

## ORIGINAL ARTICLE

### Antibacterial activity of the fruit extract of *Physalis angulata* and its formulation

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The inhibitory activity of zinc oxide-ointment formulation as well as the unformulated crude extract of fruits of *Physalis angulata* was investigated against clinical wound isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The zinc oxide-ointment formulation and the unformulated *P. angulata* crude extract were found to be ineffective against *P. aeruginosa* at all concentrations used, but potent against *S. aureus* at varying degrees. The zinc oxide-ointment (100 mg g<sup>-1</sup>, 125 mg g<sup>-1</sup> and 150 mg g<sup>-1</sup>) and *P. angulata* crude extract/zinc oxide-ointment (100 mg g<sup>-1</sup>, 125 mg g<sup>-1</sup> and 150 mg g<sup>-1</sup>) formulations were only slightly active against *S. aureus* at the highest concentration of 150 mg g<sup>-1</sup>. The unformulated *P. angulata* crude extract alone exhibited the highest inhibitory activity against *S. aureus* at all concentrations used with zones of inhibition between 34.5 mm and 50.5 mm, followed by a formulation of the extract with only oleaginous base (ointment), with zones of inhibition between 12.8 mm and 20.3 mm. A one-way analysis of variance (ANOVA) of these values compared with the activity of Chloramphenicol (positive control) indicated significant inhibitory activity by the unformulated *P. angulata* crude extract and the extract and ointment formulation against *S. aureus* thus suggesting their efficacy in treating staphylococcal infections.

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#### INTRODUCTION

Wound is defined simply as the disruption of the cellular and anatomic continuity of the tissue (Edwards and Harding, 2000). Wound may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue (Raina *et al.*, 2008). The development of wound infection depends on the integrity and protective function of the skin. It has been shown that wound infection is universal and the bacterial type varies with geographical location, resident flora of the skin, clothing at the site of wound, time between wound and treatment (Anupurba *et al.*, 2006).

Efforts are being made all over the world to discover agents that can promote healing and thereby re-

duce the cost of hospitalization and save patients from amputation or other severe complications. The continuous development of antibiotic resistance of pathogenic microorganisms and particularly *Streptococcus pneumoniae* to penicillin, *Staphylococcus aureus* to methicillin, and *Enterococcus spp.* to vancomycin is a major health concern worldwide (Melissa *et al.*, 2005). More than 80% of the world's population now depends on traditional medicine for their ailments, especially for wound management (James and Isaac, 2010).

*P. angulata* L. belongs to the Solanaceae family and includes about 120 species with herbal characteristics and perennial habits. It is distributed throughout tropical and subtropical regions of the world (José *et al.*, 2003). *P. angulata* has a broad spectrum of biological activity including antibacterial, molluscicidal, antiprotozoal, anticancer, cytotoxic and immunomodulatory activities (Bastos *et al.*, 2005; Hseu *et al.*, 2011). In Ghana, it is called "totototo"

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among the Akans. The juice is used in the treatment of earache, jaundice, fever, bladder disease to mention a few. The fruit and other aerial parts are used in the treatment of boils, sores or wounds, constipation and digestive problems (Dokosi, 1998). Zinc oxide has been shown to have therapeutic application in treating a variety of skin conditions in products such as baby powder, barrier creams to treat diaper rashes, calamine cream, anti-dandruff shampoos, and antiseptic ointments (Harding, 2007). Application of oleaginous base as a vehicle in the formulation of zinc oxide for medicinal purpose may be worthwhile. This study was conducted to validate the scientific basis of using *P. angulata* in the treatment of wounds and to verify if zinc oxide ointment in combination with the extract of *P. angulata* will result in enhancement of inhibitory activity of the extract.

## MATERIALS AND METHODS

### Source of test bacteria

Clinical isolates of *S. aureus* and *P. aeruginosa* were obtained from incision type wounds at the Microbiology Department of the Tamale Teaching Hospital in the Northern Region of Ghana, in the Month of March 2012. Bacterial isolates were stored at a temperature between 2 and 8°C in nutrient broth. Pure cultures of each of the bacterial isolates were obtained by sub-culturing the isolates on Chocolate agar (OXOID, Basingstoke, Hampshire, England).

### Plant material

The fruits of *P. angulata* L were collected from Navrongo in the Upper East Region of Ghana in the month of November, 2011 and authenticated by Dr. Walter M. Kpikpi of the Department of Applied Biology in the Faculty of Applied Sciences of the University for Development Studies, Navrongo.

### Ethanollic extract of the fruits

The fruits were air dried under shade and then powdered using surface-sterilized mortar and pestle. One hundred and twenty (120 g) grams of the powdered material was macerated in 240 mL of ethanol and refluxed exhaustively. The extract was filtered after 24 hours with the aid of sterile cotton. The filtrate was then concentrated under reduced pressure using

a rotary evaporator at 45°C to obtain a yield of 7.2 g of crude extract. This was carried out according to the method described by Tomassini *et al.*, (2009).

### Preparation of *P. angulata* crude extract-ointment formulation

An ointment base was prepared by melting 2.5 g of white wax in a beaker on a thermostatic water bath. White petroleum jelly BP (47.5 g) was added and warmed until liquefied. The mixture was stirred until it began to congeal. Three batches of the *P. angulata* crude extract - ointment containing 100 mg g<sup>-1</sup>, 125 mg g<sup>-1</sup> and 150 mg g<sup>-1</sup> respectively of the extract were prepared and used in the antibacterial activity studies.

### Preparation of zinc oxide - ointment formulation

To verify whether zinc oxide has inhibitory effect on the test microorganisms, a zinc oxide - ointment was formulated with oleaginous emulsion base in batches with concentrations of 100 mg g<sup>-1</sup>, 125 mg g<sup>-1</sup> and 150 mg g<sup>-1</sup> respectively. These concentrations were weighed into separate beakers labeled A, B and C respectively. Molten ointment base was then added to each beaker to make 1000 mg of each batch. Each of the individual batches was then homogenized by trituration to give the zinc oxide-ointment.

### Preparation of *Physalis angulata* crude extract

*P. angulata* crude extract was weighed into separate beakers labeled E, F, G respectively, and dispersed in a buffer of pH 7.2 to derive concentrations of 100 mg mL<sup>-1</sup>, 125 mg mL<sup>-1</sup> and 150 mg mL<sup>-1</sup> respectively. These concentrations were used for the antibacterial studies.

### Preparation of *P. angulata* crude extract-zinc oxide ointment formulation

*P. angulata* crude extract and zinc oxide ointment was formulated together to determine whether they could have synergistic effect on the bacterial isolates. Equal quantities (50, 62.5, and 75 mg) of *P. angulata* crude extract and zinc oxide were weighed into separate beakers labeled J, K and L respectively. The mixtures were then treated with oleaginous

base at varying concentrations of 100 mg g<sup>-1</sup>, 125 mg g<sup>-1</sup> and 150 mg g<sup>-1</sup> respectively.

### Agar Diffusion Bioassay

The modified agar well diffusion method described by Perez *et al.* (1990) was employed. Inoculum of each test organism was prepared by emulsifying in 100 mL of sterile peptone water and standardized to get a turbidity of 0.5 McFarland Standard. Within 15 minutes of its preparation, a sterile cotton swab was then dipped into the standardized inoculum suspension. Surplus moisture was removed by rotating the swab several times whilst pressed firmly against the walls of the tube at a level above the peptone water. Agar plates (Mueller Hinton Agar, Oxoid) were then inoculated with the cotton swabs by rotating the swab while rubbing taking care that the whole area is inoculated. The inoculated plates were allowed to dry and five-millimeter (5 mL) diameter wells made on the plate with a sterile cork-borer at wide enough intervals. The wells corresponded with the prepared number of concentrations of each formulation, the positive and negative controls.

For each formulation, 1 mL of each concentration was drawn into a labeled well with a sterile micropipette taking care to avoid spillage onto the surface of the culture plate. A similar volume of the negative control (98% ethanol) and positive control (Chloramphenicol at 100 mg g<sup>-1</sup>) was also introduced into a well each on the same plate. The plates were left to stand until complete diffusion of the formulations into the medium. The plates were incubated in inverted positions at 37°C for 24 hours after which they were observed for inhibitory activity depicted by zones of inhibition around the wells. Inhibition zone diameters were measured using a ruler and recorded in millimeters (mm). The experiments were repeated three times to check for reproducibility.

### Statistical Analysis

Means and standard error of the mean were calculated for the zones of inhibition measured for the three sets of experiments in each case. These means were statistically compared using the one – way ANOVA to determine if they were significantly different at P < 0.05.

## RESULTS

All the three concentrations of the *P. angulata* crude extract formulation did not show activity against *P. aeruginosa*. They were however active against *S. aureus* with recorded zones of inhibition of 12.8 mm at the concentration of 100 mg g<sup>-1</sup> which increased to 20.3 mm at a concentration of 150 mg g<sup>-1</sup> (Table 1). *P. aeruginosa* was resistant to all concentrations of the zinc oxide ointment formulation used while *S. aureus* was only susceptible to the 150 mg g<sup>-1</sup> concentration with a mean zone of inhibition of 11.8 mm as compared to 45.5 mm recorded for the control (Chloramphenicol) at 100 mg g<sup>-1</sup> concentration (Table 2).

**Table 1: Activity of *P. angulata* crude extract-ointment preparation against test organisms**

PAG extract - ointment preparation	Zone of inhibition (mm)*	
	<i>S. aureus</i>	<i>P. aeruginosa</i>
PAG 100 mg g <sup>-1</sup>	12.75 ± 1.06	0.00 ± 0.00
PAG 125 mg g <sup>-1</sup>	16.25 ± 0.35	0.00 ± 0.00
PAG 150 mg g <sup>-1</sup>	20.25 ± 0.35	0.00 ± 0.00
CLP 100 mg g <sup>-1</sup>	45.75 ± 0.35	0.00 ± 0.00

**PAG = *P. angulata* and CLP = Chloramphenicol, \*Values represent means ± SEM of three independent measurements**

**Table 2: Activity of zinc oxide-ointment formulations against test organisms**

Zinc oxide-ointment	Zone of inhibition (mm)*	
	<i>S. aureus</i>	<i>P. aeruginosa</i>
ZnO 100 mg g <sup>-1</sup>	0.00 ± 0.00	0.00 ± 0.00
ZnO 125 mg g <sup>-1</sup>	0.00 ± 0.00	0.00 ± 0.00
ZnO 150 mg g <sup>-1</sup>	11.50 ± 0.71	0.00 ± 0.00
CLP 100 mg g <sup>-1</sup>	45.75 ± 0.35	0.00 ± 0.00

**ZnO = zinc oxide and CLP = Chloramphenicol, \*Values represent means ± SEM of three independent measurements**

### Antibacterial activity of *P. angulata* crude extract

Mean diameters of zones of inhibition of *S. aureus* by various unformulated *P. angulata* crude extract increased with concentration. *P. aeruginosa* was, however, not susceptible to the unformulated crude extract of *P. angulata* (Table 3). A maximum mean zone of inhibition diameter of 50.5 mm was attained at a concentration of 150 mg g<sup>-1</sup> against *S. aureus* as compared to the standard antibiotic (Chloramphenicol) which gave a mean zone of inhibition diameter of 79.8 mm at 100 mg g<sup>-1</sup> concentration.

### Antibacterial activity of *Physalis angulata* crude extract-zinc oxide ointment formulation

The *P. angulata* crude extract-zinc oxide ointment formulation gave the lowest inhibitory activity of all the formulations prepared. While *P. aeruginosa* was resistant to all concentrations, *S. aureus* was only inhibited by the 150 mg g<sup>-1</sup> concentration of the formulation with a mean zone of inhibition diameter of 7.50 mm (Table 4). This could be probably be due to the fact that the non-concentrated bioactive compound present in the crude extract has been prevented by the oleaginous base from having much contact with the test organisms. Ethanol was used as negative control and indicated no inhibitory activity in all test models.

## DISCUSSIONS

According to Bastos *et al.*, (2005), the fruits of *P. angulata* possess steroids known as physalins, physagulins with anolides and flavonoids. The inhibitory

**Table 3: Activity of unformulated *P. angulata* crude extract against test organisms**

Unformulated PAG extract	Zone of inhibition (mm)*	
	<i>S. aureus</i>	<i>P. aeruginosa</i>
PAG 100 mg g <sup>-1</sup>	34.50 ± 0.71	0.00 ± 0.00
PAG 125 mg g <sup>-1</sup>	40.50 ± 1.41	0.00 ± 0.00
PAG 150 mg g <sup>-1</sup>	50.50 ± 0.71	0.00 ± 0.00
CLP 100 mg g <sup>-1</sup>	79.75 ± 0.35	0.00 ± 0.00

**PAG = *P. angulata* and CLP = Chloramphenicol, \*Values represent means ± SEM of three independent measurements**

**Table 4: Activity of *P. angulata*-zinc oxide ointment formulation against test organisms**

<i>P. angulata</i> -zinc oxide ointment formulation	Zone of inhibition (mm) *	
	<i>S. aureus</i>	<i>P. aeruginosa</i>
PAG + ZnO 100 mg g <sup>-1</sup>	0.00 ± 0.00	0.00 ± 0.00
PAG + ZnO 125 mg g <sup>-1</sup>	0.00 ± 0.00	0.00 ± 0.00
PAG + ZnO 150 mg g <sup>-1</sup>	7.50 ± 0.71	0.00 ± 0.00
CLP 100 mg g <sup>-1</sup>	9.50 ± 4.80	0.00 ± 0.00

**PAG = *P. angulata*, ZnO = Zinc oxide and CLP = Chloramphenicol, \*Values represent means ± SEM of three independent measurements**

effects of *P. angulata* crude extract on the *S. aureus* could be due to these bioactive phytochemical compounds which are known to possess antibacterial properties. Results obtained from this study is similar to the work of Melissa *et al.*, (2005) and Osho *et al.*, (2010) which reported strong activities for methanol/water extracts and ethanolic extracts of *P. angulata* against some Gram-positive and Gram-negative organisms respectively.

Zinc oxide has been reported to have antibacterial activities (Padmavathy and Vijayaraghavan, 2008) and therefore was expected to have shown high inhibitory activity against the test organisms. The reduced activity exhibited by the zinc oxide could partly be due to the effect of oleaginous base which has been reported to have poor drug releasing potential and hence preventing the zinc oxide from exhibiting full efficacy against the microorganisms (Shargel *et al.*, 2009). The antibacterial activity of the formulated extract-ointment was low compared with the unformulated extract. This may be attributed to the effect of oleaginous base or the direct effect of the *P. angulata* components on the microorganisms.

## CONCLUSION

The comparatively high activities shown by both the *Physalis angulata* crude extract-ointment formulation and the unformulated *P. angulata* crude extract against *Staphylococcus aureus* seem to justify the

widespread use of the plant in the treatment of boils, sores and wounds in Ghana. Isolation, purification and formulation of the bioactive compounds could lead to the development of novel drugs from *P. angulata* with maximal therapeutic activity.

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### COMPETING INTERESTS

The authors declare that they have no competing interests.

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