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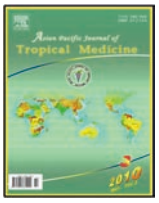

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Antimicrobial and antispasmodic activity of leaf extract and fractions of *Stachytarpheta cayennensis*

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ABSTRACT

Objective: To investigate the antimicrobial activity of the methanol leaf extract (ME), n-hexane fraction (HF), ethylacetate fraction (EF) and methanol fraction (MF), of *Stachytarpheta cayennensis* C. Rich (verbenaceae) as well as to ascertain the antispasmodic effects of the ME and the various fractions (HF, EF and MF) on acetylcholine (Ach) and histamine (H) induced contractions on isolated guinea pig ileum. **Methods:** The *in vitro* agar well diffusion method was used for the antimicrobial studies while the isolated tissue method was employed for the antispasmodic test. Organisms used were all clinical isolates of *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella paratyphi, Candida albicans* and *Aspergillus niger*. **Results:** The extract and fractions exhibited dose dependent inhibition against all the bacteria tested and also exhibited insignificant antifungal activity against *Candida albicans* and *Aspergillus niger*. The minimum inhibitory concentration (MIC) of the extract and fractions (mg/mL) on *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Salmonella paratyphi* respectively were ME 5.62, 14.12, 22.38, 2.11; EF 1.25, 6.30, 9.40, 9.40 and MF 3.98, 8.81, 39.80, 21.13. The n-hexane fraction exhibited MIC of 1.07 mg/mL against only *Bacillus subtilis*. The extract and fractions exhibited significant ($P< 0.05$) dose dependent attenuation of contractions induced by acetylcholine and histamine on isolated guinea pig ileum. Concentrations of the extract and fractions (μg/mL) which evoked 50% inhibition of maximal response exhibited by Ach were ME 0.64, HF 0.16, EF 0.08 and MF 0.15, while that of histamine included ME 5.12, HF 0.16, EF 0.04 and MF 0.64. Preliminary phytochemical studies on the extract and fractions indicated the presence of carbohydrates, alkaloids, saponins, flavonoids, steroids and terpenoids. **Conclusions:** The extract and fractions of *Stachytarpheta cayennensis* possessed both antibacterial and antispasmodic effects confirming the claimed use in folkloric medicine for wound healing and gastrointestinal ulceration.

1. Introduction

Infectious diseases are persisting as a major health problem for almost half a century now and there has been increasing incidence of resistance to currently available antibacterials [1]. There is the need for intensive studies for possible discovery of new agent with antibacterial potentials. Many drugs have been discovered by the exploitation of traditional medicine since the early dates of human existence[2]. In ethnomedicine, the plant, *Stachytarpheta cayennensis* has been reported to have the following activity; vermifuge [3], sedative and anxiolytic [4], anti-inflammatory and analgesics [5], as well as anti-diarrhea [6]. Other documented ethnomedicinal uses include asthma, itching, sores, boils, intestinal parasite, neuralgia, and eczema, cough and venereal diseases [7], as well as antiulcer [8] and anticonvulsant[9]. Due to the popularity of this plant in folk medicine, we were prompted to investigate the antimicrobial and antispasmodic activities of its leaf extracts and fractions using *in vitro* models.

2. Materials and methods

2.1. Plant material

Fresh leaves of *S. cayennensis* were collected in July 2006 from Nsukka, Enugu State, Nigeria and authenticated by Mr. A. Ozioko of Bioresources Development and Conservation Programme (BDCP) Center, Nsukka. The leaves were cleaned, dried under the sun and pulverized to coarse powder using laboratory mill (Thomas Willey, Model 4). The powdered leaves (400 g) were extracted with methanol by cold maceration for 48 hours [10]. The filtrate was concentrated in a rotary evaporator under reduced pressure to obtain the methanol extract (ME: 103 g; 25.75%

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w/w). The extract (30 g) was partitioned with n-hexane, ethyl acetate and methanol in order of increasing polarity. The n-hexane, ethylacetate and methanol fractions were also concentrated in a rotary evaporator under reduced pressure, and afforded 4.3 g of the n-hexane fraction (HF; 1.1% w/w), 6.7 g of the ethyl acetate fraction (EF; 1.7% w/w) and 35.6 g of the methanol fraction (MF; 8.90% w/w). The extract and fractions were tested for activity and subjected to phytochemical analysis using standard methods [10, 11].

2.2. Animals

Guinea pigs (250-500 g) bred in the Laboratory Animal Facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, were used in the studies. The animals were maintained under standard laboratory conditions and had free access to standard pellets (Guinea Feeds, PLC, Nigeria.) and water.

2.3. Microorganisms

Clinical microbial isolates namely *Escherichia coli, Bacillus substilis, Pseudomonas aeruginosa, Salmonella paratyphi, Staphylococcus aureus, Aspergillus niger and Candida albicans*, obtained from the Microbiology Unit of the Department of Pharmaceutics and Pharmaceutical Microbiology, University of Nigeria, Nsukka, were used.

2.4. Antimicrobial assay

500 mg each of the extract and fractions (ME, HF, EF and MF) were dissolved in 5 mL of dimethyl sulfoxide (DMSO) by shaking to obtain 100 mg/mL concentration. Subsequently, the concentration was diluted to obtain 50 mg/mL and 25 mg/mL for the determination of the minimum inhibitory concentration (MIC) at the dose levels. Agar well diffusion method [12, 13] was employed for the assay. A 0.5 Marcfaland standard was used in the preparation of the organisms used to seed the agar plates. The test organisms were sub-cultured at 37 ℃ and maintained on nutrient agar media for bacteria and sabouraud agar medium for fungi. Petri dishes containing 20 mL of respective medium were seeded with selected microbial strains which were incubated at 37 ℃ for 24 hours. Standard antimicrobial agents used as positive controls were; ampicillin (Elvis Pharm.), gentamycin (Lek, Slovakia), ciprofloxacin (Medriech, India) and tetracycline (NASSMU, Lagos). After 24 hours the inhibition zone diameters (IZD) were recorded and the mean calculated. The minimum inhibitory concentration (MIC), was then determined at various dilutions by extrapolation from the graphs of IZD squared (IZD^2) against logarithm of the concentration.

2.5. Antispasmodic effect

A modified method described by Mukherjee [14] was adopted for the antispasmodic effect. A segment of the guinea pig ileum (about 2 cm) was suspended in a 50 mL organ bath containing Tyrode solution maintained at (36±1) ℃ and connected with an aerator. The tissue was allowed to equilibrate for 30 minutes under a resting tension of 0.5 g before exposure to drugs and extracts. During equilibration period, the tissue was washed with fresh Tyrode solution every 10 minutes to prevent the accumulation of metabolic end products [15]. Non-cumulative dose responses to acetylcholine and histamine were established. The effects of the extract and fractions on the responses elicited by these agonists were recorded. The responses were recorded via a frontal writing lever on kymograph paper (Scientific and Research Instruments Ltd., England). The responses were repeated three times and mean percentage inhibition calculated.

3. Results

3.1. Phytochemical constituents of the extract and fractions

Phytochemical screening revealed the presence of saponins, carbohydrates, flavonoids and terpenoids in all the extract and fractions. Saponins had the highest abundance, whereas alkaloids, steroids, reducing sugars and glycosides were significantly present in the methanol extract and fractions (Table 1).

Table 1
Phytochemical constituents of the extracts and fractions.

Constituent	ME	HF	EF	MF
Carbohydrates	+++	+	++	++
Reducing sugar	+	–	–	+
Glycosides	+	–	–	+
Flavonoids	+	+	++	+
Saponins	+++	+++	++	+++
Alkaloids	++	–	–	++
Terpenoids	++	+	++	+
Steroids	++	–	–	+

+++ = Conspicuously present; ++ = moderately present; + = present; – = absent.

3.2. Antimicrobial assay

The result indicated that both the extract and fractions possessed significant antibacterial activity with insignificant anti-fungal activity. The result of inhibition zone diameter (IZD) and MIC revealed a dose dependent potency in antibacterial activity. The n-hexane fraction inhibited the growth of only B. subtilis organism whereas all the other fractions and the extract exhibited certain level of inhibition on all the other bacterial strains tested (Tables 2 and 3).

3.3. Antispasmodic effects

The findings showed that the extract and fractions did not evoke contraction of the guinea pig ileum at concentrations tested. However, they exhibited dose-dependent inhibition of contractions induced by acetylcholine (Ach) and histamine (H), on the isolated guinea pig ileum. The ethyl acetate fraction (EF) was the most potent while the methanol extract (ME) indicated the least inhibitory effects (Table 4, Figure 1 and Figure 2).

Table 2

Inhibition zone diameter (IZD) of extract and fractions of *S. cayennensis*.

	HF			EF			MF			ME			SA (μg/mL)		
	100	50	25	100	50	25	100	50	25	100	50	25	A	T	G
B. Subtilis	5.0	4.5	4.0	9.0	6.0	4.5	4.0	3.5	3.0	5.0	4.0	3.0	9.0	11.0	26.0
Staph aereus	+	+	+	8.0	5.0	4.0	6.0	5.0	4.0	6.0	3.0	2.0	20.0	+	26.0
Ps. Aeruginosa	+	+	+	4.5	4.0	3.0	6.0	3.0	+	4.0	3.0	+	8.0	16.0	22.0
Sal. Paratyhpi	+	+	+	4.5	3.5	3.0	4.0	3.0	+	5.0	4.5	4.0	9.0	17.0	20.0
C. albican	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–

HF = n–Hexane fraction, EF= Ethylacetate fraction, MF=Methanol fraction, ME= Methanol extract, + = no significant inhibition, SA= Standard antibiotics used. Concentrations of extracts and fractions in mg/mL, all IZD figures are in mm, A= ampicillin, T= tetracycline and G= gentamycine.

Table 3

The minimum inhibitory concentration (MIC) of extract an fractions (mg/mL).

Microorganism	HF	EF	MF	ME	AMP	TETRA	GENT
B. subtilis	1.07	1.25	3.98	5.62	5.62	1.33	0.02
Staph. aureus	–	6.30	8.81	14.12	1.00	–	0.23
Ps. aeruginosa	–	9.40	39.80	22.38	12.50	2.51	1.77
Sal. paratyphi	–	9.40	21.13	2.11	22.38	1.67	0.79

HF = nHexane fraction, EF = Ethylacetate fraction, MF = Methanol fraction, ME = Methanol extract, AMP = Ampicillin, TETRA = Tetracycline, GENT = Gentamycin.

Table 4

Concentrations of extract and fractions at 50% inhibition of maximal response exhibited by acetylcholine and histamine.

Extract/Fraction	Concentrations at 50% inhibition (μ g/mL)	
	Acetylcholine	Histamine
ME	0.64	5.12
HF	0.16	0.16
EF	0.08	0.04
MF	0.15	0.64

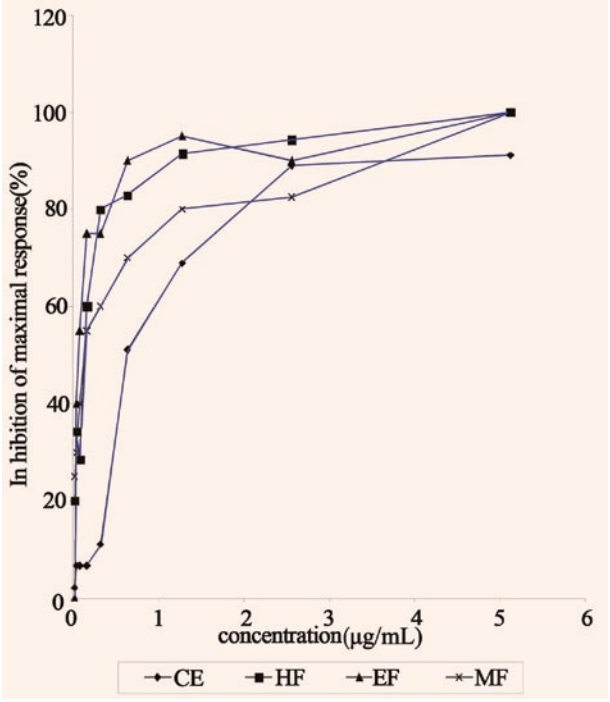

Figure 1. Effects of extract and fractions on acetylcholine induced contractions on isolated guinea pig ileum.

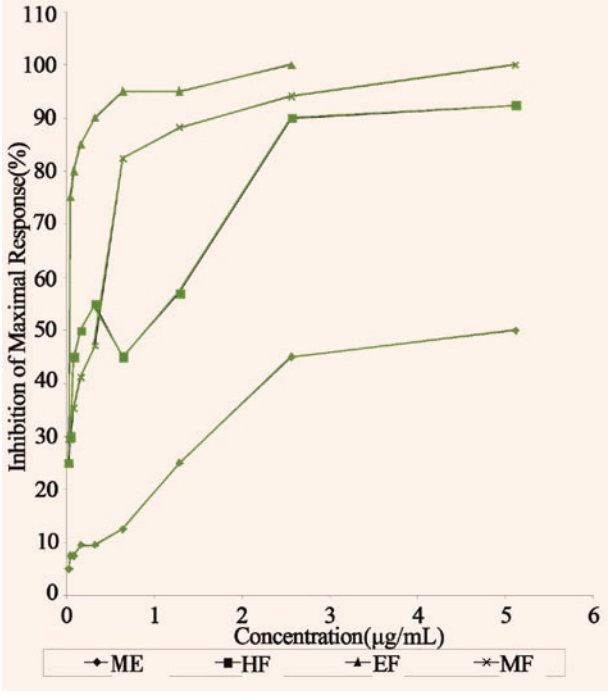

Figure 2. Effects of extract and fractions on histamine induced contractions on lsolated guinea pig ileum.

4. Discussion

The antimicrobial screenings showed that the extracts exhibited antibacterial activity against various gram–negative and gram–positive organisms but are devoid of significant fungistatic activity. The order of bacterial susceptibility was *B–subtilis*> *Staph aureus*>*Ps. aeruginosa* >*Sal. paratyphi*. The minimum inhibitory concentration data indicated that the antibacterial activity of the extracts and fractions was comparable to the standard antibiotics

employed in the study. The antibacterial activity could be attributed to the effects of bioactive phytochemical constituents such as flavonoids, saponins and terpenoids [16, 1, 10]. The phytochemical studies indicated highest concentration of terpenoids and flavonoids in EF. There is the possibility that these constituents might be responsible for the most potent antibacterial effect exhibited by EF. The result of the antibacterial study tends to support the ethnomedicinal use of *S. cayennensis* in the treatment of sores, boils and venereal diseases [7]. It also highlights the potential wound healing activity, since *P. aeruginosa* and *Staph aureus* are among microbial agents that are often associated with the progression of wounds and sores of diverse etiology [17]. The extract and fractions exhibited significant dose dependent inhibition of contractions induced by Ach and histamine on isolated guinea pig ileum. Histamine and acetylcholine are important endogenous spasmogens and agents that inhibit their contractions may have a good antispasmodic potential. There is an indication of a non-specific antagonism by the extracts [18]. This antagonism could either be through the receptor site such as the muscarinic and histaminic receptors or through other musculotropic route such as influx or out flux of calcium ions [19]. The ethylacetate fraction, which exhibited the most potent inhibition of contractions induced by the spasmogens, contains highest concentration of flavonoids bioactive constituents as depicted by the phytochemical studies. Though these effects may not be categorically been assigned to a particular constituents at this level, but flavonoids have variously been implicated and demonstrated to inhibit guinea pig ileal contraction induced by some spasmogens[20, 21]. The antispasmodic activity of the extract and fractions has further highlighted the potential antiulcer activity of the extracts of *S. cayennensis*[8]. These results showed that the extract and fractions of *S. cayennensis* possessed broad spectrum antibacterial activity with potent spasmolytic effect.

Conflict of interest statement

We declare that we have no conflict of interest.

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