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Full Length Research Paper

In vitro antimicrobial activity of *Cryptolepis sanguinolenta* (periplocaceae)

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The aim of the present study was to investigate *in vitro*, the effect of three different preparations of *Cryptolepis sanguinolenta*, obtained from 2 mg/ml each of 70% ethanol, hot and cold aqueous extract, as antimicrobial agents using agar diffusion method. The microbes used in this study consisted of one strain of *Salmonella typhimurium*, two strains each of *Proteus mirabilis, Pseudomonas aeruginosa* and *Candida albicans*, three strains each of *Salmonella typhi*, *Staphylococcus aureus* and *Klebsiella pneumo pneumoniae* and four strains of *Escherichia coli*. The ethanolic extract, inhibited the growth of 85% of the test organisms with zones of inhibition ranging from 16 - 30 mm (averaging 23.8 ± 0.88 mm). The hot water extract, inhibited 75% of the test organisms with zones of inhibition ranging from 13 - 30 mm (averaging 23.0 ± 1.07 mm) whilst the cold water extract inhibited 75% of the test organisms with zones of inhibition ranging from 8 - 23 mm (averaging 17.2 ± 1.23 mm). Thus, all the three extracts of *C. sanguinolenta* exhibited different degrees of antimicrobial activity.

Key words: Antibacterial, *Cryptolepis sanguinolenta*, periplocaceae.

INTRODUCTION

Cryptolepis sanguinolenta (Lindl.) Schltr. (Periplocaceae) also known as Nibima in Ghana is a slender, thin-stemmed climbing shrub with orange-coloured juice in the cut stem (Paulo and Houghton, 2003). It is normally found in the forest and thickets but can also be cultivated (Addy, 2003). The root of the plant is used in traditional African medicine to treat a variety of diseases, including malaria (Dokosi, 1998). Among the Fulani traditional healers in Guinea, the aqueous extract is used to treat jaundice and hepatitis B whilst in Zaire and Senegal, infusion of the roots is used in the treatment of stomach and intestinal disorders. The major alkaloid of the roots, cryptolepine (Dwuma-Badu et al., 1978, Tackie et al., 1991) is reported to possess a multiplicity of biological effects including antimicrobial, antimuscarinic, vasodilating, noradrenergic, antithrombotic, antiinflammatory, and hypoglycemic activities (Bierer et al., 1998). Some of these effects have been demonstrated in the crude extracts as well as its fractions (Addy, 2003). For example, the aqueous extracts of the roots have been reported to exhibit antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Candida albicans* (Boakye-Yiadom and Dwuma-Badu, 1977; Boakye-Yiadom and Heman-Ackah, 1979).To date the aqueous root extract of *C. sanguinolenta* has been evaluated for its antimalarial as well as anti-microbial properties, however, the potency of other solvent fractions have not been extensively investigated to the best of our knowledge. In this study, we have compared *in vitro*, the effect of ethanolic, cold and hot aqueous extracts of *C. sanguinolenta* as antimicrobial agents using Gram positive and Gram negative organisms as well as *C. albicans*, a fungus.

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EXPERIMENTAL MATERIALS

Plant material

Roots of *C. sanguinolenta* were collected from the hilly areas of Kwahu in the Eastern region of Ghana and authenticated by Mr Edwin Ofori-Lartey of the Plant Development Department of the

Table 1. Microbes used to access the antimicrobial activity of the different plant extracts.

Type of microbe	Microbe is resistant to	Source*
Salmonella typhi	AMP,CHL,COT	Clinical isolate 1
Salmonella typhi	COT,AMP,CHL	Clinical isolate 2
Salmonella typhi	CHL,COT,CXR	Clinical isolate 3
Salmonella typhimurium	COT,AMP,CHL	Clinical isolate
Escherichia coli	GEN,CXR	Clinical isolate 1
Escherichia coli	GEN,CXR	Clinical isolate 2
Escherichia coli	COT,GEN,CXR	Clinical isolate 3
Escherichia coli	AMP,GEN,COT,TET	Clinical isolate 4
Proteus mirabilis	COT,AMP,GEN	Clinical isolate 1
Proteus mirabilis	COT,TET,AMP	Clinical isolate 2
Pseudomonas aeruginosa	GEN	Clinical isolate 1
Pseudomonas aeruginosa	GEN,AMP,COT	Clinical isolate 2
Candida albicans	-	Clinical isolate 1
Candida albicans	-	Clinical isolate 2
Staphylococcus aureus	TET,AMP,PEN	Clinical isolate 1
Staphylococcus aureus	TET,GEN,PEN,AMP	Clinical isolate 2
Staphylococcus aureus	AMP,PEN	Veterinary animal
Klebsiella pneumo pneumoniae	COT	Clinical isolate 1
Klebsiella pneumo pneumoniae	COT,AMP,GEN	Clinical isolate 2
Klebsiella pneumo pneumoniae	TET,AMP,PEN	Veterinary animal

*Source: The numbered clinical isolates are from different clinical samples
AMP – Ampicillin COT – Co-trimoxazole GEN – Gentamycin
CHL – Chloramphenicol TET – Tetracycline PEN – Penicillin
CXR – Cefuroxime.

Centre for Scientific Research into Plant Medicine (CSRPM) and voucher specimens (CSRPM No. 520) kept at the CSRPM herbarium.

Microbes

The microbes used in this study were isolated from different clinical sources and consisted of one strain of *Salmonella typhimurium*, two strains each of *Proteus mirabilis, P. aeruginosa* and *C. albicans*, three strains each of *Salmonella typhi*, *Staphylococcus aureus* and *Klebsiella pneumo pneumoniae* and four strains *Escherichia coli*. The sources as well as the relevant characteristics of these test organisms are presented in Table 1.

METHODS

Antibiotic susceptibility testing

The test organisms were tested for susceptibility to ampicillin (AMP) (10 µg), cefuroxime (CXR) (30 µg), co-trimoxazole (COT) (1.25/23.75 µg), erythromycin (ERY) (15 µg), penicillin G (PEN) (10 µg), gentamycin (GEN) (10 µg) and tetracycline (TET) (30 µg) by the disc diffusion technique according to the guidelines set by the National Committee for Clinical Laboratory Standards (NCCLS, 1998).

Preparation of extracts

A cold water extract was prepared by maceration of 300 g of coarsely pulverized roots of *C. sanguinolenta*, in two litres of cold water overnight. The extract was obtained by filtration using a filter-press. The hot water extract, on the other hand, was prepared by boiling 300 g of the root powder in four litres of water for one hour and filtered using a filter press while hot to obtain a clear filtrate and allowed to cool. The hot and cold water extracts were freeze-dried and kept in a desiccator until needed. An ethanolic extract was obtained by the maceration of 300 g of root powder in two litres of 70% (v/v) ethanol overnight. The filtrate was obtained by pressing in a filter press. The alcohol was removed by evaporation under reduced pressure and the remainder of the filtrate freeze-dried and stored in a desiccator until needed.

Phytochemical screening

Phytochemical screening methods described by Fong et al. (1977) and Ciulei, (1982) were used to screen the various extracts obtained from the cold water, hot water and ethanol for the presence of alkaloids, cyanogenic glycosides, flavonoids, polyuronides, saponins and reducing sugars.

Determination of antimicrobial activity

Hundred millimeter (100 mm) Petri dishes, filled with 25 ml of Mueller-Hinton agar to a depth of 4 mm was allowed to solidify. In the case of *C. albicans*, Sabouraud dextrose agar plates were used. Each plate was then flooded with the test organisms and allowed to dry at room temperature for an hour on a level surface. Twenty milligram freeze-dried powders from the hot and cold water extracts were re-dissolved in 10 ml sterile distilled water whilst in the case of the ethanolic extract 20 mg was re-dissolved in 10 ml of

Table 2. Phytochemical screening of *C. sanguinolenta* extracts.

Extract	Polyuronides	Polyphenols	Saponins	Flavonoids	Alkaloids	Cyanogenic glycosides	Reducing sugars
Cold water	+	_	_	_	+	_	+
Hot Water	+	_	_	_	+	_	+
Ethanolic	+	_	_	_	+	_	+

+ Positive
- Negative

20% dimethyl sulfoxide (DMSO). A sterilized borer of internal diameter 6 mm was used to bore holes/wells in the media. About 100 µl of the different extracts of *C. sanguinolenta* were dispensed into the holes/wells. The plates were then kept in the refrigerator until complete absorption of the extract into the agar. The Mueller-Hinton agar plates were then incubated at 37°C for 18 - 24 h whilst the Sabouraud dextrose agar plates were incubated at 28°C for 5 - 7 days. The zones of inhibition produced were measured in millimeters using a metre rule. Water was used as the solvent control for the aqueous extracts whereas 20% DMSO was used in the case of the ethanolic extract. Chloramphenicol and Tioconazole 1% w/v were included as positive controls. All assays were carried out in triplicates.

RESULTS

Phytochemical screening

The phytochemical screening of the root of *C. sanguinolenta* showed that the constituents found in all the three extracts are alkaloids, polyuronides, and reducing sugars (Table 2).

Antimicrobial activity

All the three extracts at a concentration of 2 mg/ml were found to possess different degrees of antimicrobial activity (Table 3). The ethanolic extract, for instance, inhibited the growth of 90% of the test organisms with zones of inhibition ranging from 16 - 30 mm [23.8 ± 0.88 mm (STDEV)]. The hot water extract, on the other hand, inhibited 85% of the test organisms with zones of inhibition ranging from 13 - 30 mm [23.0 ± 1.07 mm (STDEV)] whilst the cold water extract inhibited 75% of the test organisms with zones of inhibition ranging from 8 - 23 mm [17.2 ± 1.23 mm (STDEV)]. In comparison, it was observed that even though the ethanolic extract inhibited the growth of majority of the organisms, the zones of inhibition produced were not significantly different from those produced by the hot water extract. In most cases, the zones of inhibition produced by the extracts were better than those produced by the chloramphenicol and tioconazole.

DISCUSSION

The aqueous extract of *C. sanguinolenta* is an established antimalarial in the West African sub-region (Boye and Ampofo, 1983), and there are several reports on the antimalarial activity of its major alkaloid, cryptolepine (Kirby et al., 1995; Noamesi et al., 1991; Wright et al., 1996). For the treatment of several infections in Africa, indigenous medicinal plants are often the only means (Fennell et al., 2004) and, thus, the continuous interest in laboratory screening of medicinal plants, not only to scientifically validate claims with regard to their usage, but also to discover new active principles for drug development, is indeed a laudable effort. In the present study, the antimicrobial properties of *C. sanguinolenta* were investigated using cold and hot water as well as ethanolic extracts prepared from the dried roots.

All three extracts were found to show broad spectrum activity by inhibiting both Gram-positive and Gram-negative organisms used including organisms isolated from veterinary animals as well as *C. albicans*. It has been shown that, due to the introduction of antibiotics in poultry and animal husbandry, most microbes isolated from veterinary animals have been found to be highly resistant (Kharchatourians, 1998; Sackey et al., 2001). It is therefore good news that, the preparations from *C. sanguinolenta* were all able to inhibit the isolates obtained from the veterinary animals. These inhibitions produced by the three extracts could be due to the presence of alkaloids, principally, cryptolepine. For instance, cryptolepine, the main indoloquinoline alkaloid present in *C. sanguinolenta,* has been shown to cause cell lysis and morphological changes in *S. aureus* (Sawer et al., 2005). Cryptolepine is also known to be a DNA intercalator and an inhibitor of DNA synthesis through topoisomerase inhibition (Bonjean et al., 1998; Dassonnrville et al., 2000; Guittat et al., 2003; Lisgarten et al., 2002). Thus, with these attributes of cryptolepine, it is no wonder that *C. sanguinolenta* has the ability to inhibit the growth of both Gram positive and Gram negative organisms as well as *C. albicans*.

All the test organisms used have been implicated in various diseases such as diarrhoea, dysentery, wound sepsis, pneumonia, typhoid fever, food poisoning, respiratory and urinary tract infections (http://medic.med.uth.tmc.edu/00001450.hmt, 2006). The ethanolic extract inhibited the growth of majority of the test organisms probably because it may possess some active compound(s) that might be absent in both the hot and cold water extracts or the ethanol might have extracted more of the alkaloids than the other two extracts. However, the fact that the hot water extract had comparable zones of inhibition to those of the ethanolic

Table 3. Antimicrobial activity of the crude extracts of *C. sanguinolenta.*

Test organism	Mean zones of inhibition (mm)		
	Ethanolic extract	Hot water extract	Cold water extract
Salmonella typhi	25.0 ± 1.00	18.0 ± 1.00	NS
Salmonella typhi	23.0 ± 2.65	20.0 ± 5.91	NS
Salmonella typhi	16.0 ± 1.73	18.0 ± 2.65	11.0 ± 5.91
Salmonella typhimurium	16.0 ± 1.73	30.0 ± 0.91	20.0 ± 1.73
Escherichia coli	NS	13.0 ± 2.65	8.0 ± 0.91
Escherichia coli	25.0 ± 1.73	24.0 ±	13.0 ± 2.65
Escherichia coli	25.0 ± 1.73	25.0 ± 1.73	22.0 ± 2.65
Escherichia coli	23.0 ± 1.73	20.0 ± 2.65	12.0 ± 2.65
Proteus mirabilis	28.0 ± 2.65	25.0 ± 2.65	15.0 ± 1.73
Proteus mirabilis	19.0 ± 4.58	NS	NS
Pseudomonas aeruginosa	19.0 ± 1.00	NS	NS
Pseudomonas aeruginosa	25.0 ± 2.65	25.0 ±1.73	23.0 ± 1.00
Candida albicans	NS	NS	NS
Candida albicans	27.0 ± 5.91	25.0 ± 1.73	17.0 ± 0.84
Staphylococcus aureus	20.0 ± 2.65	22.0 ± 2.65	22.0 ± 1.73
Staphylococcus aureus	23.0 ± 4.58	20.0 ± 2.65	20.0 ± 2.65
Staphylococcus aureus	22.0 ± 3.46	25.0 ± 1.73	18.0 ± 1.00
Klebsiella pneumo pneumoniae	30.0 ± 3.60	25.0 ± 0.84	20.0 ± 2.65
Klebsiella pneumo pneumoniae	30.0 ± 2.65	30.0 ± 0.84	23.0 ± 1.73
Klebsiella pneumo pneumoniae	24.0 ± 2.00	26.0 ± 1.73	14.5 ± 1.03

NS – Not Susceptible.
Results are presented as the mean zones of inhibition ± Sandard deviation (STDEV) of three (3) determination.

extract, one could conclude that the active constituents, possibly cryptolepine may be thermostable.

In conclusion, one can say that, all three extracts displayed good antimicrobial activity against majority of the test microorganisms.

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