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Full Length Research Paper

Antibacterial activity of alkaloids from *Sida acuta*

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Sida acuta **is a shrub indigenous to pantropical regions. The plant is widely used for its various pharmacological properties. Among compounds of pharmacological interest occurring in the plant, are indoloquinoline alkaloids. The aim of the present study was to investigate the antimicrobial activity of alkaloids of *S. acuta* from Burkina Faso. The alkaloids had a good antimicrobial activity against the test microorganisms. In the agar- well diffusion assay, highest inhibition zone diameters were recorded with Gram-positive bacteria. The broth microdilution assay gave minimal inhibitory concentration values ranging from 16 to 400 µg/ml and minimal bactericidal concentration values ranging from 80 to up to 400 µg/ml. The gas chromatography-mass spectrometry analysis of the same alkaloids led to the identification of cryptolepine and quindoline as the major components.**

Key words: Cryptolepine, quindoline, indoloquinolines, antibacterial, *Sida acuta.*

INTRODUCTION

Using plants for medicinal purposes is an important part of the culture and the tradition in Africa. Thus, up to 80% of the population depend directly on the traditional medicine for the primarily health care (Kirby, 1996). This traditional medicine uses numerous plants, among them, *Sida Acuta* Burm f. (Malvaceae). *S. Acuta* is a shrub indigenous to pantropical areas, widely distributed in these regions and widely used in traditional medicine. The aerial part of the plant is the most frequently used part. In central America, the plant is used to treat asthma, renal inflammation, colds, fever, headache, ulcers and worms (Caceres et al., 1987; Coee and Anderson, 1996). In Colombia the plant is known to treat snake bites. Otero et al. (2000 a,b) demonstrated that the ethanolic extract of the plant had an effective moderate activity against the venom of *Bothrox athrox*. In West Africa, particularly in Burkina Faso,*S . acuta* often called “arbre à balai in French and “zon-raaga” in *Mooré*, an indigenous language, grows around habitations in farms and in bushes. The plant is traditionally used in the treatment of malaria, diarrhoea and many other diseases (Nacoulma/ouedraogo, 1966). Research focussed on malaria led to the identification of alkaloids, principally cryptolepine the major alkaloid of the plant, as its antimalarial agent (Banzouzi et al., 2004; Karou et al., 2003). More recently, we found that polyphenol extract of the plant had a weak antioxidant activity through *in vitro* free radicals scavenging assays, on the other hand the extract was very active on pathogenic bacteria and this activity may be influenced by the polymerisation size of the phenolic compounds (Karou et al., 2005). Phytochemical screening on *S. acuta* resulted in the isolation of several alkaloids and steroidal compounds with the potential to induce quinone reductase and to inhibit 7,12-dimethylbenz-(a)anthacene-induced preneo-plastic les-ions in mouse mammary organ (Cao and Qi, 1993; Dinan et al., 2001; Jang et al., 2003).

Among the compounds isolated from *S. acuta*, its alkaloids appeared to be of great interest in pharma-cological studies. These alkaloids belong to the family of indoloquinolines. Many investigations have been done on this family of compounds and the results showed that

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they are new leads in the establishment of drugs against many diseases. For example, cryptolepine 5-méthylindolo (2-3b)-quinoline, the main alkaloid of the plant, has been well investigated for its various biological properties. First isolated from *Cryptolepis* species (*C. triangularis* and *C. sanguinolenta* (Periplocaceae)) from Africa (Clinquart, 1929; Dwuma-Badu et al., 1978), the compound has been also isolated from other plants such as *S. acuta* (Malvaceae) from Sri Lanka (Gunatilaka et al., 1980), *Microphilis guyanensis* (Sapotaceae) and *Genipa americana* (Rubiaceae) from Surinam (Yang et al., 1999). In Ghana, extracts of roots of *C. Sanguinolenta*, in which cryptolepine is the main alkaloid, have been used clinically to treat malaria, colic and stomach ulcers (Boye and Ampofo, 1983). Cryptolepine itself is found to produce many pharmacological effects such as antimicrobial (Cimanga et al., 1998), antiprotozoal (Arzel et al., 2001; Wright et al., 2001), antihyperglycemic (Bierer et al., 1998) and cytotoxic effects through GC-rich DNA sequence intercalation that provides basis for design of new anticancer drug (Bonjean et al., 1998; Dassonneville et al., 2000; Guittat et al., 2003; Lisgarten et al., 2002).

The purpose of the present study was to investigate the antimicrobial activity of alkaloids from *S. acuta* against Gram-positive and Gram-negative bacteria. The selected microorganisms included reference strains and fresh clinical strains isolated from pathologic products. We also performed the GC-MS analysis of the same alkaloid extract in order to identify the main components of the extract.

MATERIALS AND METHODS

Plant material and alkaloids extraction

Samples of the aerial part of *S. acuta* were collected in Ouagadougou in July 2003. The plant was botanically authenticated as previously indicated (Karou et al., 2003).The ground sample was made alkaline with 28% ammonia and extracted with chloroform at room temperature for a total period of 24 h and then the extract was partitioned between 5% HCl and chloroform. The aqueous phase was made alkaline again with ammonia and partitioned between water and chloroform. Finally chloroform was totally evaporated from the organic phase to form the alkaloid powder.

GC-MS analysis

Gas chromatography-Mass Spectrometry (GC-MS) analysis was performed with an auto HRGC/MS Carlo Erba Instrument using a SPB 5 (30 m / 0.25 mm / 0.5 µm) capillary column. 6 µ of 1 mg/ml alkaloids dissolved in methanol was injected in the following conditions: injector temperature, 280°C; carrier gas, helium; pressure, 150 kPa; ion voltage, 60 eV; temperature gradient, 20°C per minute from 100 to 315°C. Compounds were identified on the basis of their mass spectral data.

Microorganisms, antibiotics and media

Commercially available antibiotics discs, penicillin 10 IU/IE/UI, sulfadiazin 0.25 mg and spectinomycin 100 µg were purchased from Beckton Dickinson. All media used were from Oxoid. Microorganisms included reference strains and fresh clinical isolates. The selection of clinical microorganisms depended on their availability, thus microorganisms that have been reported to be the most frequently implicated in infectious diseases in tropical areas were well represented (Bonfiglio et al., 2002). Clinical isolates were: *Staphylococcus aureus* (n = 11), *Enterococcus faecalis* (n = 3), *Shigella boydii* (n = 4), *Shigella flexneri* (n = 5) *Shigella dysenteriae* (n = 3), *Salmonella thyphi* (n = 3), *Salmonella parathyphi* B (n = 3) and *Escherichia coli* (n = 4). All these strains were isolated from clinical samples at Laboratoire de Biologie Médicale Saint Camille in Ouagadougou. The microorganisms were identified by the use of their biochemical profiles as recommended by the manual "Bactériologie Médicale" (Leminor and Veron, 1984). The reference strains were *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 53154, *Staphylococcus carmonum* LMG 13567, *Bacillus cereus* LMG 13569, *Listeria* innocua LMG 13568, *Enterococcus faecalis* CIP 103907, *Shigella dysenteriae* CIP 54051 and *Escherichia coli* CIP 105182.

Antibacterial assays

Agar-well diffusion: The assay was conducted as described by Perez et al. (1990). Briefly, microorganisms from growth on nutrient agar incubated at 37°C for 18 h were suspended in saline solution 0.85% NaCl and adjusted to a turbidity of 0.5 Mac Farland standards (10^8 cfu/ml). The suspension was used to inoculate 90 mm diameter Petri plates with a sterile non toxic cotton swab on a wooden applicator. Six millimeters diameter wells were punched in the agar and filled with 50 µl of 2000 µg/ml alkaloids. The dissolution of the alkaloids was aided by 1% (v/v) DMSO which did not affect microorganisms growth, according to our control experiments. Commercial antibiotics were used as positive reference standard to determine the sensitivity of the strains. Discs were directly placed onto the bacterial culture. Plates were incubated in air at 37°C for 24 h. Antibacterial activities were evaluated by measuring inhibition zone diameters. The experiments were conducted twice.

Broth microdilution assay: Broth microdilution method was used to determine minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of alkaloids against the test microorganisms as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2002). The tests were performed in 96 well -plates. Alkaloids dissolved in 1% DMSO were transferred in plates to obtain a twofold serial dilutions ranging from 3.2 to 400 µg/ml. Then plates were inoculated with microbial suspensions diluted from the same 0.5 Mac Farland standards to have 10^5 cfu/ml in each well. The final volumes in wells were 200 µl. After 24 h incubation in air at 37°C, MIC was recorded as a lowest extract concentration demonstrating no visible growth in the broth. MBC was recorded as a lowest extract concentration killing 99.9% of bacterial inocula. MBC values were determined by removing 100 µl of bacterial suspension from subculture demonstrating no visible growth and inoculating nutrient agar plates. Plates were incubated at 37°C for a total period of 48 h.

Time-kill assay: Escherichia coli CIP 105182 and Shigella dysenteriae CIP 54051 were chosen arbitrary to perform time-kill assay. Thus, 0.5 Mac Farland standards suspensions of the microorganisms were diluted to have 50 ml of approximately 10^5 cfu/ml in nutrient broth, then 160 and 480 µg/ml alkaloids that corresponded to 2 MIC of *Escherichia coli CIP* 105182 and *Shigel-*

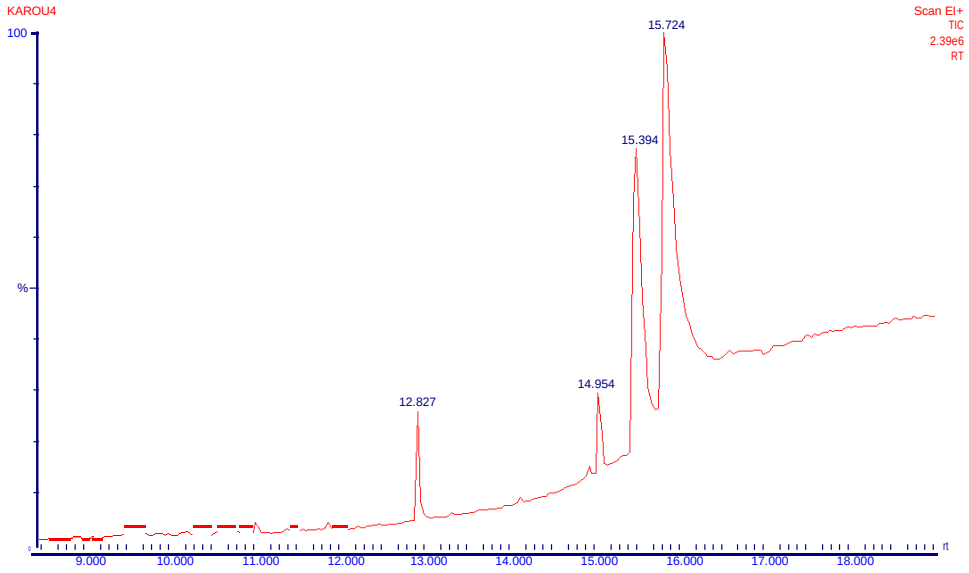
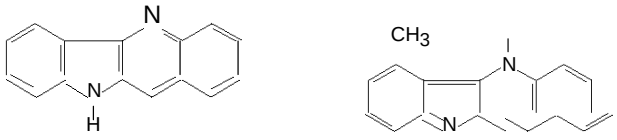


Figure 1. GC-MS chromatogram of *Sida acuta* alkaloids.



Quindoline Cryptolepine

Figure 2. Chemical structure of identified compounds from the *Sida acuta* alkaloid extract.

la dysenteriae CIP 54051, respectively, were added to the corresponding culture. The cultures were incubated in air at 37°C in INNOVA^{TM} 4000 incubator shaker. At 0, 1, 2, 3, 4, 5 and 6 h, an aliquot of 100 µl was removed and diluted with 10 ml sterile broth. The obtained suspension was used to inoculate 90 mm diameter Petri plates with a sterile non toxic cotton swab on a wooden applicator as indicated before in the agar- well diffusion assay. After 48 h incubation at 37°C, the viability of the microorganisms was evaluated by the presence of colonies on the plates. The experiment was carried out twice.

RESULTS AND DISCUSSION

Gas chromatogram as shown on Figure 1 yielded two major peaks (retention times (rt): 15.39 and 15.72) and two minor peaks (retention times: 12.83 and 14.95). The two major peaks (rt 15.39, MS (EI, m/z) 232 (100 %) and rt 15.72 MS (EI, m/z) 218 (100 %)) were identified as cryptolepine and quindoline, respectively (Figure 2), on the basis of their fragmentation data that were in agreement with reported values (Dwuma-Badu et al., 1978; Paulo et al., 1995; Pousset et al., 1995) . The two alkaloids have been found to occur in *S. acuta* and other species (Banzouzi et al., 2004; Gunatilaka et al., 1980).

For the treatment of several infections in Africa, indigenous medicinal plants are often the only means (Fenell et al., 2004; Taylor et al., 2001). This highlights the continuous interest in laboratory screening of medicinal plants, not only to determine the scientific rationale for their usage, but also to discover new active principles. African medicinal plants have been screened for their *in vitro* antibacterial activities and many described antibacterial activities have been focussed on phenolic compounds, terpenoids or essential oils (Bassole et al., 2003; Erasto et al., 2004; Viljoen et al., 2003). The plants have been found to exert good *in vitro* antimicrobial activities and some active principles have been isolated.

Examples are muzigadial isolated from *Warburgia salutaris* (Bertol. f) Chiov. (Canellaceae) (Rabe and Van Staden, 2000) and Vernodalin from *Vernonia colorata* (Willd) Drake (Asteraceae) (Reid et al., 2001). Alkaloids have been well investigated for many pharmacological properties including antiprotozoal, cytotoxic, antiinflammatory properties but there are only few reports about their antimicrobial properties. The antibacterial assays in this study were performed by the agar-well diffusion and the broth microdilution methods so that they could be qualified and quantified by inhibition zone diameters, MIC and MBC values as summarized in Tables 1 and 2. The susceptibility of the bacteria to the extract, on the basis of inhibition zone diameters varied according to

Table 1. Inhibition zone diameters (mm) recorded in agar-well diffusion assay.

Microoganisms	Gram	Pen	Sulf	Spec	Alk
S. aureus (n = 6)	+	nd	nd	14±03	25±4
S. aureus (n = 5)	+	39±09	nd	16±02	27±7
S. aureus ATCC 25923	+	nd	nd	20±00	28
S. aureus ATCC 53154	+	nd	nd	29	29
S. carmonum LMG 13567	+	40	nd	20	20
B. cereus LMG 13569	+	nd	nd	29	20
L. innocua LMG 13568	+	27	nd	20	29
E. faecalis (n = 3)	+	35±05	nd	20±00	38±02
E. faecalis CIP 103907	+	30	nd	20	35
Sh. boydii (n = 4)	-	nd	nd	18±01	17±02
Sh. flexneri (n = 5)	-	nd	nd	20±05	18±7
Sh. dysenteriae (n = 3)	-	nd	nd	21±02	18±00
Sh. dysenteriae CIP 54051	-	nd	nd	18	18
Sal. Thyphi (n = 3)	-	nd	nd	18±02	00
Sal parathyphi B (n = 3)	-	nd	nd	20±01	00
E. coli (n= 4)	-	nd	nd	16±01	16±04
E. coli CIP 105182.	-	nd	nd	20	20

Results are the means of diameters values ± standard deviations.
Pen: penicillin (10 UI), Sulf : sulfadiazin (0.25 mg), Spec: spectinomycin (100 µg), Alk: alkaloids (100 µg), nd: no detected activity.

Table 2. Means of MIC and MBC recorded in the microdilution assay using the *Sida acuta* alkaloid extract.

Microorganism	Gram	MIC	MBC
S. aureus Pen^- (n = 6)	+	240±00	240±00
S. aureus Pen^+ (n = 5)	+	80±00	80±00
S. aureus ATCC 25923	+	400	>400
S. aureus ATCC 53154	+	400	>400
S. carmonum LMG 13567	+	80	240
B. cereus LMG 13569	+	80	>400
L. innocua LMG 13568	+	16	240
E. faecalis (n = 3)	+	16±00	>400
E. faecalis CIP 103907	+	16	>400
Sh. boydii (n = 4)	-	80±00	240±00
Sh. flexneri (n = 5)	-	80±00	186±54
Sh. dysenteriae (n = 3)	-	400±00	240±00
Sh. dysenteriae CIP 54051	-	400	240
Sal. Thyphi (n = 3)	-	np	np
Sal parathyphi B (n = 3)	-	np	np
E. coli (n= 4)	-	80±00	240±00
E. coli CIP 105182.	-	80	80

Results are the means of diameters values ± standard deviations.
Pen^-: for penicillin resistant *S. aureus*; Pen^+: for penicillin susceptible *S. aureus*; np: no performed assay.

microorganisms, but globally, the highest inhibition zone diameters were recorded with Gram-positive bacteria (Table 1). Curiously, there was no inhibition with *Salmonella* species, thus these strains were not included in the microdilution assay.

The microdilution assay gave MIC values ranging from 16 to 400 µg/ml and 80 to 400 µg/ml for MBC for different strains (Table 2). Great MBC values were obtained with Gram-positive bacteria, however, these results could not show clearly weather Gram-positive bacteria or Gram-negative ones were more susceptible to the extract, but it is evidence that the extract displayed either microbiostactic (MBC 2, 3 or more times greater than MIC) or microbicide (equal values of MIC and MBC) effects on the different strains. It is reported that Gram-positive bacteria should be more susceptible since they have only an outer peptidoglycan layer which is not an effective barrier (Scherrer and Gerhardt, 1971). The Gram-negative bacteria have an outer phospholipidic membrane that make the cell wall impermeable to lipophilic solutes, while the porines constitute a selective barrier to hydrophilic solutes with an exclusion limit of about 600 Da (Nikaido and Vaara, 1985) . Many results confirmed these observations, thus some plant extracts were found to be more active against Gram-positive bacteria than against Gram-negative ones (Kelmanson et al., 2000; Masika and Afolayane, 2002).

Phytochemicals exert their antimicrobial activity through different mechanisms, tannins for example act by iron deprivation, hydrogen bounding or non specific interactions with vital proteins such as enzymes (Scalbert, 1991). In the case of indoloquinoline alkaloids the mechanism remains unclear. Sawer et al. (2005) demonstrated that the main indoloquinoline alkaloid, cryptolepine, causes cell lysis and morphological changes of *S. aureus*, but the antimicrobial effects of the alkaloid may be through another mechanism, since the compound is known to be a DNA intercalator and an inhibitor of DNA synthesis through topoisomerase inhibition (Bonjean et al., 1998; Dassonneville et al., 2000; Guittat et al., 2003; Lisgarten et al., 2002).

In order to follow the reduction of the amount of microorganisms in an inoculum as a function of the time, time-kill assay was performed with E. *coli* CIP 105182 and *Sh. dysenteriae* CIP 54051, arbitrarily selected among the test microorganisms. The results showed that after 5 h exposition there was no viable microorganism in the initial inoculum (Table 3) and the effect o f alkaloids

Table 3. Viability of microorganisms after 6 hours exposure to the *Sida acuta* alkaloid extract.

Time (h)	0	1	2	3	4	5	6
E. coli CIP 105182	+ (uc)	+ (uc)	20±05	05±02	-	-	-
Sh. dysenteriae CIP 54051	+ (uc)	+ (uc)	+ (uc)	17±04	03±02	-	-

+: for the presence of the colonies.
-: for absence of colonies.
UC: uncountable.
The results are the means of number of the colonies ± standard deviations.

was faster on *E. coli* than it was on *Sh. dysenteriae*.

In conclusion, the alkaloids displayed good antimicrobial activity against several test microorga-nisms. The GC-MS analysis of the same alkaloid extract revealed the presence of two major alkaloids; cryptolepine and quindoline. The results of the present study support the traditional medicinal use of *S. acuta* and suggest that a great attention should be paid to this plant which is found to have many pharmacological properties.

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