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Isolation and *in-vitro* assessment of two indole alkaloids from *Pericopsis laxiflora* leaf extract for their antibacterial potentials

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Abstract

Traditionally, *Pericopsis laxiflora* leaf is useful in the treatment of fevers and bacterial infections in different cultures. Qualitative and quantitative screening of the 70% ethanol leaf extract of the plant (P) revealed a strong presence of alkaloids, which were extracted and separated out as crude alkaloids (Pa). Fractionation of portion Pa using various chromatographic techniques led to the isolation and purification of two indole alkaloids whose structures were elucidated based on physical, chemical and spectral data in comparison with literature data. They were identified as 1*H*-indol-5-ol,3-[2'-(dimethylamino) ethyl (Pa1a) and 7-methoxy-1,9-dimethyl-9*H*-pyrido[3,4-b] indole (Pa2a1). *In-vitro* antibacterial and anti-typhoid assessment of P, Pa, Pa1a and Pa2a1 using the agar dilution method revealed that extract, P and alkaloidal portion, Pa at 1000 and 500 μg/cm^3 respectively displayed broad-spectrum potentials against the bacterial, typhoid and paratyphoid strains, though at higher concentrations when compared with Ciprofloxacin. For the isolated compounds, the β-carboline derivative (compound Pa_2a1) exhibited better activity than the hydroxyl tryptamine derivative (compound Pa_1a) both at 100 μg/cm^3. Generally, all test compounds (P, Pa, Pa1a and Pa2a1) displayed significant broad-spectrum antibacterial and anti-typhoid potentials, justifying the use of the plant traditionally in the treatment of bacterial and typhoid infections.

Keywords: Antibacterial, ethanol leaf extract, indole alkaloids, *Pericopsis laxiflora*

Introduction

Natural products, such as medicinal plants provide a rich source of various bioactive phytochemicals that have become an important source of new anti-microbial agents. This has led to an increased interest in the extraction, isolation, purification and structural elucidation along with biological testing of such compounds with the aim of generating novel and potent bioactive compounds, such as alkaloids. Alkaloids exist in various medicinal plants and are a common occurrence in numerous biological fluids; including the skin secretion of many amphibians [1].The United States Food and Drug Agency (USFDA) had earlier revealed that 59% of unique-small molecule drugs contain at least a nitrogen heterocycle [2]. Indole alkaloids, one of the largest and chemically intriguing classes of the alkaloids possess the indole ring moiety and are generally classified as isoprenoids (e.g. tryptamines, ergot alkaloids) and non-isoprenoids (e.g. simple derivatives of β-carboline) [3]. Alkaloids having the indole nucleus are known to be therapeutically active agents occupying a great place in drug design and development [4]. The search for new and alternative antibacterial agents from this class and other classes of phytochemicals has grown tremendously in recent years because of the increasing resistance of pathogenic bacteria to existing antibiotics and the appearance of multidrug resistant strains to commonly used antibiotics [5].

Pericopsis laxiflora (Benth. ex Baker) van meeuwen is a perennial, deciduous shrub or tree belonging to the family Papilionaceae/Fabaceae/Leguminoseae. It is commonly known as Satin wood (English), Makarfo (Hausa), Abua-ocha (Igbo) and Ayan/Sedun (Yoruba). Ethnomedicinally, the usefulness of the various organs of the plant in the treatment of hemorrhoids, headache, rheumatism, arthritis, abdominal pains, sore throat, eye problems, skin diseases, teething pains in children and other feverish conditions in different cultures is well documented [6]. It is also useful as an antidote against snakebite, intestinal worms, and guinea worms. Other uses include, in the treatment of syphilis, diarrhea, dysentery and typhoid fever [7]. It is also useful as an antibacterial, antimalarial and anti-parasitic agent [6, 8 - 9]. Biologically, the potentials of the plant as an anti-trypanosomal [10] and antimicrobial agent [9, 19, 20, 21] has been reported. Phytochemical screening of various extracts of the plant revealed the presence of alkaloids, cardiac glycosides, sterols, polyterpenes and polyphenolics [11]. Three alkaloids, namely; 5,8-dimethyl-1,2,3,4-tetrahydro-9-acridinamine,4,6-diaminopyrazolo [3,4-d] pyrimidine and 1,2,3,4,5,6-hexahydro-1,5-methano-8*H*-pyrido[1,2-a]diazocin-8-one has been isolated and characterized from the stem bark of the plant [12]. The continuous search for more bioactive alkaloids with antibacterial and anti-typhoid potentials from the plant, has therefore, prompted the isolation and *in-vitro* assessment of two indole alkaloids from the leaves of *Pericopsis laxiflora*.

Materials and Methods

Plant collection and identification

Fresh leaves of *Pericopsis laxiflora* were collected in the month of August 2018 from a farmland at Gwada village, Shiroro Local Government Area of Niger State, Nigeria. Plant was identified by Dr. (Mrs.) Jemilat Ibrahim of the Department of Medicinal Plant Research and Development of National Institute for Pharmaceutical Research and Development, NIPRD, Idu, Abuja.

Extraction of plant material

Air-dried pulverized *P. laxiflora* leaf (1 kg) was extracted exhaustively with 70% ethanol for a week by macerating with continuous shaking using a flask shaker. Extract was concentrated *in-vacuo* and brought to dryness over a water bath. Extract was labeled 'crude ethanol extract of leaves of *P. laxiflora*', P (brown gummy mass, 30.3% yield).

Test for the presence of alkaloids

The crude ethanol extract, P, (0.5 g) was hydrolyzed with 2% aqueous HCl over a steam bath for 5 minutes and the mixture filtered. The resulting filtrate (1 cm^3 each) was treated separately with 2 drops each of Hager's reagent (1 g of picric acid in 100 cm^3 of H_2O) and tannic acid. Formation of a yellow- and buff-colored precipitate respectively in each tube is indicative of the presence of alkaloids [13].

Extraction of crude (total) alkaloidal content

The total alkaloids present in crude 70 % ethanol leaf extract of *P. laxiflora*, P, was extracted using the method of Wang *et al*. [14]. The extract was suspended in 5% HCl and partitioned with dichloromethane; the acidic (aqueous) portion was then made basic (pH 9) with aq. NH_3 and further re-extracted with ethyl acetate. The EtOAc layer was dried under reduced pressure to yield an ethyl acetate-soluble portion coded 'crude alkaloids of the leaves of *P. laxiflora'*, Pa, (brownish black solid mass, 13.6%).The presence of alkaloids in the portion was confirmed by the precipitation of Hager's reagent and tannic acid solutions in separate test tubes.

Isolation and purification of two alkaloids

The crude alkaloidal portion, Pa (4 g) was subjected to a column packed with 150 g of alumina and eluted sequentially with varying proportions of increasing polarity of $CHCl_3$: MeOH (100:0 to 0:100). Eluents were collected in aliquots of 50 cm^3 and similar fractions pooled based on their thin layer chromatographic profile when sprayed with Dragendorff's reagent (potassium bismuth

iodide solution). This yielded six major fractions that were concentrated to dryness and coded Pa_1 – Pa_6. Further chromatographic purification of fraction Pa_1 (950 mg, 30 g alumina, increasing polarity of $CHCl_3$: MeOH, 10 cm^3 aliquots of eluents) gave rise to a major sub-fraction, which on further purification yielded a white crystalline solid (coded Pa_1a, compound I). Purification of fraction Pa_2 (650 mg, 30 g alumina, increasing polarity of EtOAc: MeOH, 20 cm^3 aliquots of eluents) gave rise to three major sub-fractions, Pa_2a–Pa_2c. Further purification of sub-fraction Pa_2a (88 mg, 30 g alumina, increasing polarity of $CHCl_3$: MeOH, 10 cm^3 aliquots of eluents) afforded a cream-colored amorphous solid (coded Pa_2a1, compound II).

Test for the presence of indole alkaloids [15]

(i) Van Urk's test: Each of compound I and II (0.1 g) was treated with p-dimethylaminobenzaldehyde (PDAB) reagent (Ehrlich's reagent) in 15 % H_2SO_4 containing traces of $FeCl_3$. A violet color, which changes to purple, indicates the presence of the indole alkaloids.

(ii) Marquis's test: A mixture of 100 cm^3 of 90 % H_2SO_4, 5 cm^3 of 5 % methanal and 5 % methanol were added to 0.1 g each of compound I and II. A color change to yellowish-brown or yellowish-green is taken as evidence for the presence of indole alkaloids.

(iii)To 0.1 g each of compound I and II, 10 % of $K_2Cr_2O_7$ in conc. H_2SO_4 was added, a color change to yellow or green indicates the presence of indole alkaloids.

Characterization of compounds I and II

Melting points were determined using Gallenkamp melting point apparatus and were uncorrected. IR and UV spectra of both compounds were recorded in MeOH using FT-IR 8400 spectrometer and T60 UV-Visible spectrophotometer respectively. ^{1}H-NMR, ^{13}C-NMR and DEPT-135 spectra were recorded in $CDCl_3$ on Varian Gemini spectrometer operating at 400 MHz, while, GC and MS were recorded on Agilent technologies 7890A and Agilent technologies 5975C respectively.

***In-vitro* antibacterial assay of test compounds**

The antibacterial activity of crude ethanol extract, P, its alkaloidal portion, Pa and isolated compounds Pa_1a and Pa_2a1 were tested against overnight cultures of Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi* A, B and C). All organisms were collected in a lyophilized form from the Vaccine Laboratory, Department of Microbiology, Federal University of Technology, Minna, Niger State, Nigeria. The viability test for each organism was carried out by resuscitating each microorganism on Mueller Hinton agar (MHA) and incubating at 37^{o}C for 24 h. A 0.2 cm^3 of each 24 h culture was dispensed into 20 cm^3 of Mueller Hinton broth (MHB) and incubated for 5 h at 37^{o}C to obtain 1 x 10^6 cfu/cm^3 of each test organism. The agar dilution method was adopted for the susceptibility testing [16]. 5 mg of extract P, 2.5 mg of portion Pa and 0.5 mg each of Pa_1a and Pa_2a1 were separately reconstituted in 5 cm^3 of sterile distilled water each to afford 1000, 500,100 and 100 μg/cm^3 respectively. 1 cm^3 of each reconstituted mixture was then transferred to sterile Petri dishes containing 19 cm^3 of molten MHA and allowed to set at room temperature. A loopful of each standardized bacterial culture was streaked unto each solidified agar plate. Plates were prepared in duplicates. Plates for standard control (Ciprofloxacin, 0.05 mg, 10 μg/cm^3), extract sterility control (ESC), organism viability control (OVC) and medium sterility control (MSC) were also prepared. All plates were incubated aerobically at 37^{o}C for 24h and checked for activity (+)/no activity (-).

Results and Discussion

All test compounds (extract, portion, fractions, sub-fractions and isolated compounds) gave yellow- and buff-colored precipitate each with Hager's reagent and tannic acid solution

respectively, indicating the presence of alkaloids in the plant [13]. Treatment of the alkaloidal portion, Pa, with PDAB, Marquis's reagent and a solution of acidified potassium dichromate revealed purple, yellowish-green and green colorations respectively, indicating strong presence indole alkaloids [15]. Some of the spots on TLC ($CHCl_3$: MeOH: NH_3, 9: 1: 0.1) revealed brownish colors under UV light (254 and 366 nm) and on spraying with Dragendorff's reagent, which is typical of indole alkaloids [15].

Characterization of compounds

Compound I (Pa_1a): White crystalline solid; 23 mg; melting point 144-146ºC; soluble in MeOH and EtOH, partially soluble in Et_2O, $CHCl_3$ and EtOAc and insoluble in H_2O, indicating it is a mid-polar compound. TLC, $CHCl_3$: MeOH: NH_3 (9:1:0.1, single spotted, R_f 0.67). Crystals gave positive test for indole alkaloids [15].

IR (ν cm^{-1}): 3528 (Aryl O-H stretching vibration), 3471(N-H stretching vibration), 1375 (Aryl O-H bending vibration), 1249 (C-N stretching vibration) and 1182 (Aryl C-OH stretching vibration)

UV (λmax, nm): 272 (Aryl-OH) and 300 (indole ring) [17]

MS (m/z, fragment ions): 204, $M^+[C_{12}H_{16}N_2O]^+$, 159$[C_{10}H_9NO]^+$, 146$[C_9H_8NO]^+$, 130$[C_9H_6O]^+$, 117$[C_8H_5O]^+$, 103$[C_7H_3O]^+$, 91$[C_6H_3O]^+$, 77 $[C_6H_5]^+$, 58; base peak $[C_3H_8N]^+$, 42$[C_3H_6]^+$, 41$[C_3H_5]^+$& 39$[C_3H_3]^+$. The MS revealed the molecular mass and molecular formula of the compound to be 204 $gmol^{-1}$ and $C_{12}H_{16}N_2O$ respectively.

^{1}H-NMR (δ ppm): revealed the presence of highly de-shielded doublet peaks each at low-field δ 10.1 and δ 10.2, an indication of protons attached to highly electronegative nitrogen and oxygen respectively (positions 1 and 5). Other peaks at downfield δ 7.39, 6.91, 6.99 and 6.60 are attributable to neighboring group effects resulting from such protons been attached to electronegative atoms (positions 2, 4, 7 and 6 respectively). The up-field sharp singlet at δ 2.20 is indicative of two methyl substituents bonded to a nitrogen heteroatom (position 3') as shown in Table 1.

^{13}C-NMR (δ ppm): revealed eleven proton-decoupled peaks of almost same intensities, with the exception of peak at δ 47.1 that was of greater intensity, confirming the presence of two methyl substituents in the same environment (position 3') as shown in Table 1

DEPT (δ ppm): of the eleven peaks observed in the ^{13}C-NMR spectrum, four were quaternary (disappeared in the spectrum), four were methine (above in the spectrum), two were methylene (below in the spectrum), while two were methyl (above in the spectrum, superimposed on one another) (Table 1).

Therefore, based on the physical, chemical and spectral properties obtained for **compound I** in comparison with those reported in literature, the compound was elucidated as 1*H*-indol-5-ol,3[2'(dimethylamino)ethyl]/Bufotenin/Cinobufotenine/Dimethylserotonin/5-hydroxy-N,N-dimethyltryptamine/3-(β-dimethylaminoethyl)-5-hydroxyindole**.** This indole alkaloid and its other derivatives have been reported and isolated from different sources, such as, the Leguminoseae/Fabaceae family [18].

Compound II (Pa_2a1): Cream-colored amorphous solid; 17 mg; melting point 215-221; soluble in MeOH, EtOH, partially soluble in Et_2O, $CHCl_3$ and insoluble in H_2O. TLC, $CHCl_3$: MeOH: NH_3 (9:1:0.1, R_f 0.51). Crystals also gave positive test for indole alkaloids [15].

IR (ν cm^{-1}): 3455 (N-H stretching), 3056 (C-H stretching of benzene), 2935, 2863 (C-H stretching), 1455 (CH_2 bending vibrations), 1172 (C-O-C bending vibrations) and 1140 (N-H in-plane bending)

UV (λmax, nm): 257 & 300; characteristic of an indole skeleton [17, 19].

MS (m/z, fragment ions): 226; base peak; $M^+[C_{14}H_{14}N_2O]^+$, 211$[C_{13}H_{11}N_2O]^+$, 197$[C_{13}H_{11}NO]^+$, 183$[C_{12}H_9NO]^+$, 167$[C_{12}H_9N]^+$, 154$[C_{11}H_8N]^+$, 140$[C_{11}H_8]^+$, 127$[C_{10}H_7]^+$, 113$[C_9H_5]^+$, 98$[C_8H_3]^+$,

75$[C_6H_3]^+$, 63$[C_5H_3]^+$ & 39$[C_3H_3]^+$. The GC-MS revealed the molecular mass and molecular formula of the compound to be 226 $gmol^{-1}$ and $C_{14}H_{14}N_2O$ respectively.

^{1}H-NMR (δ ppm): revealed the presence of de-shielded protons at low-fields δ 8.64, δ 7.86 and δ 8.00, an indication that the H atoms are within or close to an environment where electronegative nitrogen is present (position 3, 4 and 5 respectively). Two close doublet peaks each at δ 6.77 and δ 6.73 indicates the presence of one hydrogen atom each occurring in almost same environment, though with slight neighboring group effects (positions 6 and 8). The up-field sharp singlets at δ 2.88, δ 3.84 and δ 3.80 are indicative of protons of methyl substituents around the molecule (positions 1', 2' and 3') (Table 2).

^{13}C-NMR (δ ppm): revealed fourteen proton de-coupled peaks all of same intensities, indicating the presence of fourteen carbon atoms with the tertiary carbon atoms being the most de-shielded peaks (Table 2).

DEPT **(δ ppm):** of the fourteen peaks observed in the ^{13}C-NMR spectrum, six were quaternary (disappeared in the spectrum), five were methine (above in the spectrum), while three were methyl (above in the spectrum) carbon atoms. No peaks were recorded below (inversed) in the spectrum, indicating the absence of methylene (Table 2).

Therefore, based on physical, chemical and spectral properties obtained for **compound II** in comparison with those reported in literature, it was found to be a β-carboline derivative [17] whose structure was elucidated as 7-methoxy-1,9-dimethyl-9*H*-pyrido [3,4-b] indole/9*H*-pyrido[3,4-b]indole,7-methoxy-1,9-dimethyl/7-methoxy-1,9-dimethyl-β-carboline/9-methylharmine. β-carboline derivatives belong to a group of indole alkaloids, consisting of a pyridine ring fused to an indole ring. They are widespread in nature and have been extracted and characterized from many plant parts [20 - 21].

Table 1: ^{1}H-, ^{13}C- NMR and DEPT-135^o spectral data for compound 1 (Pa_1a) in comparison with literature values*

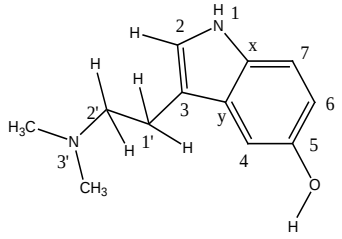
Position	δH (ppm)	δH (ppm)*	δC (ppm)	δC (ppm)*	DEPT (ppm)
1	10.1 (d)	10.1 (d)	-	-	-
2	7.39 (s)	7.47 (s)	123.3	123.0	123.3 (a)
3	-	-	108.0	107.7	D
4	6.91 (d)	6.94 (d)	103.4	103.6	103.4 (a)
5	-	-	150.9	152.4	D
	10.2 (d)	10.07 (d)			
6	6.60 (t)	6.64 (t)	111.8	112.7	111.8 (a)
7	6.99 (t)	7.05 (d)	111.2	112.5	111.2 (a)
x	-	-	130.8	131.9	D
y	-	-	128.2	128.8	D
1'	2.56, 2.48 (t)	2.55 (t)	21.7	22.6	21.7 (b)
2'	2.63, 2.55 (t)	2.63 (t)	63.8	64.2	63.8 (b)
3'	2.20 (ss)	2.25 (ss)	47.1	47.0	47.1 (a)

Keys: -: no peak observed, s: singlet, ss: sharp singlet, d: doublet, t: triplet, a: above, b: below, D: disappeared. *ACD (Product version 15)

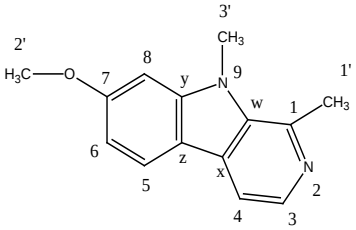
Table 2: ^{1}H-, ^{13}C- NMR and DEPT-135^{o} spectral data for compound II (Pa_2a1)

Position	δH (ppm)	δH (ppm)*	δC (ppm)	δC (ppm)*	DEPT (ppm)
1	-	-	143.0	142.1	D
2	-	-	-	-	-
3	8.64 (d)	8.69 (d)	136.8	137.6	136.8 (a)
4	7.86 (d)	7.90 (d)	112.4	112.6	112.4 (a)
5	8.00 (d)	8.05 (d)	120.9	121.8	120.9 (a)
6	6.77(d)	6.82 (d)	108.9	109.1	108.9 (a)
7	-	-	155.7	156.5	D
8	6.73 (s)	6.79 (s)	97.3	99.6	99.6 (a)
9	-	-	-	-	-
w	-	-	138.1	138.9	D
x	-	-	104.2	103.3	,,
y	-	-	136.4	137.5	,,
z	-	-	117.6	117.4	,,
1’	2.88 (ss)	2.89 (ss)	19.8	20.0	19.8 (a)
2’	3.84 (ss)	3.87 (ss)	55.2	55.8	55.2 (a)
3’	3.80 (ss)	3.82 (ss)	37.4	37.0	37.4 (a)

Keys: no peak observed, s: singlet, ss: sharp singlet, d: doublet, a: above, D: disappeared.



Compound I (Pa_1a)



Compound II (Pa_2a1)

Figure 1: Structures of compounds I and II

Antibacterial Assay

The crude ethanol leaf extract of *P. laxiflora* (P), alkaloidal portion, Pa and isolated compounds (Pa_1a and Pa_2a1) at 1000, 500,100 and 100 μg/cm^3 respectively displayed Ciprofloxacin (Table 3).

The crude extract, P and alkaloidal portion, Pa both exhibited broad-spectrum potentials against the tested strains. They also displayed activity against Gram-negative *S. typhi*, *S. paratyphi* A, B and C (the causal organisms of typhoid fever). For the isolated indole derivatives; Pa_2a1 (compound II) displayed better antibacterial activity than Pa_1a (compound I) both at 100 μg/cm^3. The significant biological activity displayed by the β-carboline alkaloid (compound II) supports the findings of other authors [22 – 23]. Such class of alkaloid reportedly bestows significant biological activities in plants in which they are present in [24].

Table 3: Antibacterial potentials of test compounds against selected pathogenic strains in comparison with Ciprofloxacin (C)

Test bacterial strains	Activity of test compounds (μg/cm^3) against test organisms				
	P (1000)	Pa (500)	Pa_1a (100)	Pa_2a1 (100)	C (10)
B. subtilis	+	+	+	+	+
S. aureus	+	+	+	+	+
P. aeruginosa	+	+	-	+	+
E. coli	+	+	-	-	+
K. pneumoniae	+	-	-	-	+
S. typhi	+	+	-	+	+
S. paratyphi A	+	+	-	+	+
S. paratyphi B	+	+	+	+	+
S. paratyphi C	+	+	+	+	+

Keys: P= crude ethanol extract of *P. laxiflora*; Pa = alkaloidal portion of *P. laxiflora*; Pa_1a and Pa_2a1 = isolated alkaloids; + = activity; - = no activity

Also, the biological activity of cinobufotenine, a quaternary derivative of 5-hydroxytryptamine (compound I) is said to be enhanced when compared with its non-hydroxylated analogues by the presence of the OH group at C-5 [25]. Previous works carried out on antibacterial potentials of alkaloids [2, 26] and their indole derivatives [23-24] have shown that they display good broad-spectrum efficacy, as revealed by this study.

Conclusions

The crude ethanol leaf extract of *Pericopsis laxiflora*, its alkaloidal portion and two of its indole alkaloids; cinobufotenine (a quaternary derivative of 5-hydroxytryptamine) and 9-methylharmine (a β-carboline derivative) all displayed significant broad spectrum antibacterial and antibacterial potentials, justifying the use of the plant traditionally in the treatment of bacterial and typhoid infections.

Declaration of Interest

The authors declare no competing interest anywhere.

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