 ± 57.74**	100.01 ± 25.70

*P < 0.05, ** P < 0.01, when compared to Cicatrin® cream (positive control), [a]P < 0.05, [b]P < 0.01 when compared to 20 % w/w *Spathodea campanulata* cream, Normal saline = negative control, Healed = 100 % wound contraction or closure

The excision wounds were left untreated for 24 hours before treatment so as to allow for the *S. aureus* infection to take root. Wound contraction, which is a part of the end-stage wound-healing process, was measured and used as an index of the extract's wound healing potential. Wounds naturally heal dependent on the presence or absence of wound infection. Excision wounds infected with *S. aureus* took a longer time to contract and heal than the uninfected ones due to the fact that wound infection leads to the formation of microbial toxins and exudates implicated in regenerating cells'killing (Houghton et al., 2005). In both infected and uninfected excision wounds, wound contraction or healing process was accelerated by the application of *S. campanulata* topical preparations. Wound-healing effect exhibited by 20 % w/w Spathodea cream was slightly more pronounced ($p > 0.05$) than that of 10 % w/w Spathodea cream. Wound healing potential of 20 % w/w Spathodea cream was comparable ($p > 0.05$) to that of Cicatrin® cream, acknowledged as a standard antibacterial cream used to the effect of wound healing.

Different physiological processes known to be associated with wound healing include anti-inflammatory action, fibroblast proliferation, effect on keratinocytes, fibroblast protein expression, collagen lattice formation, antimicrobial activity, and antioxidant properties (Houghton et al., 2005). In order to offer scientific proof of, and justification for, their traditional use as wound-healing agents, plant-based topical remedies should exhibit multiple effects on these wound healing-related physiological processes. The current study provided ample evidence of wound healing potential of *S. campanulata* stem bark extract in terms of improving the rate of wound contraction and healing in both infected and uninfected experimental rats. The wound healing potential of *S. campanulata* stem bark could be attributed to the presence of phytochemicals such as glycosides, flavonoids, and tannins. Some of these phyto-constituents are known to demonstrate various antimicrobial and antioxidant

properties. The antimicrobial effect exhibited by the extract prevents the formation of microbial toxins which tend to inhibit cell regeneration, while its antioxidant action removes excess proteases and reactive oxygen species from the wounds and protect protease inhibitors from oxidative damage (Houghton et al., 2005). Flavonoids present in methanol extract of *S. campanulata* stem bark are known to reduce peroxidation by preventing or slowing down the onset of cell necrosis, as well as by improving vascularity. Flavonoids and tannins also promote wound healing, mainly due to their astringent and antimicrobial properties, which seem to be responsible for wound contraction and an increased rate of epithelization achieved by *S. campanulata* administration (Tsuchyia et al, 1996). The effect of *S. campanulata* stem bark extract, manifested in experimental rats in an accelerated rate of wound contraction and healing, provides a scientific basis in support of traditional use of *S. campanulata* stem bark to the effect of wound healing.

Conclusions

Methanol extract of *S. campanulata* stem bark contains glycosides, flavonoids and tannins, but no saponins, coumarins, terpenoids, steroids, and alkaloids. The extract was relatively stable when stored at the room temperature for 6 months. In experimental rats, 20 % w/w *S. campanulata* cream exhibited a substantial wound healing potential comparable to that of Cicatrin® cream, i.e. a standard antibiotic used for wound healing. The findings of this study justify the use of *S. campanulata* stem bark as a wound-healing agent recognized by folkloric medicine.

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