



## Infected wound healing and antimicrobial effects of *Chenopodium ambrosioides* and *Mitracarpus scaber*

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### ABSTRACT

*Chenopodium ambrosioides* Linn. (*Chenopodiaceae*) and *Mitracarpus scaber* Zucc. (*Rubiaceae*) are herbal medicinal plants. They are commonly used in Togolese folk medicine to treat skin infections such as infected wounds, dermatoses, and scabies. The aim of this work was to evaluate the wound healing and antimicrobial properties of the two plants. Firstly, we evaluated the infected wound healing property of the two plants. We induced and treated infected wound in Wistar rats as follows. We inserted a sterile needle under the skin of the animal previously anesthetized by ether. Along the needle, we made an incision of 2 cm of length. The wound was infected with 24 hours colonies of *P.aeruginosa*. One infected wound was created per animal. 0.5 g of ointments prepared from *C. ambrosioides* and *M. scaber* leaf extracts, was applied twice a day. Secondly, we conducted antimicrobial assays with extracts prepared with the leaves of the plants. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Escherichia coli* and *Salmonella sp.* were used in this study. Infected wound in control group healed in  $12.6 \pm 0.245$  days. The ointment from *C. ambrosioides* or *M. scaber* leaf extracts, significantly and respectively reduced this mean time to  $07.04 \pm 0.245$  ( $P < 0.01$ ) and  $06.4 \pm 0.245$  ( $P < 0.01$ ) days. The extracts showed antimicrobial activities against all tested strains. *C. ambrosioides* and *M. scaber* possess wound healing and antimicrobial properties. These findings support their traditional use in treatment of skin infections.

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**Keywords:** antimicrobial activity, *Chenopodium ambrosioides*, *Mitracarpus scaber*, ointments, skin infected wounds.

### INTRODUCTION

Skin infections are very dangerous because they allow pathogens to reach other parts of the organism through the bloodstream (Robert and Kupper, 1999). These germs are involved in a large number of diseases including infectious endocardities (Entenza et al., 2004), arthritis and lung infections (Shirliff and Mader, 2002). *Chenopodium ambrosioides* Linn. (*Chenopodiaceae*) and *Mitracarpus scaber* Zucc. (*Rubiaceae*) are herbal medicinal plants, which are distributed over large areas in Togo. *C. ambrosioides* has been shown to be analgesic (Amole and

Yusuf, 1999), ascaricidal and insecticidal (Chiason and Bostanian, 2004). It is also hepatotoxic (Rufa et al., 2002), cytotoxic and genotoxic (Gadano et al., 2006). Anti-inflammatory (Ekpendu et al., 1994), antifungal (Obiodum, 1999), hepatoprotective, antiulcer (Aklidikou et al., 1995) properties have been demonstrated for *M. scaber*. The pulp of the leaves of *C. ambrosioides* is topically used for abscesses; its leaves are also used in treatment of wounds and abscesses in Ghana (Ekpendu et al., 1994). Oedema and pain are treated with the sap of the plant; the leaves are also used for treating scabies and

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other dermatoses (Ekpendu et al., 1994). The two plants are commonly used in Togolese folk medicine to treat skin abscesses, infected wounds, viral, fungal and bacterial dermatoses (Adjanon-houn et al., 1986). There is no scientific report which supports the traditional use of the plants for treatment of skin infections. Then, we aimed to evaluate the antimicrobial and infected wound healing properties of the two plants.

## MATERIALS AND METHODS

### Plant material

We collected *C. ambrosioides* and *M. scaber* in April and October 2006 respectively. They were collected at Tsevie, a locality situated at 35 km in the north of Lomé (Togo). The samples were identified at the Department of Botany and Plant Ecology of the Faculty of Sciences, University of Lomé (Togo). A voucher specimen of each plant was deposited in the herbarium of this Department. Aerial parts of the plants were washed, cut into pieces, shade dried and used for extract preparation.

### Tested microorganisms

*Staphylococcus aureus* (gram positive bacterium), *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella sp.* (Gram negative bacteria) and *Candida albicans* (fungus) were used for the *in vitro* antibacterial and antifungal tests. All of these germs were available in the Laboratory of Microbiology of the "Ecole Supérieure de Techniques Biologiques et Alimentaires, ESTBA", University of Lomé, Togo.

### Culture mediums used

We used for bacteria, Müller Hinton agar and broth. For *C. albicans*, we used Sabouraud agar, Sabouraud broth and Chloramphenicol Sabouraud.

### Drugs used

Gentamycin, nystatin and 3% aureomycin cream were used as reference drugs. They were bought in pharmacies.

### Animals

Male and female Wistar rats weighing between 80 g and 90 g were used. Animals were bred at the Laboratory of Physiology/Pharmacology of the Faculty of Sciences,

University of Lomé. They were housed under standard environmental conditions (temperature and humidity). They had a 12 hours light/dark cycle. The animals had free access to food and water.

### Ointment preparation

The ointments were formulated as 20% extracts of the plants in shea nut butter base. Sixteen (16) grams of purified shea nut butter were melted. 4 g of the extract were added to the purified shea nut butter and mixed. The purified shea nut butter was supplied by the Department of Chemistry, Faculty of Sciences, University of Lomé.

### Extract preparation

We soaked in hydroethanolic solvent (v/v) for 72 hours, one hundred grams (100 g) of the dried material. Solutions obtained were filtered and evaporated to dryness. Evaporation was made under vacuum (Buchi) at 40 °C. Yields were 13% for *M. scaber* and 9.5% for *C. ambrosioides*.

### *Pseudomonas aeruginosa* infected wound induction

We used the modified method of Gisby et al. (2002) for induction of the infected wound. The method is described below. Animals were anesthetized by inhalation of ether. The fur on the back and flanks of the animal was clipped. The skin was cleaned with 95% ethanol. A sterile needle was inserted under the skin. An incision of 2 cm was made along the needle. The wound was infected with 24 hours colonies of *P. aeruginosa*. The inoculum contained  $10^8$  cfu/ml of bacterial suspension. One infected wound was made per animal.

### *In vitro* antimicrobial activity

We added to 0.5 ml of *C. ambrosioides* or *M. scaber* extract, 10 µl of germ suspension. The same volume of germ suspension was added to 0.5 ml of gentamycin (bacteria) or nystatin (*C. albicans*). A volume of 10 µl of the germ suspension was also added to 0.5 ml of Müller Hinton or Sabouraud broth. Each suspension contained  $10^4$  cfu/ml. The tubes were incubated at 37 °C for bacteria and 30 °C for the fungus. 24 hours later, the medium was spread on the appropriate agar plates. Each medium was

duplicated. Müller Hinton and Chloramphenicol Sabouraud agars were used for bacteria and *C. albicans* respectively. Inhibition rate was calculated as follow:  $I.R = 100 (1-X/10^5)$ . I.R: Inhibition rate, X: mean number of colonies on the two assay agar plates. Müller Hinton and Chloramphenicol Sabouraud agars served as control. Colonies numbered on these plates were considered  $10^5$  (de Souza and Amegavi, 1993).

Minimum Inhibition Concentrations (MICs) were the lowest concentrations which inhibited growth of the microorganisms more than 80%.

#### Treatment of the infected wounds

We started the treatment when skin lesions were formed. 0.5 g of the ointment was applied twice a day until total wound healing. Animals were randomly divided into five groups. Each group contained five animals. Animals were individually housed in each group. Group I consisted of rats with infected wounds not treated (control group). In group II, the infected wound was treated with shea nut butter. Shea nut butter was the vehicle for the administration of the extracts. The infected wound of group III was treated with 20% *M. scaber* extract ointment. Group IV infected wound was treated with 20% *C. ambrosioides* extract ointment. Group V was the positive control group; its rats with infected wounds were treated with 3% aureomycin ointment.

#### Statistical analysis

Results are expressed as mean  $\pm$  SEM. Statistical comparisons were made using the Student *t*-test. A P value of  $<0.05$  was considered statistically significant.

## RESULTS

Infected wounds of the non treated control group and the ones of the group treated with shea nut butter healed in twelve days. The ointment from *C. ambrosioides* or *M. scaber* reduced this period respectively to  $7.4 \pm 0.245$  days ( $P < 0.01$ ) and  $6.4 \pm 0.245$  days ( $P < 0.01$ ). Infected wounds treated with 3% aureomycin ointment healed in  $4.0 \pm 0.178$  ( $P < 0.001$ ) days (Table 1). Extract activities did not significantly ( $P > 0.05$ ) differ from the one exhibited by 3% aureomycin cream.

*C. ambrosioides* and *M. scaber* extracts inhibited all tested microorganisms. The best activities were obtained with *M. scaber* extract. This extract was more potent on *S. aureus*, *C. albicans* and *P. aeruginosa*, MICs are respectively 0.03 mg/ml, 0.03mg/ml and 1 mg/ml (Table 2).

## DISCUSSION

Bacterial infections as well as diabetic skin infections, surgical wounds and orthopaedic infections are very dangerous because they rapidly cause septicaemia. Septicaemia can be fatal if it is not quickly diagnosed. Skin candidosis and these bacterial infections are more dangerous for patients with depressed immunity. We infected rats' wounds with *Pseudomonas aeruginosa* colonies. *Pseudomonas aeruginosa* is a gram negative bacterium. Gram negative bacteria like *P. aeruginosa* and *Klebsiella pneumoniae* cause skin infections (Hendricks et al., 2001). The infected wounds induced were significantly healed by the ointments prepared from *C. ambrosioides* or *M. scaber* leaf extracts. Furthermore the extracts prepared

**Table 1:** Effects of *M. scaber* and *C. ambrosioides* extracts ointments on *P. aeruginosa* skin infected wounds in rats.

Groups	Wounds healing time (days)
Group I	$12.6 \pm 0.245$
Group II	$12.2 \pm 0.374$
Group III	$07.4 \pm 0.245^*$
Group IV	$06.4 \pm 0.245^*$
Group V	$04.0 \pm 0.178^*$

Group I: Non treated control group. Group II: Treated with shea nut butter only. Group III: treated with 20% *C. ambrosioides* extract ointment. Group IV: treated with 20% *M. scaber* extract ointment. Group V: treated with 3% aureomycin ointment (positive control). Treatment consisted of 0.5 g of the ointment applied twice a day. Values are means  $\pm$  SEM of 5 rats. \* significantly different from control ( $P < 0.01$ ).

**Table 2:** Antimicrobial activities of *C. ambrosioides* and *M. scaber* extracts.

Microorganisms	Minimum inhibition concentration (mg/ml)			
	<i>C. ambrosioides</i>	<i>M. scaber</i>	Gentamycin	Nystatin
<i>S. aureus</i>	50.00	0.03	0.01	N.T
<i>E. coli</i>	50.00	12.50	0.1	N.T
<i>P. aeruginosa</i>	75	1.00	0.1	N.T
<i>Salmonella sp.</i>	75.00	20.00	0.1	N.T
<i>C. albicans</i>	50.00	0.03	N.T	0.01

NT: non tested.

from their leaves significantly inhibited the growth of *P. aeruginosa* *in vitro* in our study. Gram positive bacteria such as *S. aureus*, *S. pyogenes* are also involved in skin infections (Hendricks et al., 2001). The growth of *S. aureus* was significantly inhibited *in vitro* by the extract prepared from leaves of *M. scaber* in our study. Robert and Kupper (1999) also obtained inhibition of growth of *S. aureus* with extract from *M. scaber*. They obtained, with the extract of *M. scaber*, the inhibition of growth of other gram positive strains, which cause skin infections, such as *Streptococcus faecalis* and *Bacillus subtilis*. Furthermore, *M. scaber* has been shown to possess antimicrobial properties against gram negative bacteria involved in skin infections such as *Klebsiella pneumoniae* (Ruffa et al, 2002). *C. albicans* is also involved in skin infections (Kugulberg et al., 2005). We obtained *in vitro*, inhibition of growth of *C. albicans* with the extracts of *C. ambrosioides* and *M. scaber*. Shirlif and Mader (2002) also obtained *in vitro* inhibition of *C. albicans* with extract of *M. scaber*. *C. ambrosioides* has been shown to possess antimicrobial properties against other microorganisms involved in skin infections like *Trichophyton mentagrophytes*, *Mycobacterium tuberculosis* M37Rv29 and *Microsporium audouinii* (Kishore et al., 1999; Lall and Meyer, 1999). The rapid healing of the infected wounds in our study could be explained by inhibition of the germ *in vivo* and acceleration of cicatrisation process. The extracts could also have accelerated the defence of the rats *in vivo*.

### Conclusion

We induced in rat, *P. aeruginosa* skin infected wounds. These infected wounds were significantly healed with ointments prepared from *C. ambrosioides* and *M. scaber*

hydroethanolic extracts. Furthermore, the extracts inhibited *in vitro* growth of some strains commonly associated with skin infections such as *S. aureus*, *P. aeruginosa* and *C. albicans*. *C. ambrosioides* and *M. scaber* possess infected wound healing and antimicrobial properties. These results support the traditional use of the two plants in skin infection treatment.

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