

# Psychoactive Chemical Constituents of *Carissa edulis* (Forssk.) Vahl Roots and Its Safety Limits in Folk Treatment: Benzodiazepines Analogous

Intisar Salih Ahmed and Damra Elhaj Mustafa

Department of Chemistry and Industrial Chemistry, College of Applied and Industrial Sciences, University of Bahri, P.O. Box 1660, Khartoum 11111, Sudan

**Abstract:** *Carissa edulis* Vahl is well known in Sudanese herbal medicine, commonly used for treatment of epilepsy, headache, chest pains, rheumatism, skin lesions, mania and other psychoactive diseases. The investigations of the safety use for psychoactive purposes in Sudanese healing traditions and identifying secondary metabolites of the plant extracts are the key steps towards determination of appropriate medicinal doses. Therefore, one of the chemical constituents was isolated and structurally identified by <sup>1</sup>H-NMR and LC-MS. With the aim of evaluating *Carissa edulis* folk random uses, the isolated compound was compared with reference artificial drugs Lormetazepam, a potentially toxic compound. Structure investigations confirm that the isolated product was benzodiazepines analogous 7-chloro 1,4-benzodiazepine-2-ones. It is important to know the potential toxicity of certain plant in order to assess the therapeutic effect of it, as these are slight distinctions between the medicinal and toxic doses. In general the results obtained justify the use of the roots of *Carissa edulis* in traditional medicine for the treatment of some psychiatric diseases.

**Key words:** *Carissa edulis*, psychoactive purposes, Lormetazepam, benzodiazepines, potential toxicity.

## 1. Introduction

*Carissa edulis* (Forssk.) Vahl, belonging to *Apocynacea* botanical family is locally known as Allali. It is used for the treatment of many microbial infections such as venereal, respiratory and gastrointestinal infections. The leaves, stem and root barks are used for treatment of sickle cell anemia, oedema, toothache, chest complaints, cough, gastric ulcer and in expelling worms [1, 2]. The roots of *Carissa edulis* Vahl and *Securidaca longipedunculata* Fresen are used as a body wash to treat epilepsy in Malawi [3].

The phytochemical screenings of the stem of *Carissa edulis* have shown to contain tannins, flavonoids, cardiac glycosides, coumarins, steroids, terpenes and sesquiterpene [4, 5], whereas the roots have shown the presence of alkaloids [6]. The leaves

and fruits have been reported to contain carbohydrates, tannins, flavonoids, saponins, cardiac glycosides, terpenes and steroids [7-9].

Extract of *Carissa edulis* has been found to have analgesic activity, with fruits having the highest activity, followed by leaves, seeds, root bark and stem bark [10]. The antimicrobial activity of the water extracts of the leaves and fruits have been screened using *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*. The Gram positive organisms, *Bacillus subtilis* and *Staphylococcus aureus* have been found to be more susceptible than the Gram negative organisms, *Escherichia coli* and *Pseudomonas aeruginosa* [11].

Interestingly *Carissa edulis* has been reported to possess anticonvulsant activity on the animal models [12]. Importantly, short-term administration of the standardized ethanol extract of *Carissa edulis* root bark at doses lower than 1,000 mg/kg has been found to be safe in rats and not to exert severe toxic effects

---

**Corresponding author:** Intisar Salih Ahmed, Ph.D., assistant professor, research fields: phytochemistry.

[13]. Thus this anticonvulsant activity provides a rationale for its use in traditional medicine for the treatment of epilepsy.

Interestingly, anti-HSV active components such as lupeol, oleuropein and carissol have been isolated from the root bark of *Carissa edulis*. Specifically, lupeol has been shown to exhibit strong anti-inflammatory, anti-arthritic, anti-mutagenic, anti-malarial and anti-viral activity *in vitro* and *in vivo* systems [14].

Extracts of aerial parts of *Carissa edulis* have been found to afford benzenoids, phenyl propanoid, lignans [4, 5], 3-*O*-acetyl chlorogenic acid, kaempferol 3-*O*- $\beta$ -d glucopyranoside, quercetin-3-*O*- $\beta$ -d glucopyranoside, rhamnetin-3-*O*- $\beta$ -d glucopyranoside, isorhamnetin-3-*O*- $\beta$ -d-glucopyranoside, rhamnopyranoside, caredulis, and (+) butyl-*O*- $\alpha$ -l-rhamnoside from ethyl acetate fraction [15].

The benzodiazepines which are expected to resemble to *Carissa edulis* chemical constituent analogues are a class of psychoactive drugs whose core chemical structure is fused ring system of benzene and diazepine ring [16]. These compounds are known for the treatment of anxiety, insomnia, epilepsy, alcohol withdrawal, and anesthesia [17], and classified into 2-keto compounds, 3-hydroxy compounds, Triazolo compounds, Imidazo compounds, and 1,5-benzodiazepines; such as diazepam, lormetazepam, triazolam, midazolam, and clobazam, respectively [18].

In addition to synthesized benzodiazepines, naturally occurring ones in other plant specimens and brain samples of animals, including a human brain have been detected [19].

Benzodiazepine alkaloids, circumdatins were isolated from culture of the fungus *Aspergillus ochraceus* [20] and the deep sea derived fungus *Aspergillus westerdijkiae* [21].

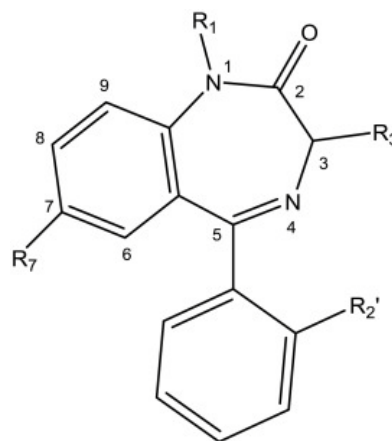
Aqueous acid extracts of wheat grains and potato tuber have been found to contain compounds belonging to the classical 5-phenyl-1,4-benzodiazepinones. In

wheat grains diazepam, N-desmethyldiazepam, delorazepam, deschloro-diazepam, delormetazepam, lormetazepam and isodiazepam have also been identified. Potato tuber has been reported to contain diazepam, N-desmethyldiazepam, delorazepam, lorazepam and delormetazepam [22].

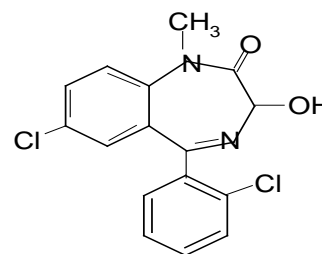
Substances with high affinity for central and peripheral benzodiazepine receptors have been determined in the pod and leaves extracts of *Ceratonia siliqua* (carob) [23].

There is increasing awareness of the adverse effects of benzodiazepines, particularly addiction and drug abuse. Nevertheless, benzodiazepines are frequently prescribed for short-term anxiety relief and some neurologic disorders [24].

Lormetazepam produces anxiolytic, muscle relaxant, sedative and hypnotic effects. The primary manifestation of over dosage ranges from drowsiness to coma and symptoms may include ataxia, hypotension, hypotonia, respiratory depression which may lead to death [25].



**Scheme 1. Benzodiazepines.**



**Scheme 2. Lormetazepam.**

Since most users perceived that *Carissa edulis* root bark extract was efficacious, evidence from characterization of chemical components could assist to direct the proper use, validate efficacy and determine safety limits. Therefore, it could contribute to the solution of a medical, cultural, or societal problem.

## 2. Experimental

### 2.1 Chemicals and Instrumentations

All the reagents were obtained from Merck and Fluka Chemical Companies and used without further purification. The column chromatography was performed on Merck silica gel 60 (230-240 mesh). The isolation of the compound was followed by TLC using silica gel 254 plates. Melting points of the isolated compounds were determined by using Gallen Kamp apparatus. The  $^1\text{H-NMR}$  (500 MHz) spectra was recorded on Bruker DMX 500 Spectrometer. The chemical shifts ( $\delta$ ) are given in ppm relative to internal TMS.

A spectrum was recorded on Agilent-LC-MS 1100 MSD single quadrupole mass spectrometer. The mass scan range was 100-800 m/z.

### 2.2 Plant Materials

*Carissa edulis* (Forssk.) Vahl root bark was purchased from the herbalist with a voucher specimen is on deposit at the MAPRI Herbarium, National research institute, Sudan.

### 2.3 Extraction and Isolation

Totally, 200 g of powdered dried root bark was extracted successively using petroleum ether and methanol. The methanol extract was dissolved in 300 mL water and then partitioned with hexane ( $3 \times 300$  mL), chloroform ( $3 \times 300$  mL) and finally ethyl acetate ( $3 \times 300$  mL).

The ethyl acetate extract was loaded into silica gel column and eluted gradient with increasing concentrations of ethyl acetate in hexane. The fractions of 10 mL each were collected and monitored

by TLC with visualization under UV ( $\lambda_{\text{max}}$  254 and 365 nm), using the chloroform/ethyl acetate solvent system. Similar fractions undergo further purification using recrystallization to collect one pure compound which was identified as benzodiazepine derivative by comparison of its spectroscopic profiles with published data, in addition to 2,4-dinitrophenyl hydrazine test for keto functional group and lassaigne's test for elemental analysis [26, 27].

### 2.4 Determination of Alkaloids

The plant extract was subjected to phytochemical test for the identification of alkaloids using Dragendroff's reagent and Mayer's reagent as visualizing reagents. The formation of orange red precipitate indicates the presence of alkaloids [28].

## 3. Results and Discussion

### 3.1 Phytochemical Tests

The result of the qualitative chemical screening detected the presence of alkaloids in the root bark extract. This positive presence of alkaloids known to have analgesic activity is relevant with the plant remedy uses, particularly psychotic diseases.

### 3.2 Isolated Compound

The purification and recrystallization of the isolated product resulted in white precipitate melt at 212-213°C.  $^1\text{H-NMR}$  spectrum  $\delta$ /ppm of the compound (Fig. 1); singlets at  $\delta$  7.59, 7.45, 7.28, 6.99 and 6.49 corresponding to aromatic protons, whereas, a singlet appeared at  $\delta$  5.51, 3.63 ppm denoted to (1H, CH) and hydroxyl group, respectively. Beside, 0.88 ppm (m, 1H, CH), 1.62 ppm (m, 2H, CH<sub>2</sub>), 2.01 ppm (t, 3H, CH<sub>3</sub>) and 1.25 (d, 3H, CH<sub>3</sub>) accounted to methyl groups. Those  $^1\text{H}$  NMR data correlate with Lormetazepam (7-chloro-5-(2-chlorophenyl)-3-hydroxy-1-methyl-3H-1,4-benzodiazepin-2-one) spectral data, in which H-3 appears as singlet at 5.81 ppm and hydroxyl group at 3.65 ppm, in addition to 7.53, 7.48-7.53, 7.32, 7.24-7.30, 6.90 assigned to aromatic protons [29, 30]. APCI-MS

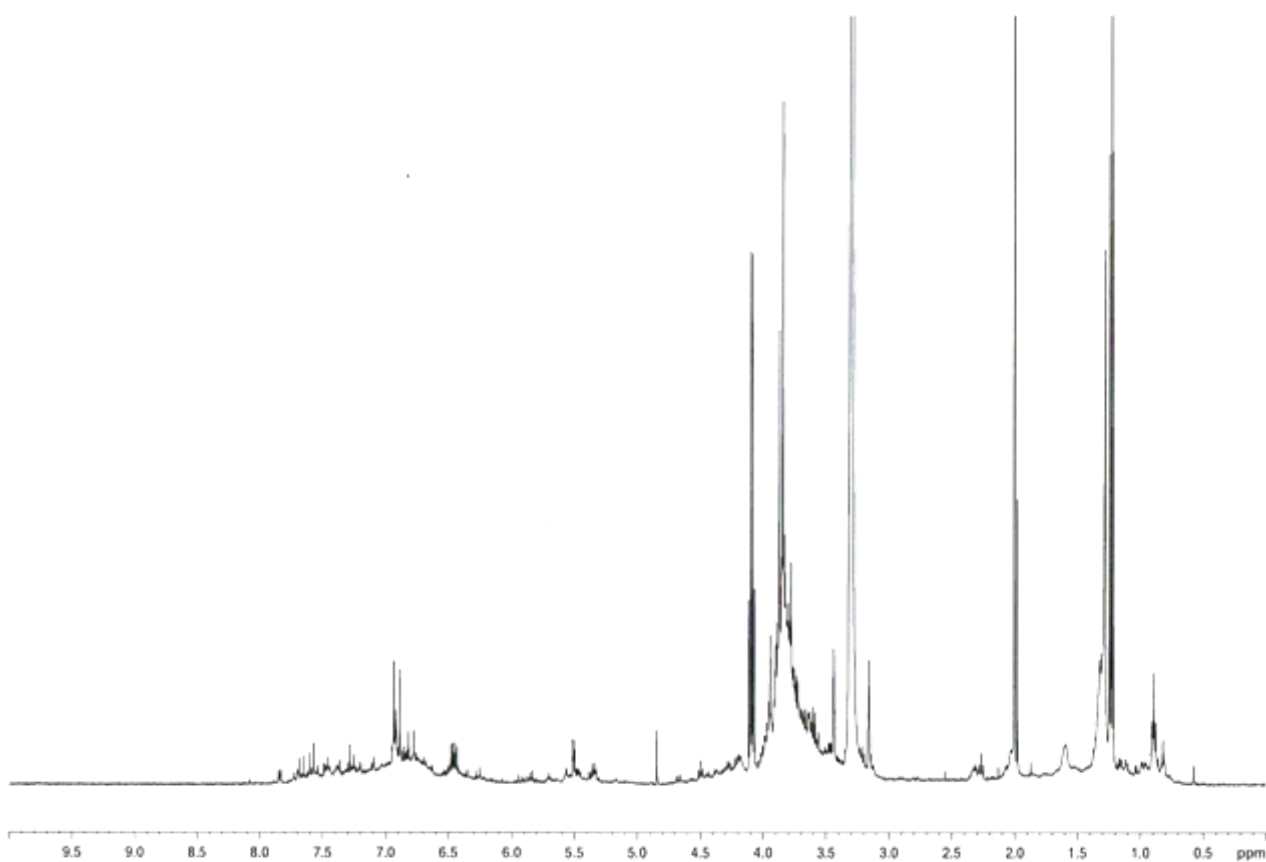


Fig. 1 <sup>1</sup>H NMR spectrum for the isolated compound.

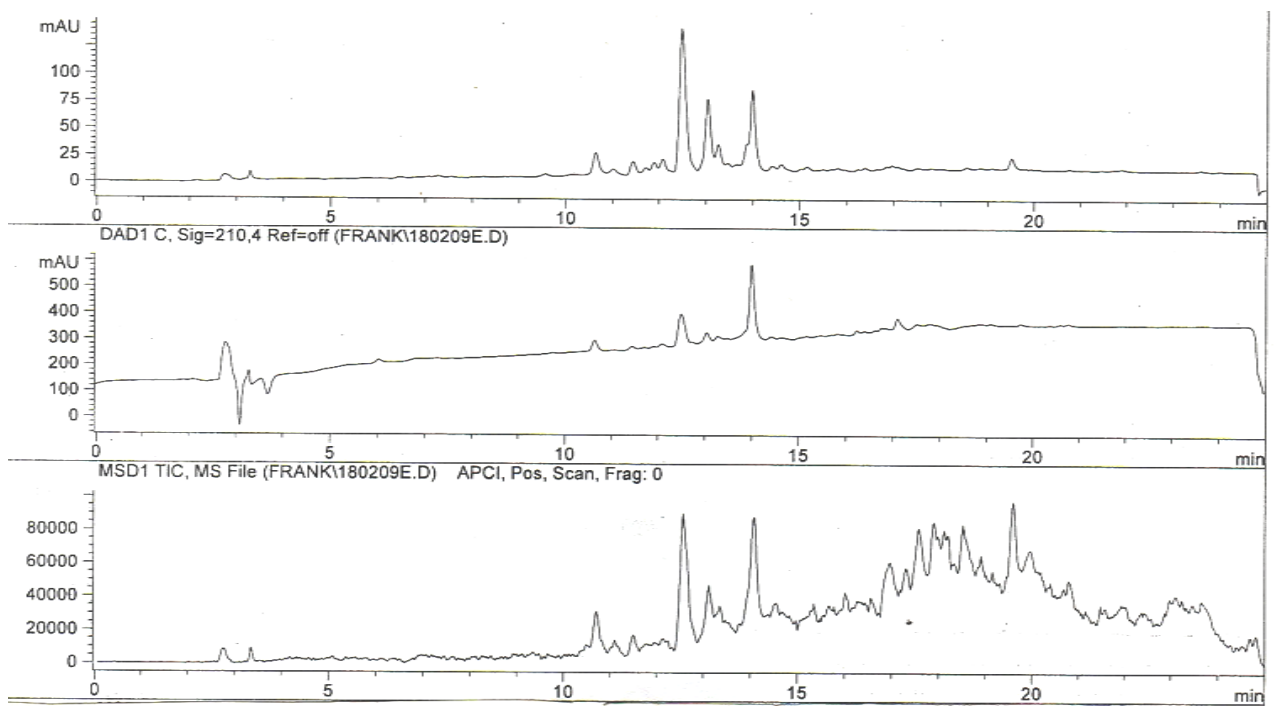
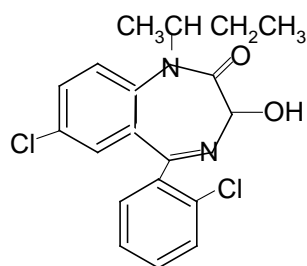


Fig. 2 LC-MS spectra for the isolated compound.



**Scheme 3.** 7-chloro-5-(2-chlorophenyl) 1-butyl, 3-hydroxy, 1,4-benzodiazepine-2-one.

spectrum (Fig. 2) showed molecular ion peak at  $m/z$  377, which corresponds to the proposed molecular formula  $C_{19}H_{18}Cl_2N_2O_2$ . Moreover, when treating an ethanolic solution of the compound with 2,4-dinitrophenylhydrazine hydrochloride in methanol, a yellow colour was formed, indicating the presence of a keto functional group as well as Lassaigne's confirmatory test positive result of the presence of both nitrogen and chlorine elements in the compound [26, 27]. Accordingly, the compound was identified as 7-chloro-5-(2-chlorophenyl)1-butyl, 3-hydroxy, 1,4-benzodiazepine-2-one and has not been separated and identified from *Carissa edulis* before.

#### 4. Conclusion

*Carissa edulis* root consists of alkaloids which can be regarded as precursor or nucleus of psycho active drugs. One compound isolated from the root extract 7-chloro-5-(2-chlorophenyl) 1-butyl, 3-hydroxy, 1,4-benzodiazepine-2-one, which was identified as benzodiazepines analogous highly addictive, as well as extremely effective for treating epilepsy, anxiety, insomnia, and alcohol withdrawal.

#### References

- [1] Achenbach, H., Weibal, R., and Addac-mensah, I. 1985. "Sesquiterpenes from *Carissa edulis*." *Phytochemistry* 24: 1056-67.
- [2] Omino, E. A., and Kokwaro, J. O. 1993. "Ethnobotany of Apocynaceae Species in Kenya." *J. Ethnopharmacol* 4: 167-80.
- [3] Gelfand, M., et al. 1985. "The Traditional Medical Practitioner in Zimbabwe." In *His Principles of Practice and Pharmacopoeia*, Vol. 17. Gweru: Mambo Press.
- [4] Achenbach, H., Waibel, R., and Addae-Mensah, I. 1983. "Lignans and other Constituents from *Carissa edulis*." *Phytochemistry* 22 (3): 749-53.
- [5] Bentley, M. D., Brackett, S. R., and Chapya, A. 1984. "2-Hydroxyacetophenone, Principal Root Volatile of the East African Medicinal Plant." *Carissa Edulis. J Nat Prod* 47: 1056-7.
- [6] El Ghazali, G. E. B., Abdalla, W. S., Khalid, H. S., Khalafalla, M. M., and Hamad, A. A.-D. 2003. "Medicinal Plants of the Sudan, Part V." In *Medicinal Plants of Ingassana Area*.
- [7] Ibrahim, H., and Bolaji, R. O. 2002. "Preliminary Phytochemical and Antimicrobial Studies of the Fruits of *Carissaedulis* Vahl." *J. Phytomed. Therapeutics* 7 (1-2): 94-101.
- [8] Nedi, T., Mekonnen, N., and Urga, K. 2004. "Diuretic Effects of the Crude Extracts of *Carissa edulis* in Rats." *Journal of Ethanopharmacology* 95 (1): 57-61.
- [9] Ibrahim, H., Bolaji, R. O., Abdurahman, E. M., Shok, M., Ilyas, N., and Habib, A. G. 2005. "Preliminary Phytochemical and Antimicrobial Studies of the Leaves of *Carrisa edulis* Vahl." *Chem Class Journal* 2: 15-8.
- [10] Ibrahim, H., Abdurahman, E. M., Shok, M., Ilyas, N., Musa, K. Y., and Ukandu, I. 2007. "Comparative Analgesic Activity of the Root Bark, Stem Bark, Leaves, Fruits and Seeds of *Carissa edulis* Vahl (Apocynaceae)." *African Journal of Biotechnology* 6 (10): 1233-5.
- [11] Ibrahim, H., Oyi, R. A., Ehinmidu, J. O., Musa, K. Y., and Bright, N. T. 2010. "Antimicrobial Activity of the Water Extracts of the Leaves and Fruits of *Carissa edulis* Vahl (Apocynaceae)." *Journal of Medicinal Plants Research* 4 (11): 1028-32.
- [12] Ya'u, J., Yaro, A. H., Abubaker, M. S., Anuka, J. A., and Hussainin, I. M. 2008. "Anticonvulsant Activity of *Carissa edulis* (Vahl) (Apocynaceae) Root Bark Extract." *J. Ethnopharmacol* 120 (2): 255-8. doi: 10.1016/J.jep.2008.08.029.
- [13] Ya'u, J., Chindo, B. A., Yaro, A. H., Okhale, S. E., Anuka, J. A., and Hussaini, I. M. 2013. "Safety Assessment of the Standardized Extract of *Carissa edulis* Root Bark in Rats." *J. Ethnopharmacol.* 147 (3): 653-61. doi: 10.1016/j.jep.2013.03.064.
- [14] Festus, T., Geoffrey, R., John, O., Quang, N., Hashimoto, K., and Asakawa, Y. 2008. "The Antiviral Activity of Compounds Isolated from Kenyan *Carissa edulis* (Forssk.) Vahl." *The Internet Journal of Alternative Medicine* 8 (1).
- [15] Al-Youssef, H. M., and Hassan, W. H. B. 2017. "Chemical Constituents of *Carissa edulis* Vahl." *Arabian Journal of Chemistry* 1 (10): 109-13.
- [16] Shorter, E. 2005. "Benzodiazepines." In *A Historical*

118 **Psychoactive Chemical Constituents of *Carissa edulis* (Forssk.) Vahl Roots and Its Safety Limits in Folk Treatment: Benzodiazepines Analogous**

- Dictionary of Psychiatry*. Oxford University Press, pp. 41-2. ISBN 978-0-19-517668-1.
- [17] Olkkola, K. T., and Ahonen, J. 2008. "Midazolam and Other Benzodiazepines." In *Modern Anesthetics. Handbook of Experimental Pharmacology*, edited by Schüttler, J., and Schwilden, H. pp. 182, 335-60. doi: 10.1007/978-3-540-74806-9\_16. ISBN: 978-3-540-72813-9. PMID 18175099.
- [18] Carlo, P., Finollo, R., Ledda, A., and Brambilla, G. 1989. "Absence of liver DNA Fragmentation in Rats Treated with High Oral Doses of 32 Benzodiazepine Drugs." *Fundamental and Applied Toxicology* 12 (1): 34-41. doi: 10.1016/0272-0590(89)90059-6. PMID 2925017.
- [19] Sand, P., Kavvadias, D., Feineis, D., Riederer, P., Schreier, P., Kleinschnitz, M., Czygan, F.-C., Abou-Mandour, A., Bringmann, G., and Beckmann, H. 2000. "Naturally Occurring Benzodiazepines: Current Status of Research and Clinical Implications." *European Archives of Psychiatry and Clinical Neuroscience* 250 (4): 194-202. doi: 10.1007/s004060070024.
- [20] Rahbæk, L., Breinholt, J., Frisvad, J. C., and Christophersen, C. 1999. "Circumdatin A, B, and C: Three New Benzodiazepine Alkaloids Isolated from a Culture of the Fungus *Aspergillus ochraceus*." *J. Org. Chem.* 64 (5): 1689-92. <https://doi.org/10.1021/jo981536u>.
- [21] Peng, J., Zhang, X.-Y., Tu, Z.-C., Xu, X.-Y., and Qi, S.-H. 2013. "Alkaloids from the Deep-Sea-Derived Fungus *Aspergillus westerdijkiae* DFFSCS013." *J. Nat. Prod.* 76 (5): 983-7. <https://doi.org/10.1021/np400132m>.
- [22] Wildmann, J., Vetter, W., Ranalder, U. B., Schmidt, K., Maurer, R., and Möhler, H. 1988. "Occurrence of Pharmacologically Active Benzodiazepines in Trace Amounts in Wheat and Potato." *Biochem Pharmacol* 37 (19): 3549-59. doi: 10.1016/0006-2952(88)90384-x.
- [23] Avallone, R., Cosenza, F., Farina, F., Baraldi, C., and Baraldi, M. 2002. "Extraction and Purification from *Ceratonia siliqua* of Compounds Acting on Central and Peripheral Benzodiazepine Receptors." *Fitoterapia* 73 (5): 390-6. doi: 10.1016/s0367-326x(02)00115-6.
- [24] Jain, K. K. 1999. *Benzodiazepines*. Accessed February 8, 2020 <https://www.medlink.com/article/benzodiazepines>.
- [25] <https://pubchem.ncbi.nlm.nih.gov/compound/Lormetazepam>.
- [26] Brady, O. L., and Elmslie, G. V. 1926. "The Use of 2,4-Dinitrophenylhydrazine as a Reagent for Aldehydes and Ketones." *Analyst* 51 (599): 77-8. doi:10.1039/AN9265100077.
- [27] Murray, P. R. S. 1977. "Identification of Organic Compound." In *Principles of Organic Chemistry: A Modern and Comprehensive Text for Schools and Colleges*, 2nd ed. Heinemann, pp. 61-4.
- [28] Harborne, J. 1973. *Phytochemical Methods: A Guide to Modern Techniques of Plants Analysis*. London: Chapman and Hall, Ltd., p. 279.
- [29] Moosmann, B., Bisel, P., and Auwärter, V. 2014. "Characterization of the Designer Benzodiazepine Diclazepam and Preliminary Data on Its Metabolism and Pharmacokinetics." In *Drug Testing and Analysis*, Wiley Online Library. <https://doi.org/10.1002/dta.1628>.
- [30] [http://www.molbase.com/en/hnmr\\_846-49-1-moldata-24702.html](http://www.molbase.com/en/hnmr_846-49-1-moldata-24702.html).