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Research Article

In vitro antimicrobial activity of ethanolic fractions of *Cryptolepis sanguinolenta*

Neetika Chahar*[1], Sandeep Deswal[1], Vijender Singh Mahalwal[2]

[1]NIMS University, Shoba Nagar, Jaipur, Rajasthan, India
[2] B.B.S. Institute of Pharmaceutical and Allied Sciences, Knowledge Park III, Greater Noida, Uttar Pradesh, India

ABSTRACT

Different solvent fractions of ethanolic extract of *Cryptolepis sanguinolenta* were evaluated against standard bacteria and clinical isolates. The solvent partitioning protocol involving ethanol, petroleum ether, chloroform, ethyl acetate and water, was used to extract various fractions of dried pulverized *Cryptolepis sanguinolenta* roots. Qualitative phyto-constituents screening was performed on the ethanol extract, chloroform fraction and the water fraction. The disk diffusion method was employed to ascertain the antibiogram of the test organisms while the agar diffusion method was used to investigate the antimicrobial properties of the crude plant extracts. The microplate dilution method aided in finding the MICs while the MBCs were obtained by the method of Nester and friends. The phytochemical screening revealed the presence of alkaloids, reducing sugars, polyuronides, anthocyanosides and triterpenes. The ethanol extract inhibited 5 out of 8 (62.5%) of the standard organisms and 6 out of 8 (75%) clinical isolates. The petroleum ether fraction inhibited 4 out of 8 (50%) of the standard microbes and 1 out of 8 (12.5%) clinical isolates. It was also observed that the chloroform fraction inhibited the growth of all the organisms (100%). Average inhibition zones of 14.0 ± 1.0 mm to 24.67 ± 0.58 mm was seen in the ethyl acetate fraction which halted the growth of 3 (37.5%) of the standard organisms. Inhibition of 7 (87.5%) of standard strains and 6 (75%) of clinical isolates were observed in the water fraction. The chloroform fraction exhibited bactericidal activity against all the test organisms while the remaining fractions showed varying degrees of bacteriostatic activity. The study confirmed that fractions of *Cryptolepis sanguinolenta* have antimicrobial activity. The chloroform fraction had the highest activity, followed by water, ethanol, petroleum ether and ethyl acetate respectively. Only the chloroform fraction exhibited bactericidal activity and further investigations are needed to ascertain its safety and prospects of drug development.

Keywords: *Cryptolepis sanguinolenta*; disk diffusion method; inhibition zones; microplate dilution method; phytochemical screening

INTRODUCTION

Cryptolepis sanguinolenta (Lindl.) Schltr. (Periplocaceae) is a plant mostly found in the tropical rain forest regions of Africa with several species. *C. sanguinolenta* is the most common in Ghana. This species is found on mountainous territories in Ghana, especially the Akwapim and Kwahu mountains (Iwu., 2000; Addy., 2003). Plant is a slender thin stemmed climbing shrub with orange-coloured juice in the cut stem (Paulo., 2003) and like most medicinal herbs or plants, the exact history on the usage of the plant is not well established, but it is confirmed that some indigenous inhabitants in the Akwapim and Kwahu mountainous areas in Ghana use the plant to manage various forms of fever, malaria and some infections caused by bacteria (Boye., 1990).

* Corresponding Author
Email: neetikadolphinsr10@rediffmail.com
Contact: +91-9254088099 Fax: +91-01748-251611
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It has also been established that, the extract of *C. sanguinolenta* has anti-muscarinic, vasodilating, noradrenergic, antithrombotic, anti-inflammatory, and hypoglycemic activities (Bierer et al., 1990). The part of the plant mostly used is the root and the extract is obtained in the aqueous form by boiling, or by alcohol extraction, popularly referred to as “bitters” in Ghana. Thus, *C. sanguinolenta* is a potential medicinal plant that must be investigated to establish its antimicrobial activity. Previous in vitro studies have compared the effect of ethanol, cold and hot aqueous extracts of *C. sanguinolenta* as antimicrobial agents using Gram positive and Gram negative organisms as well as *C. albicans*. Eighty five percent of the test microbes were inhibited by the ethanol extract while the cold and hot aqueous extracts inhibited seventy five percent of the test microbes respectively (Mills-Robertson et al., 2009).

The current study investigated the antimicrobial activity of various solvent fractions of ethanolic extract of *C. sanguinolenta*, with the aim of identifying the bioactive fractions of the extract as well as finding out the degree of activity against selected pathogenic bacteria.

Table 1: Phyto-constituents of the ethanol extract and the partitioned fractions

Phytochemical parameters	Extract/ Fraction		
	Ethanol	Water	Choloroform
Saponins	-	-	-
Reducing sugars	+	+	+
Polyuronides	+	+	+
Alkaloids	+	+	+
Triterpenes	-	+	-
Phytosterols	-	-	-
Flavanoids	-	-	-
Anthocyanosides	+	+	+

+ = Present, - = Absent

Table 2: Susceptibility of microbes to the extract and fractions of *C. sanguinolenta*

Test Organisms	Ethanol Extract	Petroleum ether fraction	Chloroform fraction	Ethyl acetate fraction	Water fraction	Chloram-phenicol
Salmonella typhi ATCC 19430	18.00 ± 0.00	0.00 ± 0.00	19.67 ± 0.58	0.00 ± 0.00	16.67 ± 1.15	0.00 ± 0.00
Salmonella typhi	0.00 ± 0.00	0.00 ± 0.00	11.33 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Salmonella typhimurium ATCC 14028	0.00 ± 0.00	0.00 ± 0.00	11.33 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Salmonella typhimurium	0.00 ± 0.00	0.00 ± 0.00	9.33 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Proteus mirabilis ATCC 49565	9.33 ± 0.58	27.00 ± 1.00	20.67 ± 0.58	14.00 ± 1.00	10.33 ± 0.58	14.67 ± 0.58
Proteus mirabilis	20.67 ± 0.58	14.67 ± 0.58	19.33 ± 0.58	0.00 ± 0.00	18.67 ± 0.58	0.00 ± 0.00
Pseudomonas aeruginosa ATCC 27853	0.00 ± 0.00	0.00 ± 0.00	11.00 ± 1.00	0.00 ± 0.00	9.67 ± 0.58	0.00 ± 0.00
Pseudomonas aeruginosa	20.00±	0.00 ± 0.00	15.67 ± 0.58	0.00 ± 0.00	24.33 ± 1.15	0.00 ± 0.00
Klebsiella pneumoniae ATCC 33495	0.00 ± 0.00	0.00 ± 0.00	10.67 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Klebsiella pneumonia	22.33 ± 0.58	0.00 ± 0.00	15.67 ± 0.58	0.00 ± 0.00	23.67 ± 0.58	0.00 ± 0.00
Escherichiacoli ATCC 25922	19.67 ± 0.58	19.670.58	14.67 ± 0.58	0.00 ± 0.00	21.33 ± 1.15	14.33 ± 0.58
Escherichia coli	9.67 ± 0.58	0.00 ± 0.00	11.33 ± 0.58	0.00 ± 0.00	8.67 ± 0.58	0.00 ± 0.00
Staphylococcus aureus ATCC 25923	35.67 ± 0.58	32.670.58	32.33 ± 0.58	24.67 ± 0.58	38.33 ± 0.58	26.00 ± 1.00
Staphylococcus aureus	18.67 ± 0.58	0.00 ± 0.00	14.33 ± 0.58	0.00 ± 0.00	17.00 ± 2.00	0.00 ± 0.00
Staphylococcus saprophyticus ATCC 15305	20.33 ± 0.58	24.33 ± 1.15	19.67 ± 0.58	17.33 ± 0.58	21.00 ± 1.00	19.33 ± 0.58
Staphylococcus saprophyticus	10.00 ± 0.00	0.00 ± 0.00	10.67 ± 0.58	0.00 ± 0.00	9.33 ± 0.58	0.00 ± 0.00

Table 3: The MIC and MBC of the extract and fractions of *C. sanguinolenta*

Test organisms	Ethanolic extract		Petroleum ether		Chloroform extract		Ethyl acetate extract		Water extract	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Salmonella typhi ATCC 19430	32.0	BST	X	X	1.0	4.0	X	X	8.0	BST
Salmonella typhimurium ATCC 14028	X	X	X	X	2.0	4.0	X	X	X	X
Proteus mirabilis ATCC 49565	32.0	BST	32.0	BST	2.0	2.0	32.0	BST	16.0	BST
Pseudomonas aeruginosa ATCC 27853	X	X	X	X	2.0	4.0	X	X	16.0	BST
Klebsiella pneumoniae ATCC 33495	X	X	X	X	4.0	8.0	X	X	X	X
Escherichia coli ATCC 25922	16.0	BST	16.0	BST	4.0	16.0	X	X	8.0	BST
Staphylococcus aureus ATCC 25923	4.0	BST	32.0	BST	2.0	4.0	32.0	BST	16.0	BST
Staphylococcus saprophyticus ATCC 15305	4.0	BST	16.0	X	2.0	4.0	32.0	BST	32.0	BST
Clinical Isolates										
Salmonella typhi	X	X	X	X	1.0	8.0	X	X	X	X
Salmonella typhimurium	X	X	X	X	2.0	16.0	X	X	X	X
Proteus mirabilis	8.0	BST	32.0	BST	1.0	8.0	X	X	32.0	BST
Pseudomonas aeruginosa	32.0	BST	X	X	1.0	8.0	X	X	32.0	BST
Klebsiella pneumoniae	32.0	BST	X	X	2.0	32.0	X	X	32.0	BST
Escherichia coli	32.0	BST	X	X	1.0	32.0	X	X	32.0	BST
Staphylococcus aureus	32.0	BST	X	X	0.5	4.0	X	X	16.0	BST
Staphylococcus saprophyticus	32.0	BST	X	X	1.0	4.0	X	X	16.0	BST

X= No test done, BST= Bacteriostatic

MATERIALS & METHODS

Ethanolic extract of *C. sanguinolenta*

One kilogram (250 gm) of dried pulverized *C. sanguinolenta* roots was macerated in 2 L of 70% ethanol in water and stored at room temperature for 48 hours. The resultant extract was filtered, concentrated.

Test organisms used

The test organisms used in this study were obtained from the Microbial Type Culture Collection, IMTCH,. The isolates used consisted of one strain each of Staphylococcus aureus, Staphylococcus saprophyticus, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi and Salmonella typhimurium.

Phytochemical screening of the extracts

The phytochemical constituents of the ethanol extracts, chloroform fraction and the water fraction were deter- mined. The phytochemical parameters assayed for, included saponins, reducing sugars, polyuronides, cyanogenic glycoside, alkaloid, triterpenes, phytosterols, flavonoids, antho- cyanosides and phenolics (Sofowora., 1993).

Antimicrobial activity of the fractions/compounds

The agar diffusion method was used to investigate the antibacterial properties of both the crude ethanolic extracts and sequential solvent fractions of *C. sanguinolenta*, (NCCLS,2000; NCID, 1999)

Determination of the Minimum Inhibitory Concentration (MIC)

The MIC values of the crude extract were determined using the microplate dilution method (13). 100 µl of 32 mg/ml of the ethanolic extract was added to 100 µl of sterile bacteriological peptone in the first well in the 96-well microplate and mixed well with a micropipette, 100 µl of this dilution was transferred to the bacterio-

logical peptone in subsequent wells yielding two-fold serial dilution in the original extract. The process was repeated for the other plant extracts in other columns of the microplate. A reference solution of chloramphenicol was also serially diluted in another column of the microplate as a positive control. 100 µl of actively growing test organisms was added to each of the wells except the negative control. Triplicate of each microplate was made and the procedure repeated for the other organisms. The microplates were incubated at 37°C for 24 hours. After the incubation, 40 µl of 0.2 mg/ml INT was added to each of the wells. The microplates were then examined after additional 60 minutes incubation.

Determination of the minimum bactericidal concentration (MBC)

The MBC values were deduced from those wells with the lowest concentrations at which no growth (color development) was observed after culturing for 24 hours of incubation. (Nester et al., 2004)

RESULT AND DISCUSSION

Phytochemical screening

Phytochemical screening performed on the ethanol extract as well as the water and chloroform fractions revealed the presence of reducing sugars, polyuronides, alkaloids and anthocyanosides. The water fraction in addition contains triterpenes (Table 1).

Susceptibility of the microbes to various fractions of *C. sanguinolenta*

As depicted in Table 2, 11 out of the 16 (68.75%) microbes used were inhibited by the ethanol extract with average zones of inhibition ranging from 9.33 ± 0.58 to 35.67 ± 0.58 mm. Another 11 out of 16 isolates were not susceptible to the petroleum ether fraction; however, both the standard and wild strains of Proteus mirabilis were susceptible with 27.00 ± 1.00 mm and 14.67 ± 0.58 mm as the average zones of inhibition respectively. The chloro- form fraction registered 100% inhibitory activity against all the sixteen isolates with inhibition zones averagely ranging between 9.33 ± 0.58 and 32.33 ± 0.58 mm. The ethyl acetate fraction inhibited the growth of 3 out of the 16 (18.75%) isolates used with average zones of inhibition ranging from 14.00 ± 1.00 to 24.67 ± 0.58 mm. The water fraction of *C. sanguinolenta* inhibited 12 out of the 16 (75.00%) microbes used with average zone diameters ranging from 8.67 ± 0.58 to 38.33 ± 0.58 mm (Table 2). Chloramphenicol inhibited the growth of Proteus mirabilis (ATCC 49565), E. coli (ATCC 25922), S. aureus (ATCC 25923) and S. saprophyticus (ATCC 15305) representing 25.00% of the total number of microbes used.

MICs and MBCs of the ethanol extract of *C. sanguinolenta* and its partitioned fractions

The MICs and MBCs of the partitioned fractions of *C. sanguinolenta* showed varying degrees of potency. These tests were performed only on the test organisms that showed inhibition during the antimicrobial screening. With the exception of the chloroform fraction which showed consistent bactericidal results in both MICs and MBCs to all the test organisms, the remaining fractions were bacteriostatic to the microbes. The ethanol extract had MIC values ranging from 8.0 to 32.0 mg/ml for the wild strains while that of the standard strains ranged from 4.0 to 32.0 mg/ml. The chloroform extract had MIC values ranging from 1.0 to 2.0 mg/ml for the standard strains and 0.5 to 2.0 mg/ml for the wild strains with MBC values from 2.0 to 32.0 mg/ml. The petroleum ether fraction exhibited MIC values ranging from 16.0 to 32.0 mg/ml among P. mirabilis, S. saprophyticus ATCC 15305, S. aureus ATCC25923, P. mirabilis ATCC 49565 and E. coli ATCC 25922, while MIC value of 32.0 mg/ml was observed among S. saprophyticus ATCC 15305, S. aureus ATCC25923 and P. mirabilis ATCC 49565 in the ethyl acetate fraction. The water fraction exhibited MIC values ranging from 8.0 mg/ml to 32.0 mg/ml in twelve of the microbes used (Table 3).

CONCLUSION

In conclusion, the study confirmed that fractions of *Cryptolepis sanguinolenta* have significant antibacterial activity. Different fractions have varying antibacterial activity against different organisms. The chloroform fraction had the highest activity, followed by water, ethanol, petroleum ether and ethyl acetate respectively. It is recommended that more research be conducted into the individual compounds in the extracts; there is promise in such to find very low MICs.

REFERENCES

Addy M: Cryptolepis: An African traditional medicine that provides hope for malaria victims. Herbal Gram 2003, 60:54–59.

Bierer DE, Fort DM, Mendez CD, Luo J, Imbach PA, Dubenko LG, Jolad SD, Gerber RE, Litvak J, Lu Q, Zhang P, Reed MJ, Waldeck N, Bruening RC, Noames BK, Hector RF, Carlson TJ, King SR: Ethnobotanical-directed discovery of the antihyperglycaemic properties of cryptolepine: Its isolation from *Cryptolepis sanguinolenta*, synthesis and in vitro and in vivo activities. J Med Chem 1998, 41:894–901.

Boye LG, Oku-Ampofo O: Medicinal plants in Ghana. Economic and Medicinal Plant research. Plants Traditional Med 1990, 4:32–33.

Eloff JN: A sensitivity and quick microplate method to determine the minimal inhibition concentration of plant extracts for bacterial. Planta Med 1998, 64(8):711–713.

Iwu MM: The Handbook of African Medicinal Plants. 200 N. W. Corporate, USA: CRC Press; 1993:221–222.

Mills-Robertson FC, Aboagye FA, Duker-Eshun G, Kaminta S, Agbeve S: In vitro antimicrobial activity of *Cryptolepis sanguinolenta* (periplocaceae). Afr J Pharm Pharmacol 2009, 3:(9)476–480.

National Center for Infectious Disease, Center for Disease Control and prevention, WHO: Laboratory Methods for the Diagnosis of Epidemic Dysentery and Cholera, Center for Disease Control and prevention. Atlanta, Georgia, USA 1999:71–72.

National Committee for Clinical Laboratory Standards (NCCLS): Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Wayne, PA, USA: Approved Standard M7-A4, NCCLS; 2000.

Nester EW, Anderson DG, Roberts CE Jr, Pearsall NN, Nester T, Hurley D: Microbiology, A Human Perspective, Volume 518, 614. Fourth Editionth edition. New York, USA: MacGraw-Hill; 2004:640–641.

Paulo A, Houghton PJ: Chemotaxonomic analysis of the genus Cryptolepis. Biochem Syst Ecol 2003, 31:155–166.

Sofowora A: Medicinal plants and traditional medicine. Ibadan, Nigeria: Spectrum books Limited; 1993:224–227.