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Original Research Article

In vitro antimicrobial and antiulcer activities of hymeglusin and septicine constituents of *Barteria nigritiana* stem bark

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Abstract

Purpose: *To identify the phytochemical contents of the bioactive fractions of Barteria nigritiana (Passifloraceae) extract using the HPLC-DAD/MS techniques and evaluate its antimicrobial and anti-ulcer activities using in vitro models.*
Method: *The phytoconstituents of the methanol extract were identified using standard methods. The extract was fractionated by solvent-solvent partitioning, vacuum liquid and column chromatographic techniques following a bioactivity-guided approach. The constituents of the most active sub-fraction were identified using HPLC-DAD/MS techniques. The antimicrobial (Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi and Candida albicans) and anti-Helicobacter pylori activities assay adopted agar well diffusion methods to determine inhibition zone diameters (IZDs) and minimum inhibitory concentrations (MICs) using gentamicin/nystatin and clarithromycin (1 µg/mL) as standards.*
Result: *Repeated bioactivity-guided chromatographic purification yielded a hymeglusin/septicine-enriched sub-fraction identified from the HPLC-DAD/MS analysis. The sub-fraction (20 µg/mL) exhibited significantly ($p < 0.05$) higher IZDs of > 20 mm against four of the six Helicobacter pylori strains tested, with MICs of 0.3906 - 1.5625 µg/mL compared with other tested samples. Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Candida albicans recorded significantly ($p < 0.05$) high susceptibility patterns to the sub-fraction compared with the control, with IZDs of 15.3 ± 2.1, 18.2 ± 0.2, 15.8 ± 1.1 and 16.9 ± 0.3 mm, but lower than that of gentamicin/nystatin which showed MICs of 3.125, 1.5625, 6.25 and 3.125 µg/mL respectively.*
Conclusion: *The hymeglusin/septicine-enriched sub-fraction shows significant inhibition of H. pylori, C. albicans, E. coli, P. aeruginosa and S. typhi and hence, is are potential lead compound for the development of potent therapeutic agents for the management of peptic ulcer and other infectious diseases.*

Keywords: *Barteria nigritiana, Antimicrobial, Hymeglusin, Septicine, Helicobacter pylori*

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INTRODUCTION

Medicinal plants have continued to play a significant role as a source of lead compounds for many diseases [1]. Many drugs in use today, such as digoxin, artemisinin, aspirin and morphine, were discovered from natural products such as marine organisms, plants, animals and

endophytes. These drug sources have continued to attract researchers and public health advocates. Despite the challenges associated with biopiracy, standardization and poor integration with modern techniques, ethnomedicine has remained the major source of bioactive molecules, herbs, or herbal formulations whose activities have been linked to their phytoconstituents [2]. However, some of these plants are underutilized in the management of diseases such as peptic ulcers and parasitic infections, considering quality control issues, regulation, knowledge gap, environmental challenges and sustainability [3].

The prevalence of *Helicobacter pylori* (*H. pylori*) infection shows that about 70 % of the global population is infected, with a higher incidence in developing countries [4]. It has remained the most significant risk factor in peptic ulcer disease (PUD), with 6 - 14 cases per 10000 of the population. The major obstacles in the management of PUD are intestinal perforation or stomach cancer due to the resistant pathogenic organism H. pylori, which accounts for 1 % mortality per year [5]. The mortality and morbidity rates of PUD have continued to decline due to a better understanding and management of risk factors. The trends have plateaued in the recent epidemiological data; however, the burden is still high, especially in developing countries [6]. The current multi-therapy with combinations of histamine-2 (H_2) receptor antagonists, proton pump inhibitors, antibiotics and bismuths is fast losing its potency due to the development of resistance and associated side effects [7,8]. Many herbal medicines have demonstrated inhibitory activity against *H. pylori* [9].

Barteria nigritiana (*B. nigritiana*) Hook (Passifloraceae) is a medicinal plant widely distributed in tropical and subtropical Africa and commonly referred to as Ukwofia (Igbo), Oko (Yoruba), or Idon zakara (Hausa) in Nigeria [10]. It grows as a tree of up to 20 m tall with alternately arranged simple leaves and showy, large flowers. The use of *B. nigritiana* in folk medicine practices for managing acute and chronic bacterial infections and sores is well documented. This has accompanied other pharmacological evidence, including anti-inflammatory, antioxidant, antifungal, analgesic, antiulcer, and antimicrobial activities [11]. In Southeast Nigeria, *B. nigritiana* is decocted and applied to the wounded area or taken to reduce gastritis resulting from *H. pylori*-induced PUD [12]. Its use for ulcer treatment could be a result of the relationship with wound healing, and its antimicrobial and anti-inflammatory properties.

The claimed use of *B. nigritiana* in ethnomedicine or the phytochemical compounds responsible for the anti-ulcer activity has not been validated or described. The study, therefore, aims to identify the phytoconstituents from the most bioactive fraction of *B. nigritiana* and assay the sub-fraction for anti-*H. Helicobacter pylori* and antimicrobial activities.

EXPERIMENTAL

General identification procedure

Silica gel (0.080 - 0.400 mm; Merck, Germany) was utilized for vacuum liquid chromatography (VLC) and column (CC) chromatographic separations. Thin-layer chromatographic (TLC) analysis was performed using a pre-coated silica gel 60F254 0.25 mm thickness plate (Merck, Germany). Identification of spots on the TLC was performed by anisaldehyde sprays on the developed plates, heated at 105 °C. The high-performance liquid chromatography with diode-array detection (HPLC-DAD; Jasco, Germany) analysis was executed on a C-18 reversed phase Reprosil 100 column (250 x 20 mm, 5 µm) using a mobile phase of water and methanol gradient, flow of 1 mL/min and a temperature of 40 °C; sample injection volume of a 100 µL loop. The HPLC-MS detection was done by HPLC/ electrospray ionization quadrupole time-of-flight mass spectrometry (HPLC/+ESI-QQTOF-MS) method with a binary mobile phase gradient of water/formic acid and acetonitrile/formic acid, a loop of 5 mL and 800 µL/mL flow rate at 200-400 nm UV wavelength [13].

Plant material

The fresh stem bark of *B. nigritiana* was collected in March 2023 in Nsukka, Nigeria (N743’ 50’, E 832 10’) and authenticated by Mr Felix Nwafor of the Department of Pharmacognosy of the University of Nigeria, Nsukka. A voucher (Number: PCG/UNN/0328) was stored at the herbarium of the Department for further reference. The stem bark was chopped, properly washed with clean water, and air dried under shade at 25 °C for 14 days. The dried stem bark was pulverized to a 1 mm mesh size using a mechanical grinder.

Microorganisms

The type cultures of bacteria; *Staphylococcus aureus* (ATCC 12600), *Escherichia coli* (ATCC 1175), *Bacillus subtilis* (ATCC 6051), *Pseudomonas aeruginosa* (ATCC 10145) and *Salmonella typhi* (ATCC 19430) and fungus *Candida albicans* (ATCC 18804) were

maintained on nutrient agar and Sabouraud dextrose agar (SDA; Hi Media, India) respectively. The microorganisms were stored in glycerol (10 %) at the Pharmaceutical Microbiology unit, University of Nigeria. The organisms were revived before use to ensure viability. Five clinical strains of *H. pylori* (Hp-Bn1, Hp-Bn2, Hp-Bn3, Hp-Bn4 and Hp-Bn5) were isolated from the gastric biopsy specimens of patients with gastritis and peptic ulcers at a tertiary hospital in Enugu, Nigeria. The isolates were cultured in Columbia agar supplemented with sheep blood (5 %) under microaerophilic conditions. The isolates were characterized using phenotypic (colony morphology, Gram staining and motility test) and biochemical (urease, catalase and oxidase) techniques [14].

Extraction and solvent partition

A 1 kg of coarse powder of the plant material was macerated in 95 % v/v methanol (2 x 5 L) successively for 48 h each at 25 °C with frequent agitation as previously reported [10]. The filtrate was dried at 45 °C under vacuum. A 15 g of the methanol extract (MEBn) was dispersed in 100 mL of 10 % v/v methanol in water, made up to 400 mL and transferred into 1 L separation funnel and was successively partitioned in 400 mL, each of *n*-hexane, ethyl acetate, *n*-butanol and distilled water to yield n-hexane (HFBn, 4.06 g), ethyl acetate (EFBn, 3.05 g), n-butanol (BFBn, 0.64 g) and aqueous (WFBn, 0.17 g) fractions after evaporation under vacuum.

VLC separation of EFBn

A gradient elution technique was employed for vacuum liquid chromatographic (VLC) separation of EFBn according to previous methods with slight modification [15]. A 5 g EFBn was dispersed in 10 mL of dichloromethane (DCM): methanol (1:1) and introduced to a 45 x 5 cm glass column containing 350 g of silica gel. The sample was eluted under pressure with 1 L gradient of DCM in methanol, starting with DCM 100 %, and increasing methanol concentration to methanol: DCM (1:1) and then 100 % methanol to obtain VBn-1 (1.95 g), VBn-2 (0.848 g) and VBn-3 (1.86 g) respectively.

Chromatographic purification of VBn-3

Based on the biological activities, VBn-3 (1.0 g) was chromatographed on a silica gel column and isocratically eluted using 3 L of ethyl acetate (EtOAc)-DCM mix (1:1) [16]. The eluates, collected in 25 L tubes at 20 mins intervals, were analysed by a TLC to obtain four CC fractions when pooled as follows: Tubes 18-39 (VBn-3a, 0.11 g), tubes 43-58 (VBn-3b, 0.50 g), tubes 61-76 (VBn-3c, 0.08 g), tubes 80-97 (VBn-3d, 0.60 g). The VBn-3d (0.5 g) was further subjected to a silica CC elution with I L ethyl acetate: MeOH (9.5:0.5). The eluates (in 10 mL tubes) were collected at 15 min intervals and analysed by TLC to furnish a (brown viscous mass (VBn-3d3, 280 mg) and two other fractions, VBn-3d1 and VBn-3d2.

Phytochemical tests

The bioactive samples were tested for the presence of alkaloids, terpenoids, flavonoids, saponins, tannins and glycosides using standard methods [1].

Antimicrobial activity assay

In vitro antimicrobial activity of *B. nigritiana* was evaluated by agar well diffusion technique using Mueller-Hinton Agar (MHA) and Sabouraud dextrose agar (SDA) as media for bacteria and fungi, respectively. The agar media were seeded with 15 mL of microbial suspension of the test organism (10^7 cfu/mL) in sterile Petri dishes and allowed to solidify (one microorganism per agar plate) [17]. Wells of 6.0 mm were created with cork borer in the agar at equidistant points. Each well was filled with 100 µL of test samples (extract, VLC fractions or CC sub-fractions; 20 µg/mL) in dimethylsulphoxide (DMSO), standard control (gentamicin or nystatin, 1.0 µg/mL) and DMSO (negative control) for each organism. After 1 h, the plates were incubated at 37 °C/24 h and 28 °C/48 h for bacteria and fungi, respectively. The plates were measured for zones of inhibition using calipers.

Anti-ulcer assay

Anti-H. pylori sensitivity test

Anti-*H. pylori* sensitivity screening was evaluated using the agar diffusion method [18]. The cell culture was diluted to produce a concentration of 0.5 to 1.0 X 10^6 CFU/mL for the assay. The test samples included MEBn, HFBn, EFBn, BFBn, VBn-1, VBn-2, VBn-3, VBn-3a, VBn-3b, VBn-3c, VBn-3d, VBn-3d1, VBn-3d2 and VBn-3d3, while the test organisms were Hp-Bn1, Hp-Bn2, Hp-Bn3, Hp-Bn4, Hp-Bn5 and a type culture, ATCC 45903 as control. *In vitro* antiulcer activity of *B. nigritiana* was evaluated by agar well diffusion technique using Columbia agar as media. The agar was seeded with 15 mL of microbial suspension of the test organism (10^6 cfu/mL) in sterile Petri dishes and allowed to solidify. Wells of 6.0 mm were created with cork borer in the agar at equidistant points. Each well was filled

with 100 µL of test samples prepared in DMSO and positive control (inoculated media but not treated), negative control (non-inoculated media) and DMSO control. After 2 h, the plates were incubated in a microaerophilic system (5 % O_2 and 10 % CO_2) at 37 °C for 72 h. The plates were measured for zones of inhibition using calipers. The inhibition zone diameters (IZDs) were categorized into 0, 1+, 2+, 3+ and 4+ for IZDs of < 6, 6 – 10, 11 – 15, 16 – 20 and > 20 mm, respectively.

Evaluation of minimum inhibitory concentration

The minimum inhibitory concentrations (MICs) of test substances were evaluated against the *H. pylori* strains and other microorganisms using a broth microdilution technique [18]. The test samples were those that showed antimicrobial effects in the determination of the IZDs. Each test sample (≤ 50 µg/mL), prepared in DMSO, was serially diluted in 2-fold increments using supplemented Columbia agar (*H. pylori*), MHA (bacteria), or SDA (fungi). A 100 µL of cell culture (10^6 cfu/mL) was introduced into the broth and incubated under the microaerophilic system at 37 °C/72 h, 37 °C/24 h and 28 °C/48 h for *H. pylori*, bacteria and fungi, respectively. The MIC was determined as the lowest test sample concentration with a clear suspension or no growth indicator [19].

Statistical analysis

Data were expressed as a mean value of IZD ± standard error of the mean (SEM; n=3). A one-way analysis of variance (ANOVA) was employed to test for the statistical significance of treatment groups compared with the control via post hoc, 2-sided Dunnett's test. A $p < 0.05$ was considered significant in each case.

RESULTS

Extraction yield of *B. nigritiana*

The cold maceration of 1 kg of *B. nigritiana* stem bark in methanol yielded 46 g of extract, representing 4.6 % w/w. Solvent-solvent partitioning in *n*-hexane, EtOAc, *n*-butanol and water yielded 12.45, 9.35, 1.96 and 0.521 g, representing 1.25, 0.94, 0.196 and 0.052 %w/1000 g dried sample of *n*-hexane, EtOAc, *n*-butanol and aqueous fractions, respectively. The VLC fractionation of 9.35 g ethyl acetate soluble in graded methanol: DCM combinations yielded three fractions of 3.64, 1.59 and 3.47 g, representing 0.36, 0.16 and 0.35 %w/ 1000 g of dried stem sample.

Phytochemical contents

Following a CC separation of the most active VLC fraction (VBn-3), four subfractions were obtained, which on further CC separation yielded a bioactive light brown viscous liquid and two other subfractions. Phytochemical analysis of MBn, EFBn, VBn-3, VBn-3d and VBn-3d3 showed consistent presence of alkaloids and flavonoids.

HPLC and liquid chromatographic-mass spectrometry findings

Two compounds were identified from the most active semi-purified sub-fraction (VBn-3d3) of the EtOAc soluble fraction. The compounds (Figure 1) were assigned based on the HPLC-DADA/+ESI-QQTOF-MS (Figure 2) properties, and comparison of the UV spectrum (showing the retention times, tR and λ_{max} data) with the literature. The compounds were identified as septicine (**1**) and hymeglusin (**2**).

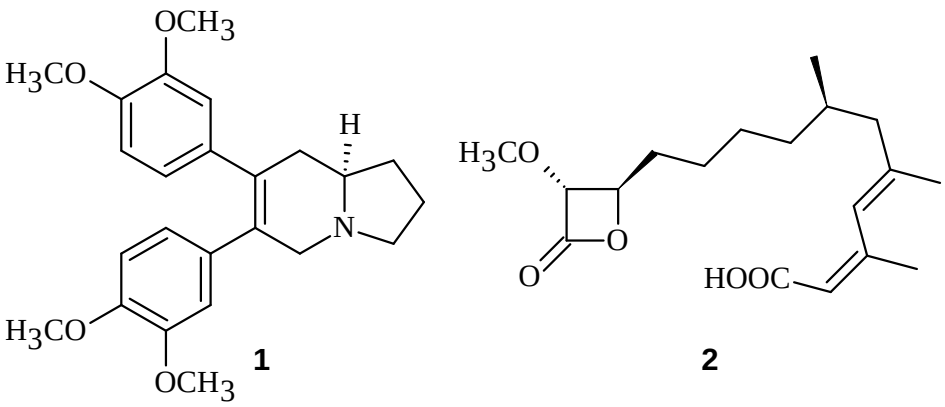

Figure 1: Chemical structures of identified compounds from Vbn-3d3

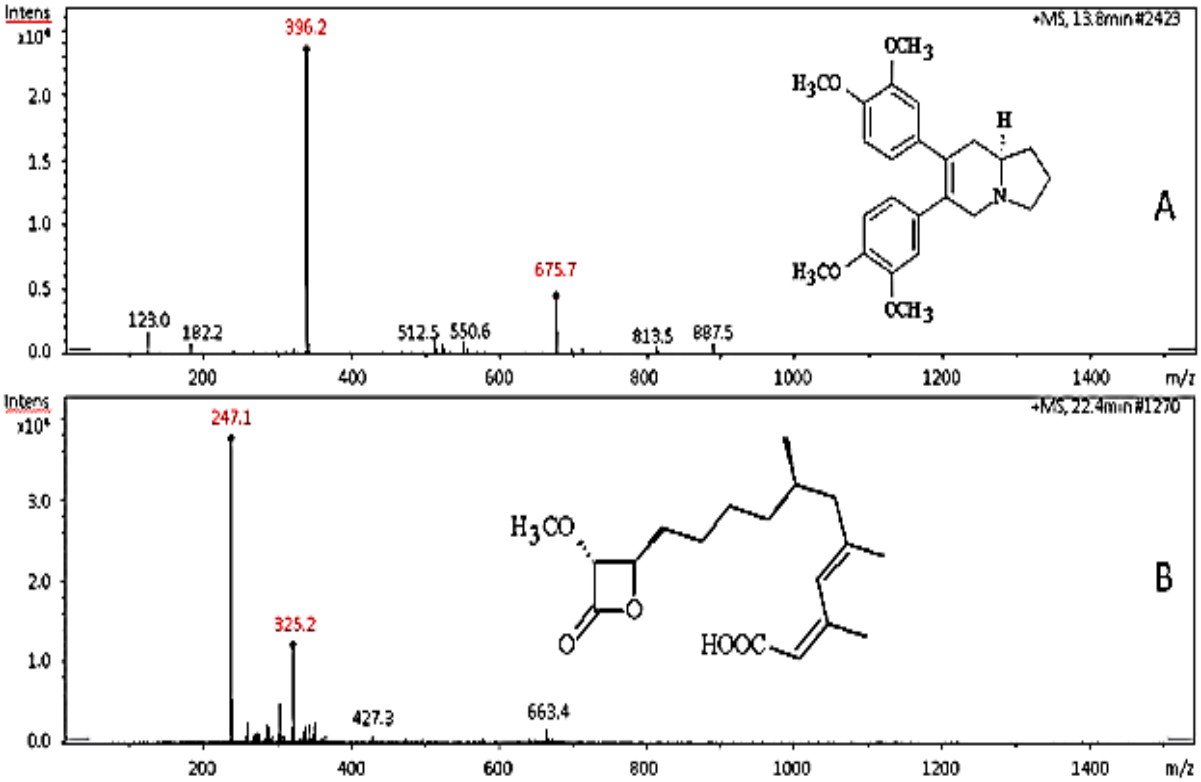

Figure 2: Base peak chromatogram of septicine (A) and hymeglusin (B)

Table 1: Inhibition zone diameters of *B. nigritiana* against *H. pylori*

Test sample/	**IZD (mm)**					
***H. pylori* strain**	***Hp-Bn1***	***Hp-Bn2***	***Hp-Bn3***	***Hp-Bn4***	***Hp-Bn5***	***ATCC 45903***
DMSO	0	0	0	0	0	0
MEBn	2+	3+	3+	3+	2+	2+
HFBn	0	0	1+	0	1+	0
EFBn	1+	3+	3+	3+	2+	3+
BTBn	1+	2+	0	0	1+	1+
WFBn	0	1+	1+	0	1+	0
VBn-1	0	1+	0	0	0	0
VBn-2	0	1+	0	1+	1+	0
VBn-3	1+	3+	4+	3+	2+	4+
VBn-3a	1+	0	1+	1+	2+	0
VBn-3b	0	0	0	0	1+	1+
VBn-3c	0	0	1+	1+	1+	1+
VBn-3d	2+	4+	3+	3+	1+	3+
VBn-3d1	1+	1+	1+	0	1+	0
VBn-3d2	0	1+	1+	2+	1+	1+
VBn-3d3	1+	4+	4+	4+	1+	4+
Clarithromycin	4+	4+	4+	4+	4+	4+

Septicine was dereplicated from the HPLC-MS chromatogram of VBn-3d3. The RF (EtOAc: MeOH: $CHCl_3$ 9.5:4:0.5) = 0.88, molecular formula $C_{24}H_{29}NO_4$, molecular weight 395.2914 Da, tR 13.8 min., UV^{MeOH} 240 nm' Hymeglusin identified as a constituent of VBn-3d3 by dereplication. The TLC RF (EtOAc: MeOH: $CHCl_3$ 9.5:4:0.5) = 0.64, molecular formula $C_{18}H_{28}O_5$, molecular weight 324.2119 Da, tR 22.4 min., UV^{MeOH} 235 nm

Antimicrobial and anti-*H. pylori* activities of *B. nigritiana*

Anti-H. pylori spectrum of B. nigritiana

The susceptibility pattern of clinical isolates of *H. pylori* to the *B. nigritiana* extract, VLC fractions and CC sub-fraction (20 µg/mL) showed that three strains and the type culture were the most susceptible to most of the tested samples (Table 1). The MEBn elicited IZD ≥ 11 mm against all the tested strains of *H. pylori*. Strains HP-Bn2,

Hp-Bn3, Hp-Bn4 and the typed culture showed the highest sensitivity to MEBn, EFBn, VBn-3, VBn-3d and VBn-3d3 with the IZD ≥ 11 mm, peaking at > 20 mm. The compounds **1** and **2** - enriched test sample, like the standard drug, caused strong inhibition of the three strains and type culture with IZDs > 20 mm.

MIC of *B. nigritiana* against *H. pylori* strains

The high susceptibility of the clinical isolates of *H. pylori* (HP-Bn2, Hp-Bn3, Hp-Bn4 and the typed culture) to test samples (MEBn, EFBn, VBn-3, VBn-3d and VBn-3d3) in a bioactivity-guided separation and fractionation of the extract motivated the determination of the MICs. The results showed that MEBn was equipotent against the tested isolates with MIC of 12.5 µg/mL (Table 2). There was a significant decrease in the MIC of hymeglusin- and septicine-enriched fractions with MIC ≤ 1.5625 µg/mL.

Antimicrobial spectrum of *B. nigritiana*

The antimicrobial spectra of *B. nigritiana* extract, fractions and sub-fractions against selected Gram-positive and negative bacteria and fungi are shown in Table 3. The results showed that MEBn, EFBn, VBn-3, VBn-3d and VBn-3d3 consistently inhibited the growth of *S. typhi, E. coli*, *P. aeruginosa* and *C. albicans* more than *S. aureus* and *B. subtilis*. Generally, *P. aeruginosa* and *E. coli* were the most susceptible to all the tested bioactive samples. The septicine- and hymeglusin-enriched fraction significantly ($p < 0.05$) inhibited the growth of *E. coli*, *P. aeruginosa, S. typhi* and *C. albicans* with IZDs of 15.3, 18.2, 15.8 and 16.9 mm, respectively.

Table 2: MICs of *B. nigritiana* against *H. pylori*

Test sample/ *H. pylori* strain	MIC (µg/mL)			
	Hp-Bn2	***Hp-Bn3***	***Hp-Bn4***	***ATCC 45903***
DMSO	-	-	-	-
MEBn	12.5	12.5	12.5	12.5
EFBn	12.5	6.25	12.5	6.25
VBn-3	6.25	6.25	12.5	12.5
VBn-3d	6.25	3.125	6.25	6.25
VBn-3d3	0.3906	1.5625	0.78125	0.3906
clarithromycin	0.097	0.097	0.097	0.097

Minimum inhibitory concentration of *B. nigritiana* against other microorganisms

The results of the MIC of the tested bioactive samples against *E. coli*, *S. typhi* and *P. aeruginosa* recorded varying MIC values (Table 4). The MEBn, EFBn, VBn-3 and VBn-3d showed equipotent activity against *P. aeruginosa* while the hymeglusin- and septicine-enriched fraction, VBn-3d3, showed the lowest MIC of 1.5125 µg/mL against *P. aeruginosa*, and 3.125 µg/mL against *E. coli* and *C. albicans*.

DISCUSSION

The emergence of antimicrobial resistance has resulted in a public health crisis, tending to reverse decades of progress achieved in antimicrobial therapy. Several resistant strains of *H. pylori,* such as clarithromycin-, metronidazole-, levofloxacin- and multidrug-resistant *H. pylori* have emerged, rendering the standard triple-drug therapy ineffective and increasing the burden of peptic ulcer disease [4-9].

Table 3: IZDs of *B. nigritiana* against selected microorganisms

Test sample/ microorganisms	Inhibition Zone Diameters (mm)					
	P. aeruginosa	***S. aureus***	***E. coli***	***B. subtilis***	***S. typhi***	***C. albicans***
DMSO	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
MEBn	14.3±0.3	7.9±0.1	12.6±0.6	7.1±0.5	11.9±0.8	10.2±0.2
HFBn	0.0±0.0	1.3±0.1	2.0±0.3	1.2±0.1	2.1±0.5	1.3±0.1
EFBn	14.8±0.8	5.2±0.2	13.9±0.4	6.0±0.9	10.2±0.3	13.4±0.3
BTBn	2.2±0.8	3.8±0.0	0.0±0.0	0.0±0.0	11.9±0,6	3.2±0.8
WFBn	3.3±0,7	1.5±0.7	0.0±0.0	0.5±0.0	1.1±0.9	0.8±0.1
VBn-1	2.9±1.2	3.8±0.9	0.8±0.0	1.1±0.4	3.2±0.2	0.2±0.0
VBn-2	4.9±0.9	4.4±0.2	0.5±0.1	9.2±0.2	1.9±0.9	7.2±0.2
VBn-3	16.8±1.1	6.4±0.9	11.8±0.9	3.4±0.0	6.8±0.9	10.0±0.0
VBn-3a	6.0±0.2	2.2±0.3	3.0±0.2	1.6±0.1	0.0±0.0	4.1±0,2
VBn-3b	3.9±1.0	1.8±0.4	2.8±0.1	2.1±0.4	0.8±0.0	0.0±0.0
VBn-3c	0.0±0.0	3.1±0.3	0.8±0.1	2.5±0.1	4.5±0.5	0.0±0.0
VBn-3d	15.7±0.5	7.4±0.8	13.8±0.3	3.2±0.4	11.9±1.3	12.3±0.4
VBn-3d1	3.8±0.7	4.3±0.1	0.0±0.0	2.2±0.2	0.0±0.0	0.0±0.0
VBn-3d2	2.2±0.3	6.3±0.2	1.2±0.2	2.8±0.3	1.7±0.1	1.7±0.1
VBn-3d3	18.2±0.2	6.4±0.2	15.3±2.1	4.2±0.3	15.8±1.1	16.9±0.3
Standards	25.1±0.5	25.4±0.6	23.9±0.2	20.6±0.8	24.8±0.4	22.7±0.1*

Note: *standard for *C. albicans* (nystatin), standard for other microorganisms (gentamicin)

Table 4: MIC of *B. nigritiana* against selected microorganisms

Test sample/ microorganisms	MIC (µg/mL)			
	P. aeruginosa	***E. coli***	***S. typhi***	***C. albicans***
DMSO	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
MEBn	12.5	6.25	6.25	12.5
EFBn	12.5	3.125	6.25	6.25
VBn-3	12.5	6.25	6.25	6.25
VBn-3d	12.5	6.25	12.5	3.125
VBn-3d3	1.5625	3.125	6.25	3.125
Standards	0.097	0.195	0.097	0.195*

Note: *Standard for *C. albicans* = nystatin; standard for other microorganisms = gentamicin

There has been a paradigm shift to natural products for the development of herbal remedies [9]. *Barteria nigritiana* provides a strong alternative considering the ethnomedicinal data, which complements other validated biological activities of the plant extracts such as the *anti-inflammatory, antioxidant, antifungal, analgesic and antimicrobial activities* [10-12].

Extraction methods and solvents play significant roles in the biological activity-guided isolation of secondary metabolites. The exhaustive cold maceration extraction of *B. nigritiana* stem bark using methanol yielded 4.6 % w/w extract, which on partitioning furnished 1.25, 0.94 and 0.248 of non-polar, mid-polar and polar constituents respectively. These separation methods yielded varying concentrations of phytochemicals, which affected the bioactivity of the tested samples in this study. Previous studies reported the presence of tannins, flavonoids, alkaloids, saponins, cyanogenic glycosides and oxalates, which have been strongly associated with its pharmacological activities [10,11].

Biological activity-guided fractionation and separation of the methanol extract using chromatographic techniques yielded a bioactive fraction containing septicine and hymeglusin, identified using HPLC-DAD and LC-MS techniques [20]. Hymeglusin is a β-lactone antibiotic that was first isolated from the culture broth of *Scopulariopsis candelabrum* strain F-244 and has not been reported in higher plants, while septicine, an alkaloid with a hexahydroindolizine core substituted with two 3,4-dimethoxy phenyl groups, has been discovered in *Tylophora indica, Gelsemium elegans* and *Ficus septica* [21]. However, their identification from the VBn-3d3 fraction of *B. nigritiana* in this study was from major peaks that matched the retention time, fragmentation patterns and/or UV spectra of hymeglusin and septicine. These structural features of hymeglusin, septicine and other unidentified constituents of VBn-3d3 could be responsible for the antimicrobial and anti-ulcer properties of *B. nigritiana*.

Ethnomedicinal data revealed the importance of *B. nigritiana* in the management of peptic ulcers and some infectious diseases, which have also been associated with anti-inflammatory and wound healing activities [9]. In this study, the antimicrobial activity of the hymeglusin- and septicine-enriched sub-fraction showed significant antibacterial activity against *H. pylori*, *E. coli*, *P. aeruginosa* and *S. typhi* as well as antifungal activity against *C. albicans* with minimum IZDs of 15 mm in an agar diffusion assay which were comparable with clarithromycin or nystatin controls. It is imperative to point out that the most significant effect of VBn-3d3 was against the Gram-negative bacteria and *C. albicans*. This suggests less involvement of the inhibition of the peptidoglycan cell wall of the bacteria in its antibacterial properties [3,15,17]. The anti-*H. pylori* activity and the strong inhibitory effect on gram-negative bacteria as well as *C. albicans,* demonstrates the extended spectrum of activity of the hymeglusin and septicine-enriched sub-fraction of *B. nigritiana* and its complementary roles in the treatment of PUD.

The strong antimicrobial activities of septicine/hymeglusin-enriched tested sample could be hypothesized considering the chemotype resemblance of the four-membered β-lactone moiety of hymeglusin with the β-lactam antibiotics. Hymeglusin, an HMG-CoA synthase inhibitor, has been isolated from *Scopulariopsis candida* strain F-244 and ethyl acetate extract of *Fusarium solani* H915 and *Fomitiporia hartigii* with demonstrated antifungal activity against *C. albicans* (MIC, 12.5 and 4.2 µg/mL), *Penicillium herquei* (MIC, 25 µg/mL), *Pyricularia oryzae* (MIC, 6.25 µg/mL), *Pestalotiopsis theae* (25 µg/mL) and *Colletotrichum gloeosporioides* (25 µg/mL) [21,22]. The significantly lower MIC obtained in this study (3.125 µg/mL vs 12.5 µg/mL or 4.2 µg/mL against *C. albicans*) could point to the additive effect of septicine and other unidentified constituents of VBn-3d3, which, however, could not be ascertained in this study. It was observed in a previous study that hymeglusin isolated from *Fomitiporia hartigii* did

not inhibit the growth of *E. coli*, *B. subtilis*, and *P. aeruginosa* at the tested concentration (67 µg/mL) [23]. This study, therefore, points to the additive antimicrobial effect of other constituents within the plant such as septicine; which has been reported to inhibit bacteria (*B. subtilis*, *S. aureus*, *Micrococcus luteus* and *P. aeruginosa*) and fungi (*Aspergillus niger*, *Aspergillus fumigatus* and *Trichoderma viridi*) in another study [21].

This study demonstrated the *in vitro* anti-*H. pylori* and antimicrobial properties of *B. nigritiana* extract and identified hymeglusin and septicine via dereplication of the most active sub-fraction via CC purification. The sub-fraction demonstrated significant activities against the tested microorganisms implicated in PUD and diverse infectious diseases, which could be attributed to the hymeglusin and septicine constituents. Despite the significant chromatographic purification of the VLC and CC fractions, the constituents of the most active fractions/sub-fractions were not isolated, characterized, or quantified in this study. The reported activity data could have resulted from synergism or antagonism of the phytochemical constituents and these activities have complemented the anti-inflammatory, antioxidant, antifungal, analgesic and antimicrobial activities of *B. nigritiana* stem bark. The isolation, characterization and quantification of hymeglusin, septicine and other constituents of VBn-3d3 are currently underway.

CONCLUSION

The anti-*H. pylori* and antimicrobial activities of *B. nigritiana* stem bark have been reported. Two important compounds, hymeglusin and septicine, have been identified by HPLC-DAD/MS from the chromatographically separated and purified bioactive sub-fraction. The hymeglusin/septicine-enriched fraction shows significant inhibition of *H. pylori, C. albicans, E. coli*, *P. aeruginosa* and *S. typhi* and is therefore a novel lead compound for the development of suitable therapeutic agents for the management of PUD and other infectious diseases.

DECLARATIONS

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Ethical approval

None required.

Availability of data and materials

The datasets used and/or analyzed during the current study are contained in this manuscript.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Concept and design- Charles Nnadi, Calister Ugwu and Edith Diovu; Data acquisition- Edith Diovu and Oboma Okonta; data analysis/interpretation- Charles Nnadi and Edith Diovu; Drafting manuscript- Oboma Okonta and Chinwe Onah; critical revision of manuscript- Charles Nnadi and Edith Diovu; statistical analysis- Edith Diovu and Chinwe Onah; final approval- all authors.

Use of Artificial intelligence/Large language models

We declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

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