

Full Length Research Paper

Antimicrobial potency of *Hyptis spicigera* leaf extracts against some pathogenic microorganisms

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Antibiotic resistance is of grave concern clinically, more so in quinolone-resistant and extended-spectrum beta-lactamase (ESBL)-producing isolates that causes complicated infections. In this study, the potency of *Hyptis spicigera* leaf extract against some pathogenic microorganisms is described. Minimum bacterial concentration of the leaf extract in five different solvents were tested at five different concentrations (0.625, 1.25, 2.5, 5 and 10 mg/ml). All the solvent extracts showed effective bactericidal potency at 2.5 mg/ml. The phytochemistry of the leaf extracts revealed the presence of tannins, sterols, alkaloids, saponin glycosides and flavonoids. *Klebsiella pneumoniae*, *Neisseria gonorrhoeae* and *Candida albicans* exhibited the greatest resistance to the bactericidal potency of *H. spicigera* leaf extract with high MBC at 5 mg/ml for all the extracts of *H. spicigera* screened.

Keywords: *Hyptis spicigera*, antimicrobial resistance, *Klebsiella pneumoniae*, *Neisseria gonorrhoea*, sensitivity, minimum bactericidal concentration.

INTRODUCTION

Plants have long been in use as medicine the world over. More recently, plant extracts have been developed and proposed for use as antimicrobials (Del Campo et al., 2000). The ability of several plants to cure several ailments is well documented (Tharke, 2004). *Hyptis spicigera* L. is an erect hairy aromatic herb from the family Labiatae commonly found in the bush lands of Southern Sudan, Northern Cameroon, Nigeria and Western Kenya. The plant leaf is commonly used to treat diarrhea, dysentery, colds, headaches and several other diseases (Othira et al., 2009). Microbial diseases remain a global issue with unacceptable high rates in developed countries, and in third world countries where they are frequently at an increase because of poor medical facilities and general hygiene issues (John, 2001). The consequences of high disease rate include; complications, morbidity and HIV (John, 2001).

The treatment of these diseases using conventional

antibiotics is made difficult because some pathogens frequently present resistance to antimicrobials through the production of extended-spectrum- β -lactamases (Livrelli et al., 1996) which are encoded mainly by SHV and/or TEM genes, (Lévèsque et al., 1995; Schumaker et al., 2000; Edelstain and Strachounski, 1998).

Antibiotic resistance increasingly compromises effective treatment of these diseases. Inexpensive treatment regimens have been rendered ineffective due to antimicrobial resistance in these microorganisms, while efficacious ones are often unaffordable. This necessitates the need for the screening of alternative and cheaper but very effective antimicrobials from plant sources.

MATERIALS AND METHODS

Plant material

The plant was collected in Zaria environs and taxonomically identified and authenticated by Mr. U. S. Gallah at the Herbarium Section, Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria and a sample deposited at the herbarium with Voucher No.528.

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Preparation of plant extract

Fresh leaves of the plant were air-dried for 72 h at room temperature. The dried samples were grounded, and 4 g was sequentially extracted with hexane, diethylether, dichloromethane, ethyl acetate and methanol and concentrated *in vacuo* to afford the various crude extracts (Yusuf et al., 2006).

Micro-organisms and media

Pure isolates of antimicrobial resistant *Staphylococcus aureus*, *Salmonella pyogenes*, *C. ulcekins* (please, provide full botanical name), *Salmonella typhi*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Neisseria gonorrhoea* and *Candida albicans* were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria and maintained in a slant of blood agar medium at 4°C until when needed.

Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) was carried out as previously described (Linda and Clyde, 1978). 0.1 g of plant extract was dissolved in each of the solvents (solvents were analar grade and obtained from Sigma) of extraction and used for the study. Plant extracts were pre-incubated in nutrient broth (Sigma) at different concentrations (10, 5, 2.5, 1.25 and 0.625 mg/ml). Normal saline was used to make a turbid suspension of the microorganisms and the solution was diluted to the Mc-Farlands scale (150×10^6 cfu/ml).

Phytochemical screening

Phytochemical screening of the extracts for saponins, alkaloids, tannins, flavonoids and saponin glycosides was carried out using standard phytochemical screening methods (Harbone, 1991) and the results are shown in Table 2.

RESULTS

Minimum bactericidal concentration

This was done to examine the antimicrobial potential of *H. spicigera* leaf extract. The solvent extracts of *H. spicigera* leaves showed significant bactericidal activity at very low concentrations of 2.5 mg/ml for the methanolic extract although *S. aureus*, *N. gonorrhoeae* and *C. albicans* were most resistant to these solvent extract with MBC of 5 mg/ml for the methanolic extract (Table 1). Di-ethyl ether extract of *H. spicigera* leaf was least effective at a concentration of 5 mg/ml against most of the microorganisms tested. *P. aeruginosa* showed the greatest sensitivity to the plant extract with MBC of 2.5 mg/ml of the leaf extract for all the solvent extract, while *N. gonorrhoea* showed the greatest resistance to the extract with MBC of 5 mg/ml for all the solvent extracts (Table 1).

Phytochemistry

The components screened for included; amino acids,

alkaloids, saponin glycosides, tannins, alkaloids, flavonoids, carbohydrates and sterols (Table 2). The methanol extract revealed a high content of saponin glycosides and flavonoids, while dichloromethane and ethyl acetate extracts had a high content of sterols. The ethyl acetate extract also showed a high content of flavonoids.

DISCUSSION

The insecticidal activity of *H. spicigera* leaf extract is documented (Othira et al., 2009; Sanon et al., 2006) though very little is known about its antimicrobial activity. Herein, the antimicrobial potential of its leaf extract against a broad spectrum of microorganisms was evaluated.

All the solvent extracts showed bactericidal activity against all the antibiotic resistant microorganisms tested, though with some variations as against the standard drug penicillin. The methanolic extract of *H. spicigera* leaf extract exhibited the highest activity against the microorganisms. This could be correlated with its high content of saponin glycosides when compared to the dichloromethane extract that also showed high content of flavonoids with a corresponding low bactericidal property. The methanolic extract has previously been shown to exhibit low insecticidal potency as demonstrated by Othira et al. (2009). The membrane lytic abilities of saponin glycosides (Babayi et al., 2004) could account for the high bactericidal activity of the methanolic extract as compared to the other solvent extracts.

The extracts were found to be bactericidal at low concentrations of 2.5 – 5 mg/ml against the microorganisms tested. This is significant in the application of this extract as a possible potent chemotherapeutic agent.

The photochemical content was similar to that obtained by other authors (Onayade et al., 1991) although different extracts revealed the different partitioning abilities of the different solvents. The methanolic extract revealed a higher quantity of saponin glycosides than the other solvents used; this could be attributable to the bipolar nature of methanol.

Antibiotic resistance in beta-lactamase producers like *K. pneumoniae* and *N. gonorrhoea* is of growing concern clinically. In this study, *H. spicigera* leaf extract showed strong bactericidal potency against antibiotic resistant strains of these organisms *in vitro* with methanol extract showing the highest activity at 2.5 mg/ml.

Conclusion

This work sought to investigate the potency of *H. spicigera* leaf extracts as an antimicrobial. The results indicate antimicrobial potency of the leaf extract on a broad spectrum of microorganisms that are resistant to ampicillin. More work needs to be done to ascertain the active component of the plant extract involved in the bactericidal mechanisms.

Table 1. Minimum bactericidal concentration of *H. spicigera* leaf extract against some pathogenic microorganisms.

Test Organisms	EAE					DCM					ME					HE					AMP					DEE														
	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	0.625 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	0.625 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	0.625 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	0.625 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	0.625 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	0.625 mg/ml										
<i>S. aureus</i>	-	*	+	++	+++	-	*	+	++	+++	-	*	+	++	+++	-	*	+	++	+++	-	*	+	++	+++	-	*	+	++	+++	++	++	++	++	++	-	*	+	++	+++
<i>S. pyogenes</i>	-	*	+	++	+++	-	-	*	+	++	-	-	*	+	++	-	-	*	+	++	-	-	*	+	++	-	-	*	+	++	++	++	++	++	++	-	*	+	++	+++
<i>C. ulcekins</i>	-	*	+	++	+++	-	*	+	++	+++	-	-	*	+	++	-	-	*	+	++	-	-	*	+	++	-	-	*	+	++	++	++	++	++	++	-	*	+	++	+++
<i>B. subtilis</i>	-	-	*	+	++	-	*	+	++	+++	-	*	+	++	+++	-	*	+	++	+++	-	*	+	++	+++	-	*	+	++	+++	++	++	++	++	++	-	-	*	+	++
<i>S. typhi</i>	-	-	*	+	++	-	-	*	+	++	-	-	*	+	++	-	-	*	+	++	-	-	*	+	++	-	-	*	+	++	++	++	++	++	++	-	*	+	++	+++
<i>E. coli</i>	-	*	+	++	++	-	*	+	++	++	-	-	*	+	++	-	-	*	+	++	-	-	*	+	++	-	-	*	+	++	++	++	++	++	++	-	*	+	++	+++
<i>P. aeruginosa</i>	-	-	*	+	++	-	-	*	+	++	-	-	*	+	++	-	-	*	+	++	-	-	*	+	++	-	-	*	+	++	++	++	++	++	++	-	-	*	+	++
<i>K. pneumoniae</i>	-	*	+	++	+++	-	*	+	++	++	-	-	*	+	++	-	-	*	+	++	-	-	*	+	++	-	-	*	+	++	++	++	++	++	++	-	*	+	++	+++
<i>N. gonorrhoea</i>	-	*	+	++	+++	-	*	+	++	+	-	*	+	++	+++	-	*	+	++	+++	-	*	+	++	+++	-	*	+	++	+++	++	++	++	++	++	-	*	+	++	+++
<i>C. albicans</i>	-	-	*	+	++	-	-	*	+	++	-	*	+	++	++	-	*	+	++	++	-	*	+	++	++	-	*	+	++	++	++	++	++	++	++	-	*	+	++	++

Key: - = no growth; * = MBC or MIC; + = scanty growth; ++ = moderate growth; +++ = heavy growth. EAE= Ethyl acetate; DCM= dichloromethane; ME= methanol; HE= N-hexane; AMP= ampicillin; DEE= diethyl ether.

Table 2. Phytochemical content of the different solvent extracts of *H. spicigera* leaf.

Solvents	EAE	DCM	HE	ME	DEE
Carbohydrate	+	-	-	+	-
Saponin glycosides	+	+	+	++	+
Sterols	++	++	+	+	+
Tannin	-	-	-	+	-
Flavonoid	++	-	-	++	-
Alkaloid	+	+	+	+	+

Key: EAE= Ethylacetate; DCM= dichloromethane; ME= methanol; HE= N-hexane; AMP= ampicillin; DEE= diethyl ether; + = present; ++ = present in high quantity; - = absent.

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