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Review

Isolation and pharmacological activities of alkaloids from *Cryptolepis sanguinolenta* (Lindl) Schlt

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Cryptolepis sanguinolenta (Lindl.) Schlt. (Periplocaceae) is a very useful medicinal plant whose aqueous decoction (prepared by Centre for Scientific and Industrial Research Into Plant Medicines- Mampong-Akwapim, Ghana) is commonly used in Ghana to treat malarial, inflammatory, hypertensive and microbial disease conditions. The plant has long been employed by Ghanaian traditional healers in the treatment of various fevers including malaria. An attempt has been made to review the phytochemical and pharmacological work done on *Cryptolepis sanguinolenta*. The current investigation yielded Quindoline and Cryptolepine from the stem of *Cryptolepis sanguinolenta*. The isolated quindoline has been investigated for its biological activities by the Agar Diffusion Assay method. The compound displayed moderate in vitro antibacterial activity against *E. coli* but no activity against *Pseudomonas aeruginosa*.

Keywords: *Cryptolepis sanguinolenta*, malaria, quindoline, cryptolepine.

INTRODUCTION

Cryptolepis sanguinolenta (Lindl.) Schlechter (Periplocaceae or Asclepiadaceae) is a shrub indigenous to West Africa. *Cryptolepis* belongs to the order Apocynales. The genus, *Cryptolepis* includes *Cryptolepis brazzei*, *C. deciduas*, *C. oblongifolia*, *C. nigritana*, *C. triangularis* and *C. sanguinolenta*. *Cryptolepis sanguinolenta* is common to West Africa. It is also known as *Pergularia sanguinolenta* (Hutchinson and Dalziel, 1963). In Ghana, the plant is given various names in different parts of the country. It is called gangamau in hausa, kadze in Ewe and nibima in Twi. The plant, *Cryptolepis sanguinolenta* grows wild along the West coast of Africa and some parts of Central Africa to be specific, Congo Brazzville. It can also be cultivated and is a plant that thrives well in areas where there is adequate supply of sunshine and water; thus it is completely absent from the mushy and salty swamps of coastal regions. In Ghana, the plant is commonly found in the month of June in certain districts of the country. It is found in Aburi Hills in the Akwapim areas and Ejura district of the Ashanti region where there is enough rainfall for optimum growth.

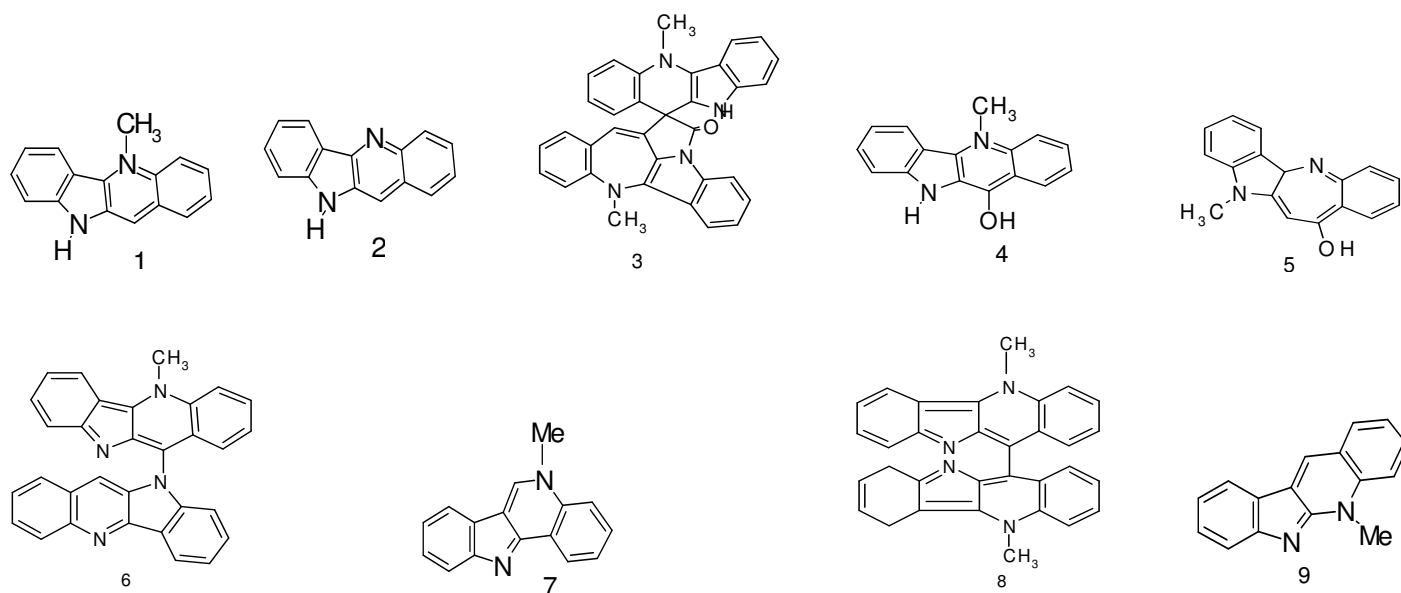
Morphology

The plant is a thin-stemmed twinning and scrambling shrub. The leaves are petiolate, glabrous, elliptic or oblong-elliptic, up to 7cm wide. The blades are acute apex and a symmetrical base. The inflorescence cymes, lateral on branch shoots, are few flowered, with a yellow corolla tube up to 5mm long. The fruits are paired in linear follicles and are horn-like. The seeds are oblong in shape, small and pinkish, embedded in long silky hairs. The dried leaves, stems and roots of *cryptolepis* have a sweet fragrance. The root varies from 0.4-6.6 cm long and 0.31-1.4 cm wide and has a bitter taste. The roots are rather tortuous and branch with little or no rootlets. The outer surface is yellowish brown and when dry, show longitudinal ridges with occasional cracks. The roots break easily with fractures leaving a smooth transverse surface, which is yellow in colour. The sap is extremely bitter and is characterized by the rapidity with which it turns deep red on exposure to air (Soforow ; Boye, 1992).

Phytochemical Investigations

Various *Cryptolepis* species have yielded several

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alkaloids. In 1906, Fichter and Boehringer described quindoline **1** as a synthetic product (Ablordepey et al., 1990; Spitzer et al., 1991)^{4,5}. It was not until 1978, that the compound was described as a metabolite of *C. sanguinolenta* (Dwuma-Badu et al., 1978).^{5,6} In 1990, the isolation of cryptolepine **2** was also reported (Ablordepey et al., 1990). This indoloquinoline alkaloid was first isolated from the extracts of the roots of *Cryptolepis triangularis* N.E.Br., a species native to the Belgian Congo, synonym to sanguinolenta in Ghana (Clinquart, 1929). This alkaloid was again isolated from the same plant species by Delvaux⁸. Cryptolepine was also isolated from a Nigerian sample of *C. sanguinolenta* (Gellert et al., 1951)⁹. About 30 years later, these alkaloids were isolated again from a Ghanaian sample of *C. sanguinolenta* (Dwuma-Badu et al., 1978)⁶.

A third alkaloid from this species, a unique spiro-nonacyclic alkaloid named cryptospirolepine **3** was reported to be isolated from this species (Dwuma-Badu et al., 1993). Alexandra Paulo et al later reported on the isolation of three new indole alkaloids: hydroxycryptolepine **4**, cryptoheptine **5**, and cryptoquindoline **6** from the roots of *C. sanguinolenta* obtained from Guinea Bissau (Ablordepey et al., 1990)⁴.

In 1995, the isolation of a new alkaloid, isocryptolepine **7** an isomer of cryptolepine, was reported in Phytochemistry (Pousset et al., 1995)^{10,11}. Not quite long, a report revealed the characterization of two new alkaloids from the root bark of the same plant *C. sanguinolenta*. These alkaloids were biscryptolepine **8** and neocryptolepine **9** (Cimanga et al. 1996).

Pharmacological screening and clinical trials

The plant has long been employed by Ghanaian healers

in the treatment of various fevers, including malaria. A root decoction prepared, bottled and labeled “Nibima” at the Centre for Scientific Research into plant Medicine (CSIR) in Ghana, has been used in the clinical therapy of both malaria and urinary and upper respiratory tract infections since 1974 (Boye and Ampofo, 1983). In central Africa (Zaire), the root bark of this medicinal plant is used as an aqueous macerate, while in parts of West Africa, especially, Ghana, Nigeria and Senegal, the entire root is used as an aqueous decoction for the same purposes. In Guinea Bissau, traditional healers have used a root decoction of this plant for the treatment of various fevers, including hepatitis; the leaves for the treatment of malaria and powdered as a cicatrizant of wounds (Feijao, 1996, Gomez and Diniz, 1993, Paulo and Gomez, 1995).

Numerous pharmacological activities have been demonstrated in extracts from the roots of *C. sanguinolenta*, as well as for the alkaloids isolated from these extracts. They include hypotensive antipyretic, anti-inflammatory, in vitro antibacterial, antimalaria, anti-viral and anti-diabetic effects (Noamesi and Bamgbose, 1980; Boakye-Yiadom and Ackah, 1979; Bamgbose and Noamesi, 1981). Traditionally, aqueous extract of cryptolepis is used by the Fulani traditional healers in Guinea-Bissau to treat jaundice and hepatitis (Silva et al., 1996). In Zaire (Democratic Republic of Congo) and Casamance district of Senegal, infusions of the roots are used in the treatment of stomach and intestinal disorders (Sofowora, 1982; Kerharo and Adam, 1974). An aqueous decoction of the root bark is used in Congo for the treatment of amoebiasis (Tona et al., 1998).

Cryptolepine, the major alkaloid in *Cryptolepis* and almost all the minor alkaloids have anti plasmodial activity. A report showed that extracts from the root bark of *C. sanguinolenta* possessed a promising antiplas-

modial activity against the *p. falciparum* chloroquin sensitive strain D-6 (Cimanga et al. 1997). In this same work the isolated compound, cryptolepine and its hydrochloride, 11-hydroxycryptolepine and neocryptolepine were assessed in vitro against plasmodium falciparum D-6 (Chloroquin-Sensitive strain), K-1, and W-2 (Chloroquin resistant strains). A strong antiplasmodial activity against *P. falciparum* chloroquin resistant strains was realized. However, quindoline, the other isolated compound was found to be less active.

More also, cryptolepine hydrochloride exhibited a significant chemosuppressive effect against plasmodium berghei yoelli and plasmodium berghei berghei. Whereas, cryptolepine exhibited the same effect against *P. berghei yoelli* only (Sofowora, 1982).

Activity of cryptolepine was not limited to only antiplasmodial effects. Cryptolepine has been found to possess other several interesting pharmacological activities. It has been reported that administration of cryptolepine HCl to dogs at a dosage of 15-30mg/kg produced marked hypothermia and decreased the hypertensive and renal vasoconstrictive activities of epinephrine. Also, ip injection (120mg/kg) of the compound was lethal to guinea pigs in about 12 hours. It has been also observed that iv administration (5mg/kg) of cryptolepine produced a marked and protracted hypotensive response in vagotomized dog with a corresponding decrease in renal volume (Raymond-Hamet, 1937).

Antimicrobial Screening

The crude extracts of the roots of *C. sanguinolenta* were found to inhibit the growth of *Neisseria gonorrhoeae*, *Escherichia coli*, and *Candida albicans* (Boakye-Yiadom, 1979).

However, the extract was not effective against *Pseudomonas aeruginosa* (Silva et al., 1996).

Present investigation

The present phytochemical investigation on the stems of *Cryptolepis sanguinolenta* yielded two known alkaloids quindoline (1) and cryptolepine (2) hitherto isolated from the other parts of the same plants. The structure elucidations were made possible by direct comparison (1H-NMR, mass spectra and TLC) with authentic samples of quindoline and cryptolepine.

The result from the biological activity carried out on quindoline showed that the higher concentrations of the isolated sample inhibited the growth of *Escherichia coli*. However, *Pseudomonas aeruginosa* was resistance to all the concentrations of the isolated sample in an agreement with what has been reported in literature (Boakye-Yiadom, 1979; Silva et al., 1996).

EXPERIMENTAL

General: M.p.s were determined on Electro-thermal melting point apparatus and are reported uncorrected. The UV spectrum was taken on Perkin-Elmer 202 spectrophotometer in methanol. The IR spectrum was taken on FTIR. NMR was recorded on 500MHz Perkin Elmer spectrometer using deuteriochloroform (CDCl_3) as solvent. Column chromatography utilized neutral Al_2O_3 ; TLC was over silica gel with the solvent system CHCl_3 – MeOH (9:1). All solvents were evaporated under reduced pressure at 40°C.

Plant material

The plant material *C. sanguinolenta* used in this study was collected at the Centre for

Scientific Research into Plant Medicine (CSRPM), Mampong-Akwapim, Ghana and was identified by Mr. Ameyaw of the Centre where a herbarium specimen was deposited.

Extraction and Isolation

Powdered, oven-dried (60°C) stems of *C. sanguinolenta* (500g) were defatted with petroleum ether for 8 hrs. The marc was extracted by soxhlet with methanol (2x2 litres) for 8 hrs. The extract was evaporated under reduced pressure to give a residue (34g) which was thoroughly mixed with AcOH (5%, 1L), diluted with water (500ml) and allowed to stand overnight. The mixture was filtered affording a clear solution and some insoluble material. The aqueous filtrate was made basic with aqueous ammonia to pH 9.5 and extracted with CHCl_3 (5x200ml). The combined CHCl_3 extracts were dried over anhydrous MgSO_4 , filtered and evaporated to afford a dark alkaloidal residue (4.7g).

The alkaloidal residue was dissolved in CHCl_3 (50ml), adsorbed onto Al_2O_3 (20g) and chromatographed over Al_2O_3 (200g). Elution was conducted successively with petroleum ether 40-600 (500ml) followed by petroleum ether- CHCl_3 (500ml) and CHCl_3 (500ml) and CHCl_3 -MeOH (9:1) 500ml and finally MeOH (500ml).

Quindoline **1** was obtained as a yellow solid after column chromatography and preparative tlc on Al_2O_3 in chloroform. It gave an orange colouration with Dragendorff's reagent, which is evidence for **1** as an alkaloid (Boye, 1992). The spectral data of quindoline **1** are summarized as follows: UV λ_{max} (methanol)nm 272, 339, 399. IR ν_{max} (KBr) cm^{-1} 3689, 3628, 3557, 3028, 2972, 1534, 1416, 1233, 1200, 1050, 916, 783, 700, 616.

¹H-NMR (400MHz, CDCl_3) δ ppm, 6.5(1H, d, aromatic), 6.4(2H, q, aromatic), 6.2(1H, t, aromatic), 5.9(3H, m, CH_3), 5.5(1H, m, C-H), 5.4(2H, s, NH_2), 3.0(3H, s, N-CH_3), 2.0(6H, s, 2x CH_3), 1.3(3H, s, C-CH_3). CMR

100MHz, CDCl₃) 128, 127, 126, 64, 40.8, 33.7, 28.3, 26.9, 24.3, 23.6, 20.6, 18.5, 17.2, 14.4, MS (M+) m/z (%): 218(100),

Elution of the column with MeOH [(9:1) afforded a violet residue. It crystallized from aqueous ethanol as long violet needles (2.2 g), MS. (M+) m/z (%): 232(100), 231(10), 217(30), 116(15), 89(12). m p 165-168o. Rf = 0.3 identical with an authentic sample of cryptolepine by direct comparison (mass spectra and TLC).

Agar Diffusion Assay: A bunsen burner was lighted and allowed to burn for five minutes to create a clean sterilized working environment. With a sterilized microbiological loop, colony from pure culture isolation plate was taken into peptone water. The inoculated peptone water was poured on the sensitivity agar: *Escherichia coli* and *Pseudomonas aeruginosas* and the excess discarded. Four different susceptibility discs (5mm in diameter) were impregnated with 10, 5, 2.5 and 1.0µg of the isolated quindoline compound dissolved in MeOH (10ml) and placed on the sensitivity agar. This was incubated at 37°C and the growth pattern observed after 18 hours.

The control experiment was done with methanol (10ml) without the quindoline compound.

CONCLUSION

The plant *C. sanguinolenta* has been found to be a well-known resourceful medicinal plant. Scientific researches revealed that besides the crude extracts, the major secondary metabolite, cryptolepine and all other minor compounds have remarkable and numerous pharmacological activity. They were proven to have a broad spectrum of antipathogenic activity.

The present investigation confirmed that cryptolepine and quindoline compounds are not only present in the roots or the leaves but also present in the stem of *Cryptolepis sanguinolenta*. Moreover, the isolated quindoline proved to have moderate in vitro activity against *E. coli* but not against *Pseudomonas aeruginosas*.

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