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Isolation of alkaloids Chabamide I, Piperine and Chingchengenamide: A from *Piper chaba* H. Leaves and their *in vitro* antimicrobial activities

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Abstract

A known alkaloids Chabamide I, Piperine and new alkaloids Chingchengenamide A were isolated from the methanol extract of dichloromethane fraction of *Piper chaba* leaves. The chemical structure of these compounds were established by detailed spectral data and in comparison with their spectral data reported earlier. The study was also performed to determine the *in vitro* antimicrobial activity by disc diffusion method. Among them, the pure compound 1 showed good antimicrobial activity against all of the test micro-organisms, fungi and standard Kanamycin.

Keywords: *Piper chaba*, piperine, chabamide, chingchengenamide, antimicrobial activity, NMR, IR, UV

Introduction

Piper species are widely distributed in the tropical and subtropical regions of the world [1]. The plant *Piper chaba* Hunter (*Piperaceae*) is a climbing, glabrous shrub available in various parts of India and Malay Islands [1]. In Bangladesh, it is grown in plenty in the southern part particularly in Jessore, Khulna, Satkhira and Bagerhat areas [2]. Popularly known as choi, it is used as spices in meat curry and other dishes and believed to have medicinal value in a wide variety of disease conditions including arthritis, asthma, bronchitis and piles. The crude extract was found to possess antibacterial, carminative, expectorant, analgesic, hypotensive and smooth muscle relaxant properties [2-4]. Recently, 80% aqueous acetone extract from the fruit of *Piper chaba* as well as some isolated alkaloids were found to be protective against ethanol and indomethacin induced gastric lesions in rats [5]. Leaves of *Piper chaba* produced a significant anti-inflammatory effect in rat model [6]. Piperine has been isolated from the bark of this plant. In this study, is described the isolation and structure elucidation of one new compounds Chingchengenamide A (which was first isolated from China piper species *Piper ottomoids*) together with isolation of two known compound Chabamide, piperine from the leaves of *Piper chaba*. Along with antimicrobial activity are performed for pure compounds [1-3].

Materials and Methods

General experimental section

Melting points were determined with a Köfeler type melting point apparatus. UV and IR spectra were obtained on a Shimadzu UV-1601, UV spectrophotometer and a Shimadzu IR prestige-21(FT-IR) spectrometer (Organic Research Laboratory, Department of Chemistry, University of, Dhaka). ¹H and ¹³C NMR spectra were recorded in CDCl₃ and CD₃OD on a BRUKER NMR DPX-400 MHz instrument (Bangladesh Council of Scientific and Industrial Research Lab, Dhaka, Bangladesh) at 400 MHz for protons and 100 MHz for carbons using TMS as the internal standard. All NMR spectra were obtained using the standard Bruker software. The ultraviolet lamp was used in visualizing TLC plates was a Mineralight® device, multiband UV-254/366 nm obtained from UVP, Inc., USA. Column chromatography (CC) was carried out over silica gel (230-400 mesh, ASTM, Merck) and TLC was carried out with silica gel 60 pre-coated plates F-254 (Merck).

Plant material

The fresh plant leaves were collected from Kurigram (Bangladesh) on July, 2018. The taxonomy of the plant was confirmed by Department of Botany, University of Dhaka.

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Extraction and isolation

The air dried fresh Leaves (~500 g) of *Piper chaba* were exhaustively extracted with MeOH at room temperature. A solid residue (11.11 g) was obtained after removal of the solvent using rotary vacuum evaporator and fridge dryer. The dried methanol extract was then suspended in H₂O and partitioned by separating funnel successively with *n*-hexane, dichloromethane (DCM), EtOAc and MeOH to give *n*-hexane (2.86 g), DCM (4.0 g), ethyl acetate (0.64 g), methanol (2.7 g) and aqueous (2.4 g) part.

Isolation of compounds 1 to 3 from DCM part

The DCM (~ 4.0 g) part, was subjected to silica gel column eluted with *n*-hexane and increasing the polarity with dichloromethane till 100% dichloromethane then with ethyl acetate and methanol, 67 fractions were collected (100 ml each), the significant fractions were then repeated CC(column chromatography) on silica gel eluted with *n*-hexane as eluent,

then increasing the polarity by using dichloromethane, ethyl acetate and methanol to give three different components from different fractions. These components were purified by small glass column afforded compound PC-1 (5 mg) in white crystalline solid from 10% EtOAc in DCM, compound PC-2 (6 mg) in white crystalline solid from 20% EtOAc in DCM and compound PC- 3 (7.5 mg) in a powdered solid from 10% EtOAc in DCM.

Compound 1: White crystalline solid (~5 mg); R_f = 0.65 (90% dichloromethane: 10% ethyl acetate); ¹H and ¹³C NMR (TMS) data were similar to reported data⁷ in Table 1.

Compound 2: White crystalline solid (~6 mg); R_f = 0.63 (80% dichloromethane: 20% ethyl acetate); ¹H and ¹³C NMR (TMS) data were similar to reported data⁷ in Table 1.

Compound 3: Powdered solid (4.8 mg); R_f = 0.36 (90% dichloromethane: 10% ethyl acetate); ¹H and ¹³C NMR (TMS) data were similar to reported data⁷ in Table 1.

Table 1: ¹³C and ¹H NMR spectral data of compounds 1,2 and 3 (100, 400MHz, DMSO-d₆, TMS):

Position no	Compound 1		Compound 2		Compound 3	
	δ _c	δ _H (J in Hz)	δ _c	δ _H (J in Hz)	δ _c	δ _H (J in Hz)
1	162.77	-	165.47	-	165.68	-
2	55.80	Overlapping(1H)	119.96	6.39(1H, d)	121.19	5.94(1H, d)
3	122.29	4.14(1H, dt)	142.62	7.39(1H, dd)	141.02	7.19(1H, dd)
4	130.44	6.05(1H, ddd)	125.37	6.71(1H, dd)	129.48	6.14(1H, m)
5	55.93	Overlapping(1H)	138.31	6.74(1H, d)	142.63	6.14(1H, m)
6	131.38	-	-	-	35	2.42(2H, q)
7	111.69	6.28(1H, br,s)	-	-	35	2.64(2H, t)
8	147.92	-	-	-	-	-
9	149.10	-	-	-	-	-
10	107.95	6.56(1H, d)	-	-	-	-
11	120.65	Overlapping(1H)	-	-	-	-
1'	46.93	3.85(2H, m)	-	-	45.85	3.57(2H, t)
2'	28.95	1.32-1.79(2H, m)	45.36	3.56(2H, s)	29.71	1.4-1.8(1H, m)
3'	28.65	Overlapping(2H)	26.18	1.57(2H, m)	24.65	0.92(3H)
4'	28.73	Overlapping(2H)	24.66	1.65(2H, m)	24.65	0.92(3H)
5'	41.25	3.85(2H, m)	24.66	1.58(2H, m)	-	-
6'	-	-	45.36	3.56(2H, s)	-	-
1''	178.53	-	131.04	-	135.17	-
2''	38.3	3.15(1H, t)	105.70	6.96(1H, s)	108.84	6.64(1H, br,s)
3''	34.79	2.94(1H, dd)	148.14	-	147.59	-
4''	54.68	-	148.14	-	145.74	-
5''	109.49	6.24(1H, br,s)	108.49	6.76(1H, d)	108.17	6.72(1H, d)
6''	146.49	-	122.5	6.88(1H, d)	119.03	6.60(1H, d)
7''	143.15	-	-	-	-	-
8''	105.40	6.46(1H, d)	-	-	-	-
9''	120.21	6.07(1H, br,s)	-	-	-	-
1'''	46.48	2.57(2H, m)	-	-	-	-
2'''	29.34	Overlapping(1H)	-	-	-	-
3'''	20.13	0.92(3H, d)	-	-	-	-
4'''	19.89	0.92(3H, d)	-	-	-	-
Methylene di-oxy	100.92 101.06	5.93(2H, d) 5.82(2H, d)	101.27	5.95(2H, s)	100.8	5.90(2H, s)

Antimicrobial activities

Agar diffusion method [8]: The total ethyl acetate extract and pure compound 1-3 of *P. chaba* H. were dissolved in sufficient amount of respective solvents and evaluated by agar diffusion method at a dose of 300 µg/disc and the results were reported as shown in Table-2. The tested fungi were *Candida albicans*, *Aspergillus niger*, and *Sacharomyces cerevacaee*; gram positive bacteria were *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Sarcina Lutea*, and

Staphylococcus aureus while gram negative bacteria were *Escherichia coli*, *Salmonella paratyphi*, *Salmonella typhi*, *Shigella boydii*, *Shigella dysenteriae*, and *Vibrio mimicus*. Kanamycin (30µg/disc) disc was used as standard antifungal and antimicrobial agents; the diameter of zone of inhibition was measured in mm. The microorganisms were obtained from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh.

Table 2: Antimicrobial activities of ethyl acetate extract and pure compound 1-3 of *P. chaba* H.

Test bacteria and fungi	Ethyl acetate extract	Comp 1	Comp 2	Comp 3	Kanamycin
	300 (µg/disc)				30 (µg / disc)
Gram-positive bacteria					
<i>Bacillus cereus</i>	6.5	7	---	---	32
<i>Bacillus megaterium</i>	6.5	13	---	---	32
<i>Bacillus subtilis</i>	13	---	---	---	32
<i>Staphylococcus aureus</i>	11	14	---	---	33
<i>Sarcina lutea</i>	12	---	---	---	33
Gram-negative bacteria					
<i>Escherichia coli</i>	11	---	---	---	33
<i>Salmonella paratyphi</i>	10	---	---	---	33
<i>Salmonella typhi</i>	11	---	---	---	33
<i>Shigella boydii</i>	9	---	---	---	33
<i>Shigella dysenteriae</i>	11	---	---	---	33
<i>Vibrio mimicus</i>	9	---	---	---	32
<i>Vibrio parahemolyticus</i>	10	---	---	---	33
Fungi					
<i>Candida albicans</i>	10	---	---	---	32
<i>Aspergillus niger</i>	18	14	---	---	32
<i>Sacharomyces cerevaca</i>	9	---	---	---	32

“---” Indicates ‘No activity’

Result and Discussion

The MeOH extract from the leaves of *Piper chaba* was suspended in water and partitioned between n-hexane, dcm, ethyl acetate and methanol successively. DCM fraction was being subjected to silica gel column chromatography (CC) afforded compounds 1 to 3.

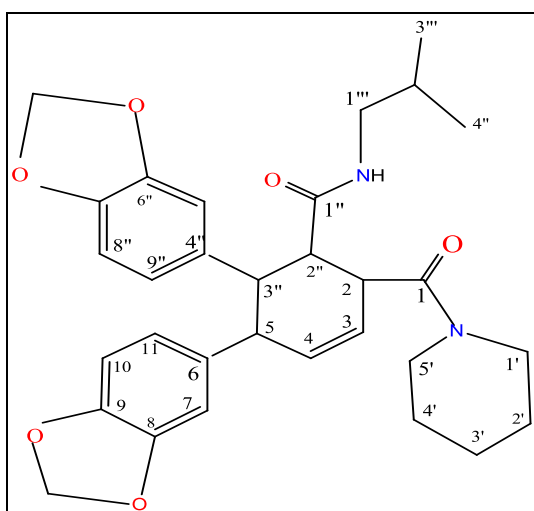


Fig 1.

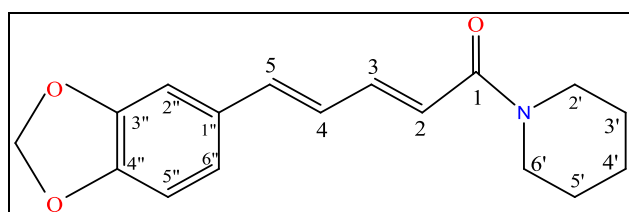


Fig 2.

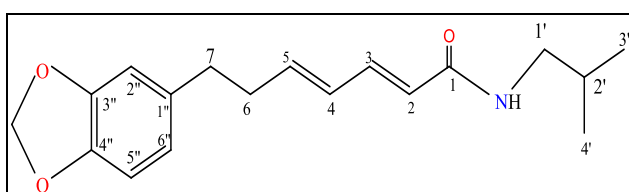


Fig 3.

Compound 1(PC-1) (Figure-1), was obtained as a white crystalline solid, m.p. 130-132^o C. The ¹H NMR (400 MHz, in CDCl₃) spectrum (Figure-1) of compound displayed the aromatic proton signals at 6.28 (br,s), 6.56(d), 6.24(br,s), 6.46(d) and 6.07(br,s) ppm. The signal of proton of 11 position overlapped with signal of proton of 10 position at 6.56 ppm. The other signals for PC-1 at δ 0.92 (6H, d) ppm for isopropyl methyl [(CH₃)₂CH-]. Another methylene protons of C-1''' position gave multiplate at 2.57 ppm. Two methylene di-oxy gave doublet at 5.93 and 5.82 ppm for 4H. The 4H attached to methylene(-CH₂-) carbon (attach to nitrogen) at position 1' and 5' gave multiplate at 3.85 ppm. The signals for tertiary carbon protons of 2'' and 3'' position were at 3.15(t) and 2.94(dd) ppm which were upfields because of single bond carbon (shielded) than the signal of protons of methine (-CH-) of position 3 and 4 at 4.14(dt) and 6.05(ddd)ppm, which were downfields because of double bond carbon (deshielded). The signal of two protons of 2 and 5 tertiary carbons overlapped at 3.85ppm as multiplates. For nitrogen atom we got a quadrupole broadening at 5.5ppm and the presence of nitrogen were also detected by lessaigne test.

The ¹³C NMR (100 MHz, in CDCl₃) spectrum of compound PC-1 explained the presence of two aromatic rings. There were total six quaternary carbon atom in two benzene rings, among which four quaternary carbons were oxygenated and they gave downfield signals than other carbon atoms of benzene rings. Signal for quaternary carbons of 8, 9, 6'', 7'' position were 147.92, 149.10, 146.49 and 143.15 ppm respectively and other two quaternary carbons signals were obtained at 131.38 and 54.68 ppm. Another six carbons of two benzene ring were methine (-CH-) carbon which signals were observed at 111.69, 107.95, 123.7, 109.49, 105.40 and 120.21 ppm. Two methylene di-oxy (-OCH₂O-) groups were attached to two benzene rings for which we obtained signals at 100.92 and 101.06 ppm.

There were also two carbonyl group signals at 162.77 and 178.53 ppm. The carbons adjacent to nitrogen were gave signals at 46.48, 46.93 and 41.25 ppm all of them were methylene (-CH₂-) carbons. Another three methylene carbons in ring containing nitrogen gave signals at 28.95, 28.65 and 28.73 ppm. There was a isopropyl [(CH₃)₂CH-] group in PC-1, which presence was confirmed by the signals of two -CH₃ carbon at 20.13 and 19.89ppm and one -CH- carbon at 29.34

ppm. Remaining six carbon atoms were in a ring with a double bond, among them two methine (-CH-) carbon were in the double bond contact gave signals downfield at 122.29 and 130.44 ppm. Rest four tertiary carbons signals were observed at 55.80, 55.93, 38.30 and 34.79 ppm. Which were upfield than methine (-CH-) protons. The ^1H NMR and ^{13}C NMR (100 MHz, in CDCl_3) values were compared to the published ^1H NMR and ^{13}C NMR values of Chabamide I and the structure of Figure-1 was proposed.

Compound 2 (PC-2) (Figure-2), The ^1H NMR (400 MHz, in CDCl_3) spectrum of compound -2 explained the aromatic proton signals at 6.96 (br,s), 6.76(d) and 6.88(d) ppm. One methylene di-oxy signal at 5.95(s) ppm. Three methylene (-CH₂-) protons gave multiplates signals and overlapped at 1.57, 1.65, 1.58 ppm for 6H. Rest two methylene (-CH₂-) adjacent to nitrogen gave signal at 3.56 (s) ppm for four protons. The presence of four methine (-CH-) protons was confirmed by the signals at 6.39(d), 6.71(d), 6.74(d) and 7.39(dd) ppm. -The ^1H NMR (400 MHz, in CDCl_3) values were compared to the published ^1H NMR values of Piperine. The ^{13}C NMR (100 MHz, in CDCl_3) spectrum (Figure 4.10) of compound-2 (Figure-2) identified the presence of one aromatic ring. Two carbon atoms of the benzene ring were oxygenated and they gave downfield signal at 148.21 and 148.14 ppm than other carbon atoms of benzene rings. Signal for quaternary carbon atom of the benzene ring was found at 131.04 ppm. Another three carbons of benzene ring were methine (-CH-) carbon which signals were obtained at 108.49, 122.5 and 105.70 ppm. Methylene di-oxy (-OCH₂O-) group attached to benzene ring gave signal at 101.27 ppm. There was a carbonyl group signal at 165.47 ppm. For nitrogen atom we got signal of the two methylene (-CH₂-) carbons adjacent to nitrogen at 45.36 ppm.

Rest three methylene (-CH₂-) group in the ring containing nitrogen chain gave signal at 24.66 and 26.18 ppm. Presence of nitrogen was proved by lessaigne test. In the side chain there were four methine (-CH-) carbon, their signals at 119.96, 142.62, 125.37 and 138.31 ppm. The ^1H NMR and ^{13}C NMR (100 MHz, in CDCl_3) values were compared to the published ^1H NMR and ^{13}C NMR values of Piperine and the structure of PC-2 compound was proposed in (Figure-2).

Compound 3 (PC-3) (Figure-3), The ^1H NMR (400 MHz, in CDCl_3) spectrum of compound-3 showed that the aromatic proton signals at 6.64 (br,s), 6.72(d) and 6.60(d) ppm. The other signals for PC-3 at δ 0.92(6H) ppm for isopropyl methyl [(CH₃)₂CH-]. One methylene di-oxy signal was found at 5.90(s) ppm. Two methylene (-CH₂-) protons of the side chain gave signals at 2.42 ppm as quatret and 2.65 ppm as triplet. The presence of four methine (-CH-) protons in side chain was confirmed by the signals at 5.94(d), 7.19(d), 6.14(m) ppm. Remaining one methylene (-CH₂-) adjacent to nitrogen gave signal at 3.57(t) ppm for two protons. Presence of nitrogen atom was confirmed by a quadrupole broadening at 1.81 ppm.

The ^{13}C NMR (100 MHz, in CDCl_3) spectrum of compound PC-3 proved the presence of an aromatic ring. Two carbon atoms of the benzene ring were oxygenated and they were found to give downfield signal at 147.59, 145.74 ppm than other carbon atoms of benzene rings. Signal for quaternary carbon atom of the benzene ring was found at 135.17 ppm. Rest three carbons of benzene ring were methine (-CH-) carbon which signals were obtained at 108.84, 108.17 and 119.03 ppm. Other four methine (-CH-) carbons in long side chain attached with each other by double bond and the signals was found at 121.19, 141.02, 129.48 and 142.63 ppm. Signal at 100.8 ppm showed the presence of methylene di-oxy (-OCH₂O-) groups attached to the benzene ring. There was a

carbonyl group signal at 165.68 ppm. For nitrogen atom we got signal of the methylene (-CH₂-) carbon adjacent to nitrogen at 45.85 ppm.

Presence of nitrogen was proved by lessaigne test. Rest two methylene (-CH₂-) group in the long side chain were observed at 34.83, 38.39 and 29.72 ppm. In the side chain there were two methylene group (-CH₂-) carbon, their signals at 35 ppm. There was a isopropyl [(CH₃)₂CH-] group in PC-3, which was confirmed by the signals of two -CH₃ carbon at 24.65 ppm and one CH carbon at 29.71 ppm. The ^1H NMR and ^{13}C NMR (400 MHz, in CDCl_3) values were compared to the published ^1H NMR and ^{13}C NMR values of Chingchengenamide A and the structure of PC-3 compound was proposed in (Figure-3).

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References

- Kirtikar KR, Basu BD. Indian Medicinal Plants. 3rd ed. International Book Distributors, 1987, 2130-2131.
- Ghani A. Medicinal Plants of Bangladesh. 2nd ed. Dhaka, Asiatic Society of Bangladesh, 2003, 267-268.
- Yusuf M, Chowdhury JU, Begum DJ. Medicinal Plants of Bangladesh. Bangladesh Council of Scientific and Industrial Research; 1994, 192.
- Haque ME, Rahman A, Amin A, Shekher HU. Alkamide and lignan from the stem bark of *Piper chaba* (Piperaceae). Dhaka University Journal of Pharmaceutical Sciences. 2004; (3):1-3.
- Morikawa T, Matsuda H, Yamaguchi I, Pongpiriyadacha Y, Yoshikawa M. New amides and gastroprotective constituents from the fruit of *Piper Chaba*. Planta Medica. 2004; (70):152-159.
- Rahman MTU, Shilpi JA, Ahmed M, Hossain CF. Preliminary pharmacological studies on *Piper chaba* stem bark. Journal of Ethnopharmacology. 2005; (99):203-209.
- HC. NMR data, occurrence, biosynthesis and biological activity of Piper amides; Víctor Gómez-Calvario, María Yolanda Rios. Centro de Investigaciones Químicas, IICBA, Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico Investigaciones Químicas, IICBA, Universidad Autónoma del Estado de Morelos, Avenida Universidad 1001, Col. Chamilpa, Cuernavaca, Morelos 62209, Mexico. Email:myolanda@uaem.mx.onlinelibrary.wiley.com/doi/abs/10.1002/mrc.4857
- Perez C, Pauli M, Bazerque P. An antibiotic assay by agar diffusion method. Acta. Biol. Med. Expart. 1990; (15):113-115.
- Mabry TJ, Markham KR, Thomas MB. Systematic Identification of Flavonoids (Springer Verlag, New York), 1970, 82.
- Noriko Komoto, Takahisa Nakane, Sachiko Matsumoto, Shusuke Hashimoto, Osamu Shiota, Setsuko Sekita *et al.* Shul flavonoids, biflavones, and flavonoids from *Cephalotaxu harringtonia* var. *nana*. The Japanese Society of Pharmacognosy and Springer Japan. J Nat Med. 2015; 69:479-486.
- Xiu-Ling Wang, Kwang-Hee Shin, Hor-Gil Hur, Su-Il Kima. Enhanced biosynthesis of dihydrodaidzein and dihydrogenistein by a newly isolated bovine rumen anaerobic bacterium. Journal of Biotechnology. 2005; 115:261-269.