Isolation and Structure Characterization of a new Bisbenzylisoquinoline Alkaloid, from *Cocculus Pendulus*

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Abstract

This paper describes phytochemical studies on aerial part of the *Cocculus pendulus*, which led to the identification of a new ingredient namely, 1,2-dihydrokurramine (**1**) along with four reported compounds 1,2-Dehydrokohatine (**2**), Kohatine (**3**), and 5′Hhydroxyapateline (**4**). Structures of all the Compounds were elucidated by using 1D and 2D NMR and mass spectroscopy. High Resolution Mass Spectroscopy (HRMS) afforded the exact mass to be 534.2160, which corresponded to the formula $C_{33}H_{30}N_2O_5$ (calc.534.2154). The mass spectrum exhibited molecular ion peak M^+ at m/z 534. The characteristics of M^+-212 peak at m/z 322 resulted from double benzylic cleavage.

1. INTRODUCTION

Cocculus pendulus (Forst. & Diels) is a climbing shrub that grows in both the north and south of Pakistan. In Pakistan, the plant's roots are frequently used to cure intermittent fever and as a tonic [1]. In recent years, a number of bisbenzylisoquinoline alkaloids with anticancer, anti-inflammatory, and antitubular properties were identified [1-2]. Guha et al. published the first comprehensive review of bisbenzylisoquinoline alkaloids in 1979 [3], which listed 186 alkaloids. Several reviews have been written since then [4-8]. Cocculus pendulus extracts have also been studied for its hypotensive and anticancer properties [9], as it is a rich source of bisbenzylisoquinoline alkaloids, which may be the primary reason for its biological activity. Tetrandrine derived from cocculus sarmentosus is known to contain anticancer, anti-inflammatory, and antitubular properties; it also protects against silicosis and histamine-induced effects and lowers blood pressure [2]. Oxycanthine and cepharanthine were discovered to be particularly effective against tuberculosis and leprosy in humans [10].

Buck provides a tabular listing of bisbenzylisoquinoline alkaloids in alphabetical order in his review of Manske's "Alkaloids" series [11]. These tables provide an exhaustive overview of the pharmacological action exhibited by bisbenzylisoquinoline alkaloids.

The discovery of new Bisbenzylisoquinoline alkaloids continues to be tied to the study of the metabolic pathways that regulate their manufacture, as well as the future identification of the enzymes/genes that regulate specific processes. This will result in the development of novel pharmacological tools, in addition to pharmaceutical and agricultural products. [12-13]

2. EXPERIMENTAL DETAILS

2.1 General Experimental Details

The optical rotations were measured using a digital JASCO 360 polarimeter. On the polatronic D instrument, certain optical rotations were also observed. Using $CHCl_3$ as a solvent, infrared spectra were acquired with a Shimadzu IR-460 FT Spectrophotometer or a JASCO IRA-1 IR Spectrophotometer. Additionally, several samples were tested using KBr disc. The pH levels were determined using a model 25 pH metre (Shanghai Kanchon, Peoples Republic of China). The melting points were determined with a Buchi 510 device. Using analytical grade methanol, UV tests

were conducted using a Hitachi U-3200 spectrophotometer or Shimadzu UV 240 equipment (Merck). On a Varian MAT 312 double focusing mass spectrometer, studies including Electron Impact (EI), peak matching, Field Desorption (FD), and Fast Atom Bombardment (FAB) were conducted. The 1H-NMR spectra were recorded on a Bruker AMX-300, AM-400, and AM-500 Nuclear Magnetic Resonance Spectrometer in CDCl3, CD3OD, or a combination of both, whereas C_5D_5N was used in some cases. The device was linked to a MAT 188 data system and PDP 11/34 DEC computer system. On a Jeol-JMX HX-110 mass spectrometer, High Resolution Electron Impact Mass Spectra (HREIMS) were captured. The chemicals and solvents utilised in various studies were acquired from E. Merck and Fluka for Thin-Layer Chromatography (TLC), which was performed on E. Merck (Art 5715) DC precoated silica gel 60-F254 preparative plates (20 x 20 cm) (20 x 20 cm). RP-DC Fertig plate RP-18 F254 S (5 x 10 cm). UV light at 254 and 366 nm was used to observe chromatograms. Dragendorff's reagent was employed for alkaloid detection. For the column chromatography, E-Merck Silica gel type-60 (Article 7734, 70-230 mesh) was utilised. Flash chromatography was carried out using silica gel (type-60, Art. 9385, 230-400 mesh).

2.2 Plant Material

The current investigation involved 40 kg of the plant's aerial parts and leaves, all of which were gathered from the Malir District on Karachi's outskirts. The University of Karachi's Department of Botany has received a herbarium specimen (Voucher # 12760).

2.3 Fractionation

40 kilogrammes of the plant's aerial parts and leaves were air-dried for 15 days, leaving 15 kilogrammes of material. The substance was diced and then crushed with an ultra-turex before being immersed in 60 litres of methanol. Under vacuum, the mixed filtrates were evaporated to produce a crude gum (1.5kg).

The resultant methanolic extract was diluted in 10 litres of 10% HCL aqueous solution and defatted with n-hexane (15 litre overall). The resulting acidified aqueous layer, which included chloride salts of the bases, was neutralised to a pH of 11 using NH_4OH. The free alkaloids were then extracted with $CHCl_3$ (20 litres) and ethyl acetate (10 litres), the resultant organic extracts

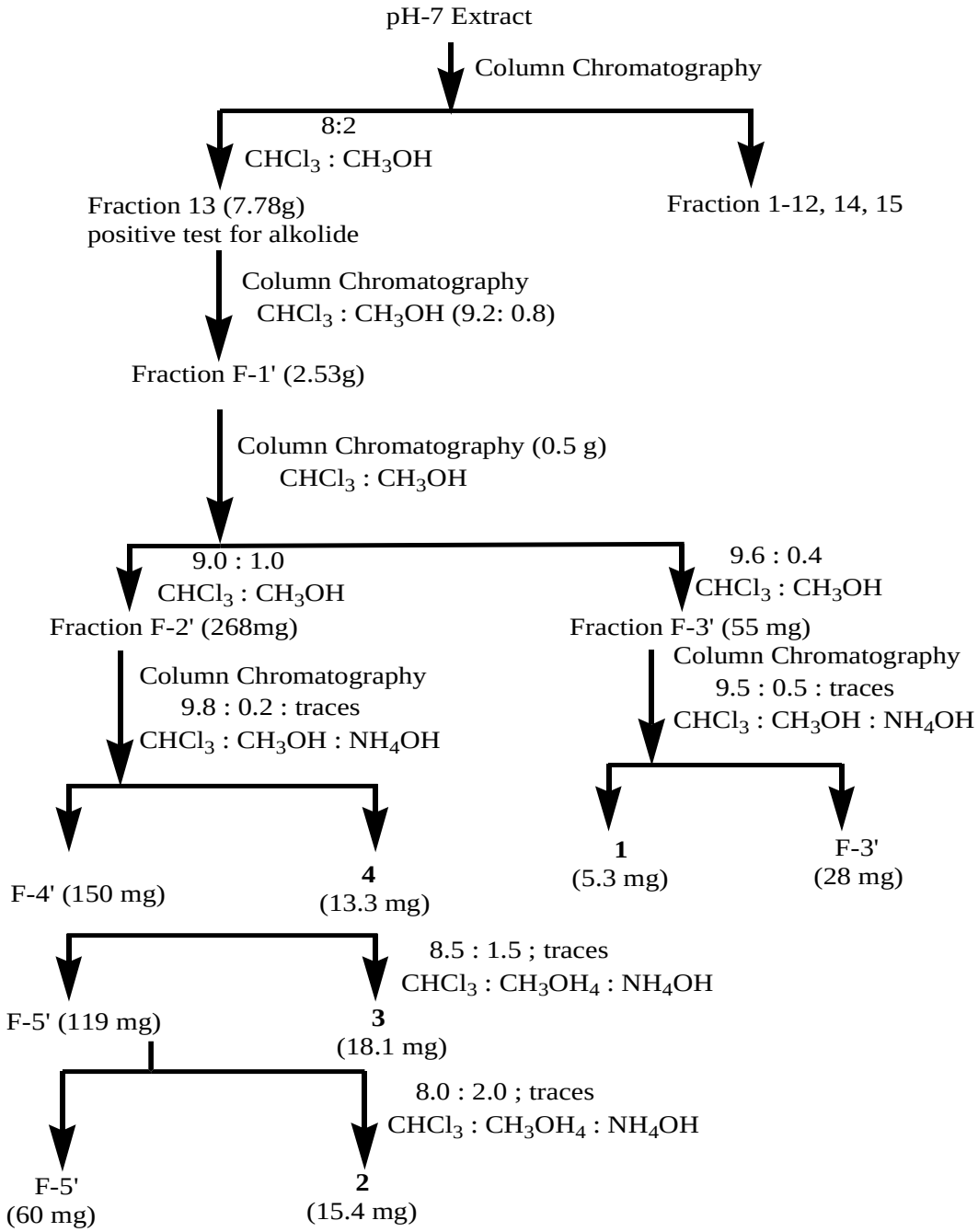

Figure 1: Scheme for the Purification of Bisbenzylisoquinoline Alkaloids.

(From $CHCl_3$ and EtOAc) were mixed, and the solvents were evaporated to produce the crude alkaloids (70 g). Re-dissolving the alkaloids in a 5% acetic acid solution (3 liter). Adjusting the pH with ammonium hydroxide and extracting alkaloids with $CHCl_3$ at pH-3 (12.29), pH-5, and pH-7

(27 g). Separate the different chloroform layers from the aqueous layer and dry them with anhydrous sodium sulphate. Under decreased pressure, chloroform was evaporated, and pH-7 fraction was subjected to repeated column chromatography (Figure **1**).

2.4 Purification of Alkaloids 1, 2, 3 and 4

The pH 7 extract (27g) was adsorbed onto silica gel (70-230 mesh, 81g) and placed into a silica gel-packed column (70-230 mesh, 1kg). To get 15 fractions, it was eluted using different solvent gradients of n-hexane, chloroform, and chloroform methanol. With Dragendorff's reagent, fraction 13, which was prepared with 8:2 chloroform: methanol (7.78 g), yielded the most promising findings. The fraction was submitted to repeated column chromatography, which yielded fraction F-2′ (268 mg) containing four alkaloids **1**, **2**, **3**, and **4**, as well as trace impurities, at a chloroform: methanol ratio of 9.6:0.4.

F-2′ was again adsorbed on flash silica (0.85 g, 230-400 mesh) and chromatographed on a flash silica-packed column (230-400 mesh, 13.5 g). The column was run in a solvent mixture of 9.0:1.0 (chloroform:methanol) with traces of aqueous ammonia. **1**, was the less polar compounds distinct from compound **2-4**. Slow-moving, highly polar alkaloids **4**, was distinct from **2**, and **3** from fraction F-2′ which was merged to create fraction F-4′ (150 mg). Once more, the latter was absorbed and put onto silica gel (230 – 400 mesh, 450 mg and 7.25 g). The column was run in chloroform:methanol (9.85:1.5) including traces of NH_4OH. The rapidly moving component **3** (18.1 mg) was eluted first, and then components **2** was separated by raising the polarity to 8.0: 2.0, running the column extremely slowly, and adding traces of NH_4OH.

3. RESULTS AND DISCUSSION

Compound **1** was purified from the fraction F-3′ (Figure 2,). The UV spectrum of this compound was typical for bisbenzylisoquinoline system, showing absorption at λ_{max} 207 (log ε 3.68), 232 (log ε 3.50) and 274 nm (log ε 2.93). The IR spectrum showed bands at 1563cm^{-1} and 1457cm^{-1} (Aromatic C=C), 2924cm^{-1} and 2854cm^{-1} (N-H), and 3407cm^{-1} (OH). High Resolution Mass Spectroscopy (HRMS) afforded the exact mass to be 534.2160, which corresponded to the formula $C_{33}H_{30}N_2O_5$ (calc.534.2154). The mass spectrum exhibited molecular ion peak M^+ at m/z 534. The characteristic M^+-212 peak at m/z 322 resulted from double benzylic cleavage. The loss of a proton from this 66 gave rise to the base peak at m/z 321 (obs. 321.1298, calculated 321.1239),

corresponding to the formula $C_{19}H_{17}N_2O_4$. Doubly charged ion peak of the bisisoquinoline unit, corresponding to the base peak at m/z 161 (24%) was another distinguishing feature in the EIMS (Figure **2**).

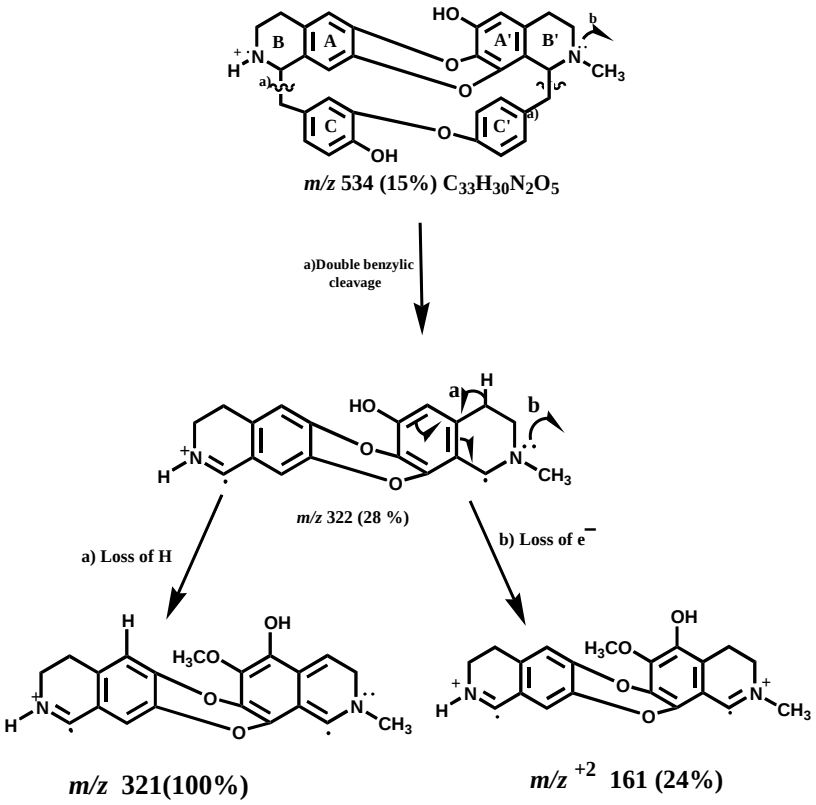

Figure 2: Mass Fragmentation pattern of 1,2-Dihydrokurramine (**1**)

The 1H-NMR spectrum (400 MHz, CD_3OD) of 1,2-dihydrokurramine (**1**) exhibited a sharp 3H-singlet at δ 2.53 assigned to 2′N-CH_3 group (Figure **3**). The H-1′ resonated as a broad doublet centered at δ 3.92 ($J_{1, \alpha'}$ = 1.93 Hz), whereas H-1 was found resonating at δ 3.72 as a broad doublet ($J_{1, \alpha'}$ = 1.95 Hz). The assignment of N-CH_3 group at 2′ was proposed by these δ values for H-1 and H-1′, because otherwise H-1′ would have been present at around δ 4.2 and H-1 close to δ 3.3-3.6. The signal at δ 6.57 (2H-multiplet) was assigned to H-10 and H-14. A double doublet resonating at δ 6.69 ($J_{13, 14}$ = 8.0 Hz) was assigned to H-13. Thus, OH in ring C could only be assigned at C-12. Four double doublets appearing at δ 6.79 ($J_{11', 10'}$ = 8.2 $J_{11', 13'}$ = 2.3 Hz), δ 7.08 ($J_{10', 11'}$ = 8.1 Hz, $J_{10', 14'}$ = 1.5 Hz), δ 7.19 ($J_{13', 14'}$ = 8.2Hz, $J_{13', 11'}$ = 2.3Hz) and δ 7.52 ($J_{14', 13'}$ = 8.2 Hz, $J_{14', 10'}$ =

1.5 Hz) were assigned to H-11′, H-10′, H-13′ and H-14′, respectively. Another three aromatic singlets in the 1H-NMR were assigned to H-8 (δ 6.21), H-5 (δ 6.61) and H-5′ (δ 6.29).

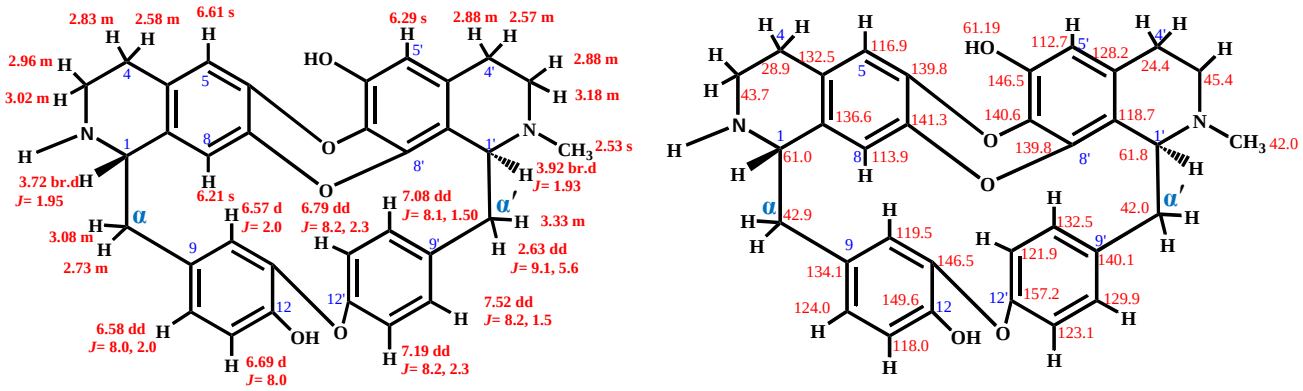

Figure-3: Molecular structure of Compound-**1** with ^{1}H and ^{13}C-NMR assignment.

The chemical shift value for H-8 (δ 6.21) was also in agreement with the placement of N-CH_3 at 2′ position[14]. The H-5′ is always found to resonate at around δ 6.29 when the adjacent C-6′ has a phenolic function. A positive optical rotation and chemical shifts for H-1 and H-1′ is also in agreement with the assigned stereochemistry C-1 and C-1′ as S, S′.

1,2-Dihydrokurramine (**1**): white amorphous powder, $[\alpha]_D^{25}$: +100, UV ν_{max} (MeOH) nm (log ε): 207 (3.68), 232 (3.50), 274 (2.93), IR, λ_{max} (CHCl3) cm^{-1}: 3407 (OH), 1563 (Aromatic C=C), EIMS m/z: 534 (M^+, 15), 322 (28), 321 (100), 307 (7), 161 (24), HREIMS m/z (formula, calcd.): 534.2160 (M^+ $C_{33}H_{30}N_2O_5$, 534.2154), 322.1148 ($C_{19}H_{18}N_2O_3$, 321.1317), 321.1298 ($C_{19}H_{17}N_2O_3$, 321.1239), 1H-NMR spectrum (400 MHz, CD_3OD, δ, ppm, Hz): 7.52 dd (1H, *J* = 8.2, 1.5, H-14′), 7.19 dd (1H, *J* = 8.2, 2.3, H-13′), 7.08 dd (1H, *J* = 8.1, 1.5, H-10′), 6.79 dd (1H, *J* = 8.2, 2.3, H-11′), 6.69 d (1H, *J* = 8.0, H-13), 6.61 s (1H, H-5), 6.58 m (2H, *J* = 8.0, 2.0, H-14, H-10), 6.29 s (1H, H-5′), 6.21 s (1H, H-8), 3.92 br.d (1H, *J* = 1.93, H-1′), 3.72 br.d (1H, *J* = 1.95, H-1), 2.56 s (1H),

1,2-Dehydrokohatine (**2**): Yellowish amorphous powder, $[\alpha]_D^{25}$: +57, UV, λ_{max} (MeOH) nm (log ε): 202 (4.59), 233 (4.32), 253 (4.12). 289 (3.73), 340 (3.40), IR, ν_{max} ($CHCl_3$) cm^{-1}: 3616 (OH), 1693 (C = N), 1510 (Aromatic C = C), EIMS, *m/z* (%):562 (M^+, 90%), 561 (100), 547 (15), 531 (7), 350 (16), 349 (44), 281 (22), 175 (26), HREIMS *m/z* (formula, calcd.): 562.2170 (M^+, $C_{34}H_{30}N_2O_6$,

562.2103) 561.2064 ($C_{34}H_{29}N_2O_6$, 561 1988) 547.1809 ($C_{33}H_{27}N_2O_6$, 547.1874)349.0440 ($C_{20}H_{17}N_2O_4$, 349.1197).

Kohatine (**3**): off white amorphous powder, $[\alpha]_D^{25}$: +179, UV, λ_{max} (MeOH) nm (log ε), 203 (4.06), 235 (3.80), 290 (2.98), IR, ν_{max} ($CHCl_3$) cm^{-1}: 1590 (aromatic C= C), 3460 (OH), EIMS, *m/z* (%): 564 (M^+, 55), 352 (29), 351 (100), 337(21), 281 (21), 176 (50), HREIMS *m/z* (formula, calcd.): 564.2278 (M^+, $C_{34}H_{34}N_2O_6$, 564.2260) 351.1360 ($C_{20}H_{19}N_2O_4$, 351.1345), 337.1196 ($C_{19}H_{17}N_2O_4$, 337.1188).

5′ Hhydroxyapateline (4): off white amorphous powder, $[\alpha]_D^{25}$: +187, UV, λmax (MeOH) nm (log ε): 232 (4.14), 203 (4.39), 268 (3.58), IR, ν_{max} ($CHCl_3$) cm^{-1}: 3743 (OH), 1601(Aromatic C=C), EIMS, m/z (%):564 (M^+, 59), 352 (35), 351 (100) 337 (27), 282 (3), 176 (54)., HREIMS m/z (formula, calcd.): 564.2278 (M^+, $C_{34}H_{32}N_2O_6$, 564.2260) 351, 1360 ($C_{20}H_{19}N_2O_4$, 351.1345) 337.1059 ($C_{19}H_{17}N_2O_4$, 337.1188).

4. CONCLUSIONS

The 1H-NMR spectrum (400 MHz, CD_3OD) of 1,2-dihydrokurramine (**1**) exhibited a sharp 3H-singlet at δ 2.53 assigned to 2′N-CH_3 group. The H-1′ resonated as a broad doublet centered at δ 3.92 ($J_{1,\alpha'}$ = 1.93 Hz), whereas H-1 was found resonating at δ 3.72 as a broad doublet ($J_{1,\alpha'}$ = 1.95 Hz). The assignment of N-CH_3 group at 2′ was proposed by these δ values for H-1 and H-1′, because otherwise H-1′ would have been present at around δ 4.2 and H-1 close to δ 3.3-3.6. The chemical shift value for H-8 (δ 6.21) was also in agreement with the placement of N-CH_3 at 2′ position. The H-5′ is always found to resonate at around δ 6.29 when the adjacent C-6′ has a phenolic function.

REFERENCES

1. Haq, Aminul, et al., *Ethnobotanical study of medicinal plants of Pashat Valley, Bajaur, along Pakistan–Afghanistan border: a mountainous region of the Hindu Kush Range.* Nordic Journal of Botany, 2022: e03580.
2. Barbosa-Filho, JoséMaria, Emidio Vasconcelos Leitão da-Cunha, and Alexander Irvine Gray., *Alkaloids of the Menispermaceae*. (2000): 1-190.

3. Guha, K., B. Mukherjee, and R. Mukherjee, *Bisbenzylisoquinoline alkaloids--a review.* Journal of Natural Products, 1979. 42(1): p. 1-84.
4. Schiff Jr, P.L., *Bisbenzylisoquinoline alkaloids.* Journal of Natural Products, 1991. 54(3): p. 645-749.
5. Schiff, P., *Bis-benzylisoquinoline alkaloids from plants.* Journal of Natural Products, 1983. 46(1): p. 1-43.
6. Schiff, P.L., *Bisbenzylisoquinoline Alkaloids.* Journal of Natural Products, 1987. 50(4): p. 529-599.
7. Schiff, P.L., *Bisbenzylisoquinoline alkaloids.* Journal of Natural Products, 1997. 60(9): p. 934-953.
8. Bharathi Priya, L., et al., *An updated review on pharmacological properties of neferine—A bisbenzylisoquinoline alkaloid from Nelumbo nucifera.* Journal of Food Biochemistry, 2021. 45(12): p. e13986.
9. Bhakuni, D.S., et al., *Screening of Indian plants for biological activity: Part II.* Indian Journal of Experimental Biology, 1969. 7: p. 250-262.
10. Oliver-Bever, B. E. P., *Medicinal plants in tropical west Africa II. Plants acting on the nervous system.* Journal of Ethnopharmacology 7.1 (1983): 1-93.
11. Buck, K.T., *The bisbenzylisoquinoline alkaloids*, in *The Alkaloids.* Chemistry and Pharmacology. 1987, Elsevier. p. 1-222.
12. Wang, Shan, et al., *Metabolic reprogramming by traditional Chinese medicine and its role in effective cancer therapy*. Pharmacological Research 170 (2021): 105728.
13. W. Huang *et al.*, *Biosynthesis Investigations of Terpenoid , Alkaloid , and Flavonoid Antimicrobial Agents Derived from Medicinal Plants*. Antibiotics, pp. 1–32, 2022.
14. Herath, W.H., et al., *Nine bisbenzylisoquinoline alkaloids from Thalictrum cultratum.* Journal of Natural Products, 1987. 50(4): p. 721-725.