

Chemotherapeutical evaluation of *Borreria verticillata* extracts

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Abstract: In the Guinean traditional medicine, *Borreria verticillata* is widely used in the treatment of skin diseases. Extracts of the plant species were found to exhibit *in vitro* moderate to weak antimicrobial activity against *Staphylococcus aureus*, *Streptococcus viridans*, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, *Mycobacterium fortuitum*, *Gardnerella vaginalis*, human immunodeficiency virus (HIV), *Candida albicans* and *Candida tropicalis*. The antimicrobial action is closely related to the presence of alkaloids.

Keywords: *Borreria verticillata*, Antibacterial, Antiviral, Anti-Human Immunodeficiency Virus, Antifungal

1. Introduction

Borreria verticillata (L.) G.Mey (synonym: *Spermacoce verticillata* L.) is widely distributed in tropical areas in Africa, Asia and America. In Guinea, it is common in the four regions particular in savanna, wastelands, in non cultivated fields, on crevices of rocks very common in humid areas, mainly found in the wet season (June-November). As well in the Guinean traditional medicine as in other western African countries, the extracts and/or the juice of the aerial parts is topically applied for the treatment of various skin diseases including psoriasis, eczema-*Tinea versicolor*, ring worm-*Tinea capitis*, scabies, skin itches, various infectious dermatitis (leprosy, furuncles, wounds etc.). The essential oil extracted from leaves has been shown to inhibit *Escherichia coli* and *Staphylococcus aureus*. The macerate of the roots is used against diarrhoea. The infusion of the whole plant or the aerial plants is used to improve the stomach digestion [1-4].

Studies have confirmed that extracts from *Borreria* and *Spermacoce* species as well as their isolated compounds possessed diverse biological activities, including analgesic, anti-inflammatory, antitumor, antimicrobial, larvicidal, antioxidant, gastrointestinal, anti-ulcer, and hepatoprotective, with alkaloids and iridoids as the major active principles [5-8]

2. Material and Method

2.1. Plant Material

B. Verticillata was collected in July 1988 in the Southern part of Guinea-Conakry. The plant sample was taxonomically identified by Dr. F. Camara, department of Botany in the Research Center of Medicinal Plants-Sérédou, Macenta, Guinea. Voucher specimens have been deposited at the herbarium of the Centre. Moreover, voucher material of the species examined has been deposited in the herbarium of the National Botanic Garden of Belgium (BR).

2.2. Preparation of the Extracts

2.2.1. Ethanolic and Hexane Extracts

Each extract was prepared exhaustively from 50g of the dried aerial parts (BVT), roots (BVR), or the whole plant (BV). BVT1, BVR1 and BV1 were obtained by percolation with 80% ethanol to yield 2.61%, 4.32% and 3.70% w/w dry weight, respectively.

BVT2, BVR2 and BV2 were obtained by percolation with hexane and the yield was 0.63%, 0.08% and 0.37% w/w dry weight, respectively. Each extract was evaporated *in vacuo* below 40°C to dryness.

2.2.2. Totum of Alkaloids

The dried aerial parts of *B. verticillata* (100g) were percolated to exhaustion with petroleum ether. The extract was concentrated *in vacuo* at 40°C. The residue was suspended in 20% Na₂CO₃ (pH 9) and extracted with 3×500ml ethyl acetate (EtOAc). The EtOAc extract was evaporated *in vacuo* below 40°C to give the residue BVA1 (0.01%) which gave a positive Dragendorff reaction. After defating the plant material with petroleum ether, the marc was extracted with methanol till exhaustion. The methanol solution was evaporated to thick syrup and the residue portioned between EtOAc (300ml) and 2% tartaric acid (300ml). After repeated extraction with the organic solvent, the aqueous phase is made basic with 20% Na₂CO₃. The basic aqueous solution is then extracted with chloroform (3×500ml). The alkaloid-containing solution was dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo* to afford the crude alkaloid residue BVA2 (0.34%).

2.3. Preparation of Samples

10mg of each lipophilic fraction (BVT2, BVR2, BV2, BVA1 and BVA2) was dissolved in 0.5ml of polyethyleneglycol 400 (PEG400), then suspended in physiological tris-buffer (pH 7.4) to give a concentration of 1mg/ml. Each ethanolic fraction (BVT1, BVR1, and BV1) was dissolved in 0.5ml of dimethylsulfoxide (DMSO), and then suspended in physiological tris-buffer to give a concentration of 1mg/ml. The first negative control was obtained by dissolving 0.5ml PEG400 in 9.5ml of physiological tris-buffer, and the second negative control by mixing 0.5ml DMSO with 9.5ml physiological tris-buffer.

2.4. Antimicrobial Testing

The qualitative evaluation of the antimicrobial activity of the extracts was accomplished using the hole plate diffusion method for bacteria and yeasts whereas the solid dilution method was employed for antifungal testing using

dermatophytes.

The following microorganisms were used : gram positive cocci including *Staphylococcus aureus*, *Streptococcus viridans*, *Str.pneumoniae*; gram negative cocci including *Neisseria gonorrhoeae*; acid-fast bacilli including *Mycobacterium fortuitum*; yeasts including *Candida albicans* and *C. tropicalis*; the gram-variable (facultative anaerobic bacteria) *Gardnerella vaginalis*.

2.5. Antiviral Testing

The antiviral testing of the plant extracts was carried out by means of the previously described endpoint titration technique. The activity was determined *in vitro* against Poliomyelitis, *herpes simplex*, and *Semliki forest* viruses. The *in vitro* anti-human immunodeficiency virus (HIV) was realized by means of the colorimetric MTT (tetrazolium) and the Reverse Transcriptase assays described by Schwartz and co-workers (1988).

3. Results and Discussion

3.1. Antimicrobial Activity

The antimicrobial properties of the extracts of *B. verticillata* are summarized in table1, and compared with those of neomycin, penicillin or nystatin. The extracts of the aerial parts, the root and the whole plant inhibited more or less the growth of the tested microorganisms. But, the aerial part was more active than the whole plant which was also more effective than the root. Except for *N. gonorrhoeae*, the antibacterial effect and spectrum of the ethanol extract of the aerial parts were more important than those of the hexane ones. *S. aureus* and *N.gonorrhoeae* were the most sensitive bacteria to both the ethanol and hexane extracts of the aerial parts, the whole plant or the root. The apolar extracts were inactive against *Gardnerella vaginalis* and *M. fortuitum*. *Candida albicans* and *C. tropicalis* were weakly sensitive to the aerial parts and the whole plant extracts.

Table 1. Antimicrobial activity of the extracts (1mg/ml) of *B. verticillata*.

Extr.	Inhibition zone width (mm)							
	<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>	<i>Neisseria gonorrhoeae</i>	<i>Gardnerella vaginalis</i>	<i>Streptococcus viridans</i>	<i>Mycobacterium fortuitum</i>	<i>Candida albicans</i>	<i>Candida tropicalis</i>
BVT1	10	2	12	5	2	5	3	3
BVR1	6	0	10	0	3	0	0	0
BV1	8	0	13	5	5	5	3	3
BVT2	5	0	14	0	0	0	0	0
BVR2	3	0	13	0	6	0	0	0
BV2	5	3	14	0	5	0	3	0
BVA1	5	0	9	0	0	0	0	0
BVA2	16	11	14	11	9	20	8	8
A	0	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0
C	22	20	20			40		
D		30	30	25	30			
E							20	10

Legend: A: 1%PEG400; B: 1%DMSO; C: Neomycin (500µl/ml); D: Penicillin (50µg/ml); E: Nystatin (5000E/ml)

At a concentration of 1mg/ml, the total alkaloid extract (BVA2) was shown to possess the highest and widest antimicrobial properties. The antimicrobial activity of the crude alkaloidal fraction was significant against *S. aureus*, moderate against *N. gonorrhoeae*, *Str. Pneumoniae*, *M. fortuitum*, *Gardnerella vaginalis*, *C. albicans*, *C. tropicalis*, and weak against *Str. viridans*. However, these activities were moderate as compared with the standard active compounds against Gram-positive and Gram-negative cocci and acid-fast bacilli. It should be noticed that the lack of zone of inhibition does not necessarily indicate a lack of activity since some compounds do not diffuse well into agar. On the other hand, it must be mentioned that the alkaloidal extracts precipitated when they were diluted with a solution of physiological tris buffer. Such phenomenon probably diminishes the activity.

In this preliminary investigation, the extracts of *B. verticillata* have shown a moderate antibacterial along with a weak anti-yeast activities. Such antibacterial potency could be correlated to the presence of alkaloids (BVA2) which showed the strongest and widest spectrum antimicrobial activity. These results could support the traditional use of the plant in the treatment of various skin diseases.

3.2. Antiviral Activity

None of the extracts of *B. verticillata* were active against Poliomyelitis, *Semliki forest* and herpes viruses. Both the ethanol and hexane extracts showed cytotoxicity at the level of 50-100 µg/mg. The highest cytotoxicity (6-25 µg/ml) among the tested extracts was observed with the crude alkaloids extract.

Only BVA2 extract was tested against human immunodeficiency virus and the results are summarized in Table 2. The anti-HIV activity was determined at non toxic concentration by calculating the infected/uninfected cell absorbance ratio (MTT) and by dosing the Reverse Transcriptase (RT) [9]. BVA2 was tested at concentrations ranging from 0.05 to 50 µg/ml and appeared to be toxic for CEM-C113 cells at concentration of 50 µg/ml. The extract was devoid of any activity neither with 0.05 or 0.5 µg/ml. At day-7, BVA2 (5µg/ml) was not toxic and decreased the viable cells (32%) while that of 3'-azido-2', 3'-dideoxythymidine (AZT; 2.5 µM) showed 75% of survival cells. But conspicuous inhibition of the Reverse Transcriptase activity was observed with BVA2 (RT: 26599) as compared with the virus control (RT: 76468). However, this inhibition was not significant as that of AZT (9755) which was 2.73 (26599/9755) time more efficient than BVA2.

In order to find out the constituents responsible for the observed antibacterial, anti-yeast and anti-HIV activities, a bioassay-guided fractionation has to be performed. Moreover, due to the antimicrobial effect of the crude alkaloid extract of *B. verticillata*, along with a previous recorded significant activity of the alkaloid borreverine against *Vibrio cholerae* (MIC: 6µg/ml) [Maynard *et al.*, 1980], the isolation and antimicrobial evaluation (HIV, *S. aureus* and *N. gonorrhoeae*) of pure alkaloids are of importance.

Table 2. Toxicity and Anti-human Immunodeficiency Virus of the Total Alkaloid Extract of *B. verticillata* (BVA2) with MTT Assay [9].

Samples	Virus	MTT Dosage Cell survival %; Day + 7	RT Dosage; Day + 7
BVA2 50 µg/ml	–	Toxic	Toxic
	+	Toxic	Toxic
BVA2 5 µg/ml	–	79	1101
	+	32	26599
BVA2 0.5 µg/ml	–	83	Not toxic
	+	50	Not toxic
BVA2 0.05 µg/ml	–	72	Not toxic
	+	65	Not toxic
Cell control	–	100	815
Virus control	+	45	76468
Ethanol control	–	85	1009
	+	36	59552
Methanol control	–	73	2696
	+	39	91061
AZT 2.5 µM	–	100	1221
	+	75	9755

4. Conclusion

The present investigation showed a conspicuous antimicrobial activity of *B. verticillata* which is closely related to the presence of alkaloids. It also showed the scientific rationale behind the traditional use of the plant in the treatment of various skin diseases.

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