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Full Length Research Paper

Wound healing and antibacterial activities of the extract of *Dissotis theifolia* (Melastomataceae) stem formulated in a simple ointment base

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In this study, we investigated a herbal ointment containing *Dissotis theifolia* extract for wound healing and antibacterial activities against clinical wound isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The ointment batches containing different concentrations of the *D. theifolia* extract was applied topically to both infected and uninfected wounds inflicted on rats and the rate of wound closure assessed by wound area measurement. *In vitro*, the *D. theifolia* extract inhibited the different clinical wound isolates of *S. aureus and P. aeruginosa* with MICs ranging from 3.0 mg/ml for 3 of the 5 clinical strains of *S. aureus* to 8.0 mg/ml for all the 3 clinical strains of *P. aeruginosa* tested. In the study of uninfected wounds, incorporation of *D. theifolia* extract (60, 90, and 120 mg/g) into the applied ointment enhanced the rate of wound closure and reduced the epithelialization period from 14.98 ± 0.46 days for the control group treated with blank ointment to 8.8 ± 0.2 days for the group treated with 120 mg/g of *D. theifolia* ointment. Similarly, the rate of wound healing of excision wounds infected with clinical isolates of *S. aureus* were higher for the groups treated with *D. theifolia* ointment. In this study, the epithelialization period was equally reduced in a dose-related manner from 16.8 ± 0.3 for the blank ointment control to 9.0 ± 0.32 for the 120 mg /g *D. theifolia* ointment treated group. Phytochemical studies show that crude *D. theifolia* stem contains saponins, tannins, glycosides, flavonoids, terpenoids, carbohydrates, alkaloids and steroids.

Key words: *Dissotis theifolia*, herbal ointment, wound, wound healing, wound infections.

INTRODUCTION

Wound healing processes are well organized biochemical and cellular events leading to the growth and regeneration of wounded tissue in a special manner. Healing of wounds involves the activity of an intricate network of blood cells, cytokines, and growth factors which ultimately leads to the restoration to normal condition of the injured skin or tissue (Clark, 1991). The aim of wound care is to promote wound healing in the shortest time possible, with minimal pain, discomfort, and scarring to the patient and must occur in a physiologic environment conducive to tissue repair and regeneration (Bowler et al., 2001).

However, several clinical conditions and factors are known to impair wound healing and these include hypoxia, infection, tumors, metabolic disorders such as diabetes mellitus, the presence of debris and necrotic tissue on the wound, certain drugs, and dietary deficiencies of protein, vitamins, or minerals (Stadelmann et al., 1998). Wound infection resulting from impaired immunity and exposure or poor hygiene is one of the most commonly encountered and clinically important impediments to wound healing (Whaley and Burt, 1996; Stadelmann et al., 1998). The injured skin remains vulnerable to invasive

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microbial infections of all kinds and subsequent development of wound sepsis until complete epithelial repairs has occurred. Bacteria directly invade wounds producing inflammation and fluid exudation which interferes with the healing process. The bacteria toxins cause tissue damage and delay fibroplasia as well as collagen synthesis (Thomas and Howes, 1997)

Topical antimicrobial therapy is one of the most important methods of wound care (Rodeheaver et al., 1980; Veerapur et al., 2004). The goal of topical antimicrobial therapy in wound care is to control microbial colonization and subsequent proliferation thus promoting the healing of the wounds (Veerapur et al., 2004). Some medicinal plants have been employed in folk medicine for wound care (Udupa et al., 1995; Taranalli and Kuppast, 1996; Saha et al., 1997; Carson et al., 1998; Chitra et al., 1998; Veerapur et al., 2004; Rathi et al., 2004). Some of these plants either promote direct wound repair or exhibit antimicrobial and other related properties which are beneficial in overall wound care. Antimicrobial principles have been isolated from some of the medicinal plants used in folk medicine for wound care. *D. theifolia* is one of such plants employed by herbalists in the treatment of sores and boils.

D. theifolia is a shrub growing up to six 2 m high with long, narrow leaves which are oppositely situated on the bract membranous stem with plain or finely saw-edges (Harold, 1966). Extracts from the leaves, stem and roots of *D. theifolia* plant popularly called “aziza ohia” are used locally by communities in Nsukka area of south eastern Nigeria to threat inflammations, wound, and sores. The stem of the plants are often converted to chewing stick to treat gum infections. The antibacterial property of *D. theifolia* against common bacteria pathogens has been studied (Ofokansi et al., 2004).

This present study was carried out to assess the wound healing properties of the methanol extract of *D. theifolia* stem formulated in a simple ointment base and to ascertain its effect on clinical isolates obtained from infected wounds.

MATERIALS AND METHODS

Plant material

The stems of *D. theifolia* (Melastomataceae) were collected from a bush in Nsukka, Enugu State, Nigeria in August, 2006 and were authenticated by Mr. P.O. Ugwuozor of the Department of Botany, University of Nigeria, Nsukka, Nigeria. The stems were cut into smaller pieces, air-dried and then pulverized. About 200 g of pulversied stem was extracted with methanol by maceration for 72 h and concentrated *in vacuo* to afford 13.4 g (1.67 %w/v) of the dry extract. Phytochemical studies was carried out on the stem powder and on the methanol extract of *D. theifolia* according to the methods of Harbourne (1998)

Animals

White albino rats (180 - 250 g) obtained from the animal house of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka were used in the studies. They were allowed to acclimatize in the research laboratory for 5 days before the commencement of the study and were fed with standard livestock pellets (Guinea Feed Nigeria Limited). The animals were allowed unrestricted access to clean drinking water.

Test microorganisms

The test organisms used for this experiments study include 5 clinical isolates (A, B, C D, E, and F) of *S. aureus* and 3 clinical isolates (A, B, and C) of *P. aeruginosa* obtained from wounds and sores of patients undergoing treatment at the National Orthopedic Hospital (NOH), Enugu, Nigeria. Laboratory isolates of *c, Escherichia coli, P. aeruginosa, S. aureus, Klebsiella spp, Salmonella paratyphi* obtained from the Pharmaceutical Microbiology Laboratory, University of Nigeria were also used in the *in vitro* studies. Identification of bacterial isolates was performed according to standard bacteriological techniques previously established (Cowan et al., 1993; Baron and Finegold, 1990).

Drugs and reagents

The following materials were used in the study: liquid paraffin (BDH), methanol (Fluka, Germany), nutrient agar (Oxoid), soft white paraffin and emulsifying wax (Kindly supplied by the Formulation Unit, Department of Pharmaceutics, University of Nigeria, Nsukka, Nigeria).

Antimicrobial sensitivity and minimum inhibitory concentration (MIC) determination

A solution of the methanol extract (10 mg/ml) was prepared in DMSO. This solution was introduced into equidistant wells of 6 mm bored on the surface of nutrient agar seeded with the laboratory isolates of test organisms. Blank DMSO were also placed in separate wells and served as controls. The plates were incubated at 37°C for 24 h after a pre-diffusion period at room temperature. Inhibition zone diameter of 5 mm and above was taken as significant susceptibility of each test micro-organism to the extract.

The MICs of the *D. theifolia* extract against the 5 clinical isolates (A, B, C D, E, and F) of *S. aureus* and 3 clinical isolates (A, B, and C) of *P. aeruginosa* obtained from sores of different patients were determined using a modification of the agar dilution technique (NCCLS, 1990). Serial concentrations of the extract (0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, and 16 mg/ml) were incorporated into nutrient agar plates. Thereafter, a 24 h actively growing culture of the isolates were then streaked on the plates. MIC for each organism was taken as the lowest concentration of the extract in the nutrient agar that inhibited the visible growth of the organism after 24 h of incubation at 37° C.

Preparation of *D. theifolia* ointment

Simple ointment containing the methanol extract of *D. theifolia* was prepared by trituration method in a ceramic mortar and pestle using soft paraffin ointment base. Three batches of the ointment containing 60, 90 and 120 mg/g of the extract were prepared for the studies. Another batch of commercial gentamicin ointment, Gentalek cream® (Taylek Nigeria Limited.) containing 1% gentamicin was used as a positive control treatment. The ointments were stored in the refrigerator until they were used.

Infliction and infection of wounds

The rats were divided into five groups, each containing 5 animals. A round seal of 20 mm diameter was impressed on the two sides of the central trunk depilated and sterilized with ethanol. Excision wound was inflicted on the rats according to methods described by Morton and Mal-

Table 1. Phytochemical constituents of the crude powdered and the methanol extract of *Dissotis theifolia* stem

Phytoconstituent	Crude powdered stem of *D. theifolia* stem	Methanol extract of *D. theifolia* stem
Saponin	+	-
Tannins	+++	++
Carbohydrate	++	+++
Glycosides	++	+++
Flavonoids	++	++++
Terpenoids	+++	+++
Alkaloids	+	+
Steroids	+++	+++

Table 2. Preliminary anti-microbial screening of methanol extract of *D. theifolia* (10 mg/ml) against some laboratory isolates

Microorganism	IZD (mm) ± SEM
B. subtilis	15.5 ± 0.5
P. aeruginosa	19.5 ± 0.5
E. coli	21.5 ± 0.5
S. aureus	21.0 ± 1.0
Klebsiella spp	23.0 ± 0.0
S. paratyphi	20.0 ± 0.0

IZD = mean inhibition zone diameter, n = 3.

one (1972) under light ether anaesthesia. Full skin thickness was excised from the marked area to get a wound measuring about 314 mm^2. After achieving complete haemostasis by blotting the wound with cotton swab soaked in warm saline, the wound on the right side of each animals was inoculated with an overnight (18 h old) *S. aureus* culture obtained from one of the patients. The animals were placed singly in individual cages. The infected and uninfected wounds on each animal were treated as follows. Group one was treated with blank soft paraffin base (negative control); group 2 was treated with 1 mg/g gentamicin ointment (standard treatment); groups 3, 4, and 5 were treated with 60, 90 and 120 mg/g of the *D. theifolia* ointment. Treatments of the infected wounds commenced on the 3rd day to allow for the establishment of the infection on the wound.

The wound area was measured with a translucent paper and thereafter estimated on a 1 mm^2 graph sheet on day 3, day 5 and thereafter at 3 days intervals until completed wound closure was recorded. Wound contraction was calculated as a percentage of the original wound size.

Statistical analysis

The data were analyzed using one way analysis of variance (ANOVA) and data subjected to LSD post hoc test. Differences in mean between paired observations were accepted as significant at $P<0.05$.

RESULTS

Phytochemical studies show that crude *D. theifolia* stem contains saponins, tannins, glycosides, flavonoids, terpenoids, carbohydrates, alkaloids and steroids. The methanol extract contains similar phytoconstituents, but did not show positive reaction for saponins (Table 1).

All the laboratory strains of the different microorganisms screened were susceptible to 10 mg/ml *D. theifolia* methanol extract with an inhibition zone diameter ranging from 15.5 mm (for *B. subtilis*) to 23 mm (for *klebsiella spp*) (Table 2). The *D. theifolia* extract inhibited the different clinical wound isolates of *S. aureus and P. aeruginosa* with MICs ranging from 3.0 mg/ml (for A, B and F clinical strains of *S. aureus*) to 8.0 mg/ml (for all the 3 clinical strains of *P. aeruginosa*) (Table 3).

In the study of uninfected wounds, incorporation of *D. theifolia* extract (60, 90, and 120 mg/g) into the applied ointment enhanced the rate of wound closure and reduced the epithelization period from 14.98 ± 0.46 days for the control treated with blank ointment to 8.8 ± 0.2 days for the group treated with 120 mg/g of *D. theifolia* ointment (Table 4). Similarly, the rate of wound healing of excision wounds infected with clinical isolates of *S. aureus* were higher for the groups treated with *D. theifolia* ointment. In this study, the epithelisation period was equally reduced in a dose-related manner from 16.8 ± 0.3 for the blank ointment control to 9.0 ± 0.32 for the 120 mg /g *D. theifolia* ointment treated group (Table 5).

DISCUSSION

Traditionally, medicinal plants have been used for many years as topical and internal preparations to promote wound repair. Current researches are devoted to validating their efficacy and to uncover the mechanisms responsible for this activity. Medicinal plants have great potentials and have been shown to be very beneficial in wound care, promoting the rate of wound healing with minimal pain, discomfort, and scarring to the patient (Mackay and Miller, 2003). Some of these plants owe their effects to direct effect on the wound healing processes and some to their anti-inflammatory and anti-microbial properties. A combination of these properties is also possible in some of the medicinal plants used in wound

Table 3. Minimum inhibitory concentration (MIC) of the methanol extract of *D. theifolia* against some clinical isolates.

Organism	Clinical Isolates (Strains)	MIC ± S.E.M (mg/ml)
Staphylococcus aureus	A	3.0 ± 0.0
	B	3.0 ± 0.0
	C	6.0 ± 0.0
	D	7.0 ± 0.0
	E	7.0 ± 0.0
	F	3.0 ± 0.0
Pseudomonas. aeruginosa	A	8.0
	B	8.0
	C	8.0

n = 3

Table 4. The effect of *D. theifolia* ointment on the rate of wound closure of uninfected excision wound in rats

Treatment group	Wound closure (mm^2) (percentage wound closure in parenthesis)¶						Epithelization period (Days)
	Day 3	Day 5	Day 8	Day 11	Day 14	Day 17	
D. theifolia ointment (60 mg/g)	172.04±11.74 (54.79)*	281.53±2.85 (89.66)*	289.82±1.04 (92.3)*	314±0.05 (100.0)*	314±0.00 (100.0)	314±0.00 (100.0)	10.66±0.25
D. theifolia ointment (90 mg/g)	218.14±3.57 (69.47)*	294.16±8.53 (93.68)*	302±1.54 (96.18)*	314±0.12 (100.0)*	314±0.00 (100.0)	314±0.00 (100.0)	9.00±0.32*
D. theifolia ointment (120 mg/g)	116.8±13.6 (37.2)*	274.72±8.65 (87.49)*	312.4±0.18 (99.49)*	314±0.00 (100.0)*	314±0.00 (100.0)	314±0.00 (100.0)	8.80±0.20*
Gentamicin ointment (1.0 mg/g)	97.15±5.59 (30.94)	218.67±6.36 (69.64)*	277.67±3.15 (88.43)*	291.64±0.96 (92.88)	314±0.00 (100.0)	314±0.00 (100.0)	9.80±0.37*
Blank ointment	67.86±11.72 (21.61)	136.28±6.04 (43.4)	197.95±4.64 (63.04)	238.33±4.58 (75.9)	279.46±3.83 (89.0)	314±0.00 (100.0)	14.98±0.46

¶ Calculated on the original wound size of 314 mm^2; *$P < 0.05$, n = 5

Table 5. The effects *D. theifolia* ointment on the rate of wound closure of infected excision wounds in rats.

Treatment group	Wound closure (mm^2) (Percentage wound closure in parenthesis)¶						Epithelization period (Days)
	Day 3	Day 5	Day 8	Day 11	Day 14	Day 17	
D. theifolia ointment (60 mg/g)	92.00±5.69 (29.3)	206.53±3.34 (65.77)*	272.21±1.37 (86.69)*	310.74±0.00 (98.96)*	314.0±0.00 (100.0)*	314.0±0.00 (100.0)	11.8±0.37*
D. theifolia ointment (90 mg/g)	110.10±55.16 (35.06)	242.98±9.54 (77.38)*	287.39±5.65 (91.53)*	310.00±3.99 (98.73)*	314.0±0.00 (100.0)*	314.0±0.00 (100.0)	10.0±1.22*
D. theifolia ointment (120 mg/g)	198±17.59 (63.06)*	279.70±8.93 (89.08)*	310.74±0.00 (98.96)*	314.0±0.00 (100.0)*	314.0±0.00 (100.0)*	314.0±0.00 (100.0)	9.0±0.32*
Gentamicin ointment (1.0 mg/g)	115.12±6.29 (36.66)	226.99±6.96 (72.29)*	298.06±4.03 (94.92)*	313.46±0.31 (99.83)*	314.0±0.00 (100.0)*	314.0±0.00 (100.0)	8.8±0.20*
Blank ointment	54.74±5.96 (17.43)	103.93±6.80 (33.10)	173.67±5.00 (55.30)	217.58±5.64 (69.29)	262.04±3.26 (83.45)	307.6±3.12 (97.96)	16.8±0.37

¶ Calculated on the original wound size of 314 mm^2; *$P < 0.05$, n = 5

care.

In this study, topical application of *D. theifolia* methanol extract incorporated into the ointment on both the infected and uninfected wounds of the rats caused a significant ($P<0.05$) and faster rate of wound closure and reduced the epithelialization period. Wound healing is a natural process of regenerating dermal and epidermal tissue. Whenever there is a wound, a set of overlapping events takes place in a predictable fashion to repair the damage (Iba et al., 2004). The process has been conveniently categorized into phases such as the inflammatory, proliferative, and remodeling phases (Stadelmann et al., 1998). In the inflammatory phase, bacteria and debris are phagocytosed and removed and factors are released that cause the migration and division of cells involved in the proliferative phase. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction (Midwood et al., 2004). In epithelialization, epithelial cells crawl across the wound bed to cover it (Garg, 2000). The wound is closed by a combination of all these and by the process of wound contraction. During wound contraction, the wound is made smaller by the action of myofibroblasts, which establish a grip on the wound edges and contract themselves using a mechanism similar to that in smooth muscle cells. In the maturation and remodeling phase, collagen is remodeled and realigned along tension lines and cells that are no longer needed are removed by apoptosis. Although wound treatment with the ointment containing the extract of *D. theifolia* showed a pro-healing activity, the exact step and mechanism in wound repair processes affected by the extract was not established.

In our *in vitro* studies, the extract inhibited the clinical wound isolates of *S. aureus* and *P. aeruginosa* obtained from sores of patients. This antibacterial property of *D. theifolia* extract is very beneficial in wound care. Wounds are known to be easy portals for infections and provides suitable medium for the proliferation of microbial organisms. Wound infection has been identified as one of the most important factors that delays wound repair processes and outcome (Bowler et al., 2001). A number of microorganisms have been found to infect wounds among which are *P. aeruginosa, S. aureus, S. faecalis, E. coli, Clostridium perfringens, Clostridium tetani, Coliform bacilli* and enterococcus (Emele et al., 1999; Bowler et al., 2001). The antibacterial activity of the ointment batches containing the *D. theifolia* extract may contribute remarkably to the faster wound healing rate shown by these treatment groups. The inhibition of microbial contaminants of wounds allows the normal tissue repair processes to occur. For an infected old wound the eradication of the colonizing organisms will also create a suitable environment for wound healing to take place. It is interesting to note that the ointment formulation of *D. theifolia* recorded similar effectiveness when compared to the group treated with a commercial brand of gentamicin ointment.

Phytochemical studies showed that the crude *D. theifolia* stem powder and the methanol extract contain saponins, tannins, glycosides, flavonoids, terpenoids, carbohydrates, alkaloids and steroids. Some of these phytoconstituents are known to have anti-inflammatory, anti-infective and pro-wound healing activities which could be responsible for the results of these studies..

Conclusion

This study shows that *D. theifolia* has antibacterial and wound healing effect when formulated as ointment and could therefore explain the successes claimed in the folk use of the plant in the treatment of sores, boils and wounds.

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