

# Alpha–Bungarotoxin, Beta–Bungarotoxin and Kappa–Bungarotoxin Time-Resolved Absorption and Resonance FT-IR and Raman Biospectroscopy and Density Functional Theory (DFT) Investigation of Vibronic-Mode Coupling Structure in Vibrational Spectra Analysis

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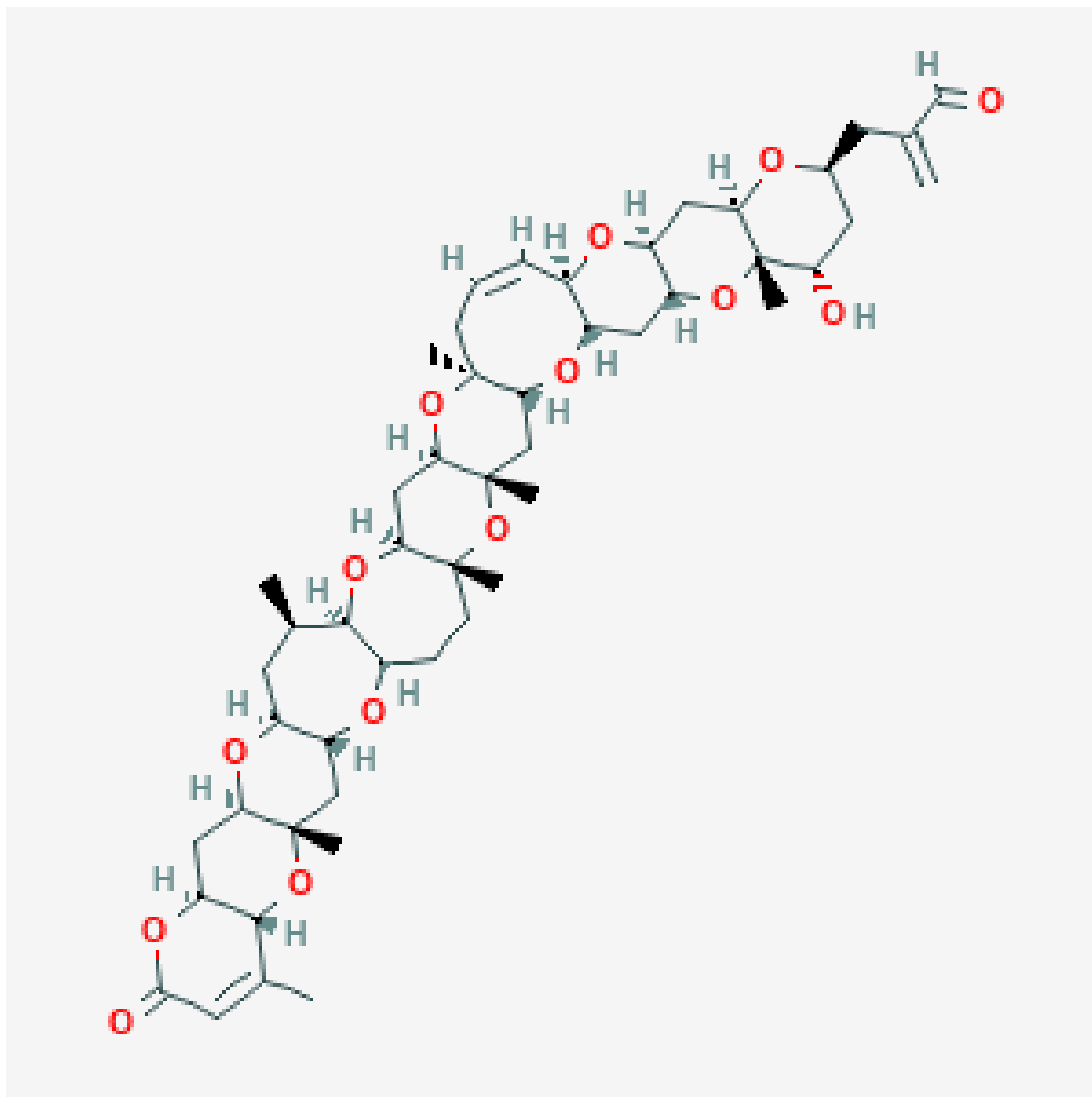
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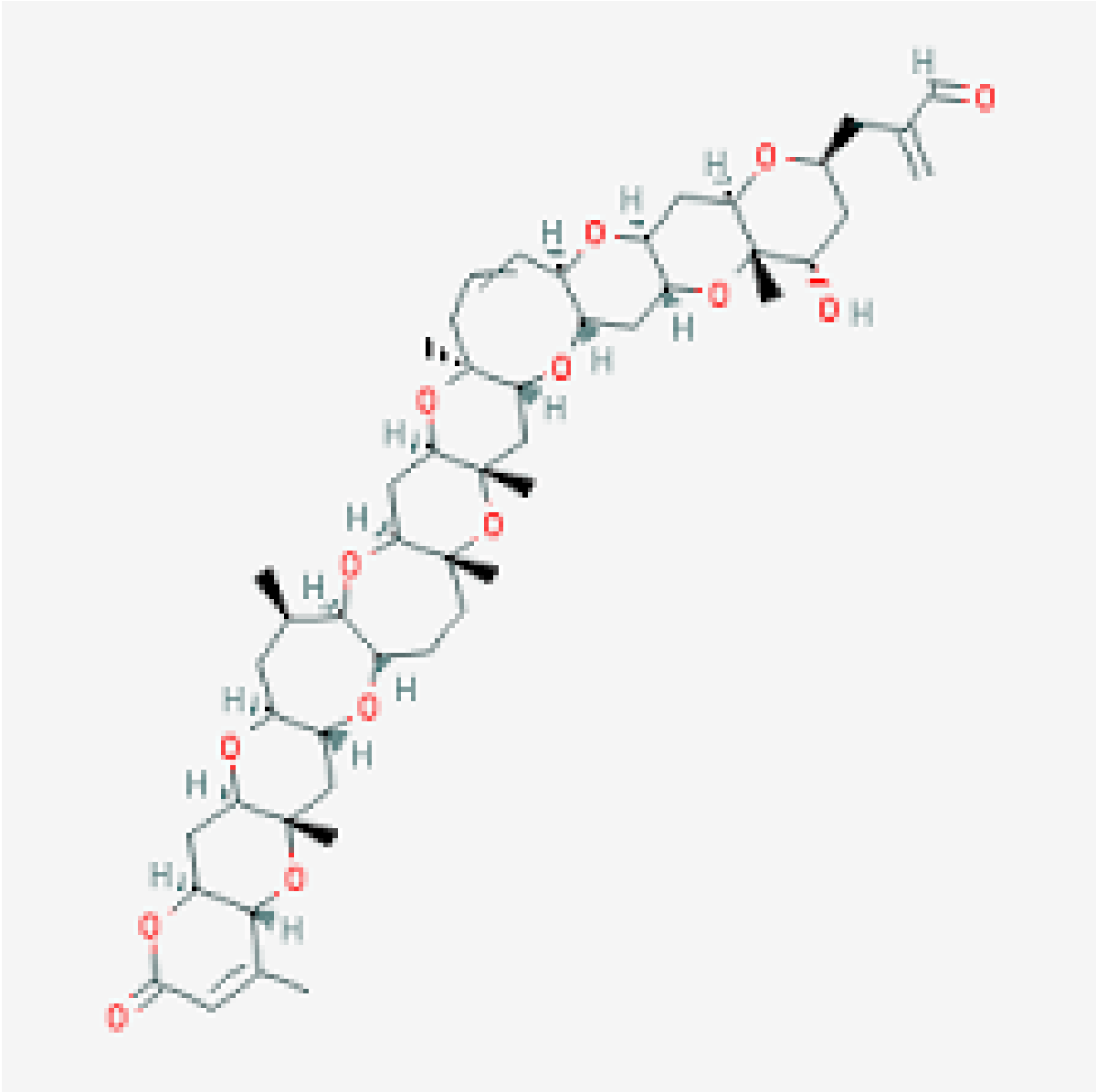
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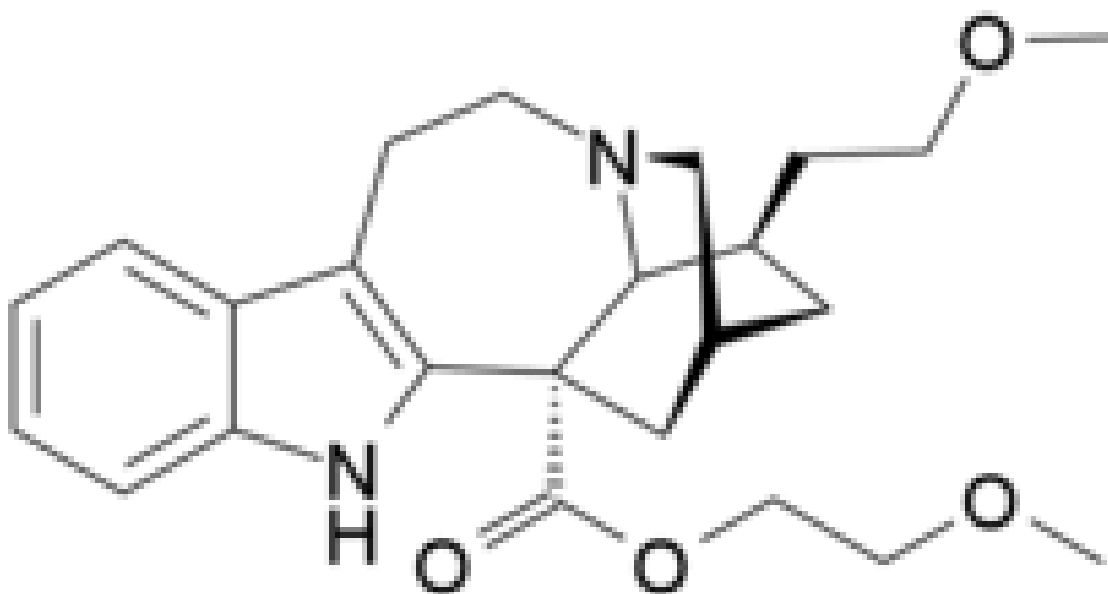
## Graphical Abstract

$\alpha$ -Bungarotoxin ( $\alpha$ -BTX) is one of the bungarotoxins, components of the venom of the elapid Taiwanese banded krait snake (*Bungarus Multicinctus*). It is a type of  $\alpha$ -neurotoxin, a neurotoxic protein that is known to bind competitively and in a relatively irreversible manner to the nicotinic acetylcholine receptor found at the neuromuscular junction, causing paralysis, respiratory failure, and death in the victim. It has also been shown to play an antagonistic role in the binding of the  $\alpha 7$  nicotinic acetylcholine receptor in the brain, and as such has numerous applications in neuroscience research. Kappa–bungarotoxin (often written  $\kappa$ -Bgt; historically also called toxin) is a protein neurotoxin of the bungarotoxin family that is found in the venom of the many-banded krait, a snake found in Taiwan. Kappa–bungarotoxin is a high affinity antagonist of nicotinic acetylcholine receptors (nAChRs), particularly of CHRNA3; it causes a post–synaptic blockade of neurotransmission. Although there is significant variability in the clinical effects of snake bites, neuromuscular paralysis and respiratory failure are associated with krait bites. Parameters such as FT-IR and Raman vibrational wavelengths and intensities for single crystal Alpha-Bungarotoxin, Beta-Bungarotoxin and Kappa-Bungarotoxin are calculated using density functional theory and were compared with empirical results. The investigation about vibrational spectrum of cycle dimers in crystal with carboxyl groups from each molecule of acid was shown that it leads to create Hydrogen bonds for adjacent molecules. The current study aimed to investigate the possibility of simulating the empirical values. Analysis of vibrational spectrum of Alpha–Bungarotoxin, Beta–Bungarotoxin and Kappa–Bungarotoxin is performed based on theoretical simulation and FT–IR empirical spectrum and Raman empirical spectrum using density functional theory in levels of HF/6–31G\*, HF/6–

31++G\*\*, MP2/6-31G, MP2/6-31++G\*\*, BLYP/6-31G, BLYP/6-31++G\*\*, B3LYP/6-31G and B3LYP6-31-HEG\*\*. Vibration modes of methylene, carboxyl acid and phenyl cycle are separately investigated. The obtained values confirm high accuracy and validity of results obtained from calculations.







Molecular structure of Alpha-Bungarotoxin (upper), Beta-Bungarotoxin (middle) and Kappa-Bungarotoxin (lower) [1-42].

**Keywords:** Alpha- Bungarotoxin; Beta- Bungarotoxin and Kappa- Bungarotoxin; Correlation Functions of Lee-Yang-Parr; Density Functional Theory (DFT); FT-IR and Raman Biospectroscopy; Time-Resolved Absorption and Resonance; Vibronic Structure; Vibrational Spectra Analysis

## Introduction

$\alpha$ -Bungarotoxin ( $\alpha$ -BTX) is one of the bungarotoxins, components of the venom of the elapid Taiwanese banded krait snake (*Bungarus Multicinctus*). It is a type of  $\alpha$ -neurotoxin, a neurotoxic protein that is known to bind competitively and in a relatively irreversible manner to the nicotinic acetylcholine receptor found at the neuromuscular junction, causing paralysis, respiratory failure, and death in the victim. It has also been shown to play an antagonistic role in the binding of the  $\alpha 7$  nicotinic acetylcholine receptor in the brain, and as such has numerous applications in neuroscience research. Kappa-bungarotoxin (often written  $\kappa$ -Bgt; historically also called toxin) is a protein neurotoxin of the bungarotoxin family that is found in the venom of the many-banded krait, a snake found in Taiwan. Kappa-bungarotoxin is a high affinity antagonist of nicotinic acetylcholine receptors (nAChRs), particularly of CHRNA3; it causes a post-synaptic blockade of neurotransmission. Although there is significant variability in the clinical effects of snake bites, neuromuscular paralysis and respiratory failure are associated with krait bites. Density Functional Theory (DFT) is one of the most powerful calculation methods for electronic structures [5-7]. Numerous results have been previously studied and indicate successful use of these methods [8-10]. The theory is one of the most appropriate methods for simulating the vibrational wavenumbers, molecular structure as well as total energy. It may be useful to initially consider the calculated results by density functional theory using HF/6-31G\*, HF/6-31++G\*\*, MP2/6-31G, MP2/6-31++G\*\*, BLYP/6-31G, BLYP/6-31++G\*\*, B3LYP/6-31G and B3LYP6-31-HEG\*\* approach [11-6]. It should be noted that

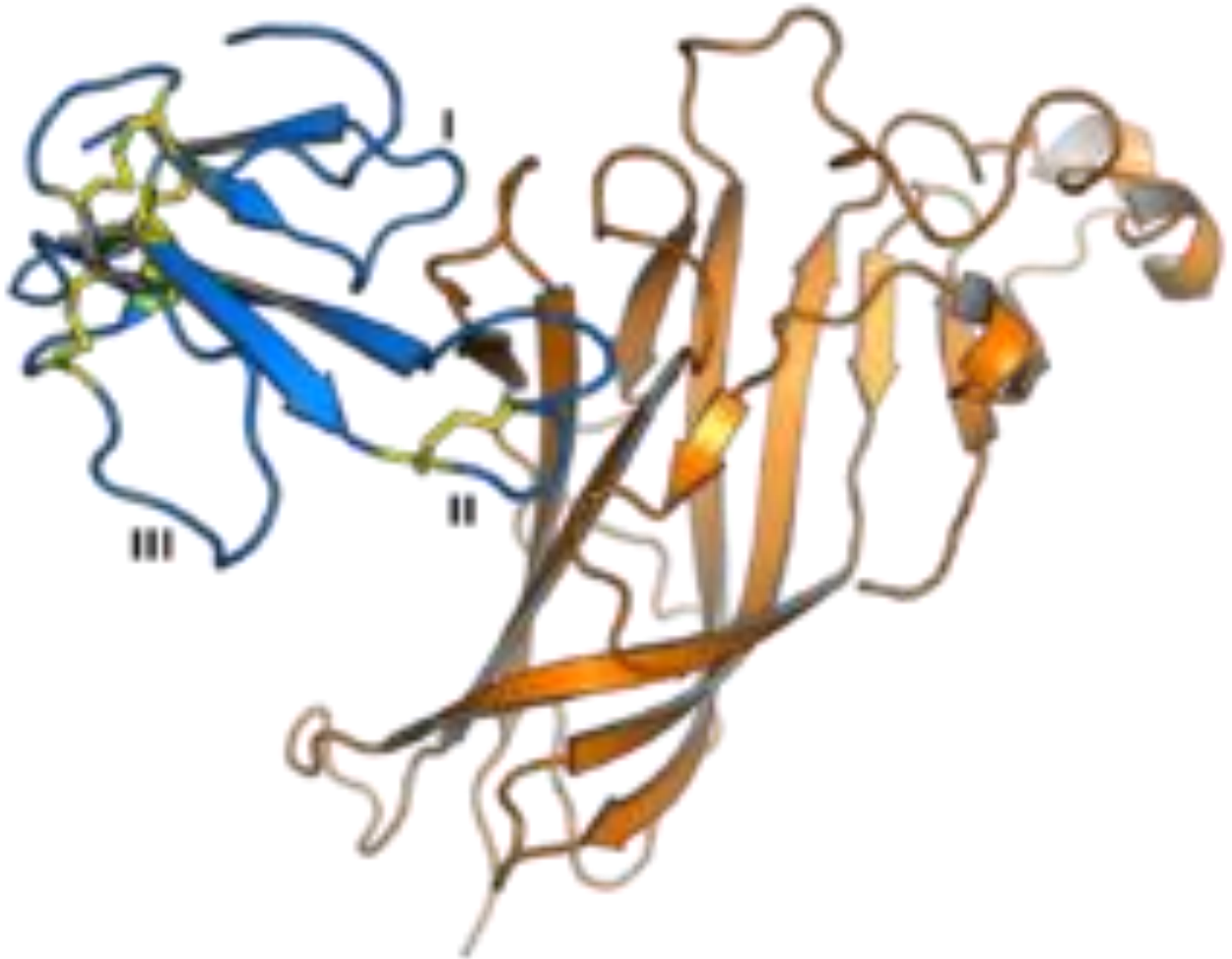
calculations are performed by considering one degree of quantum interference as well as polarization effects of 2d orbitals in interaction [17-47].

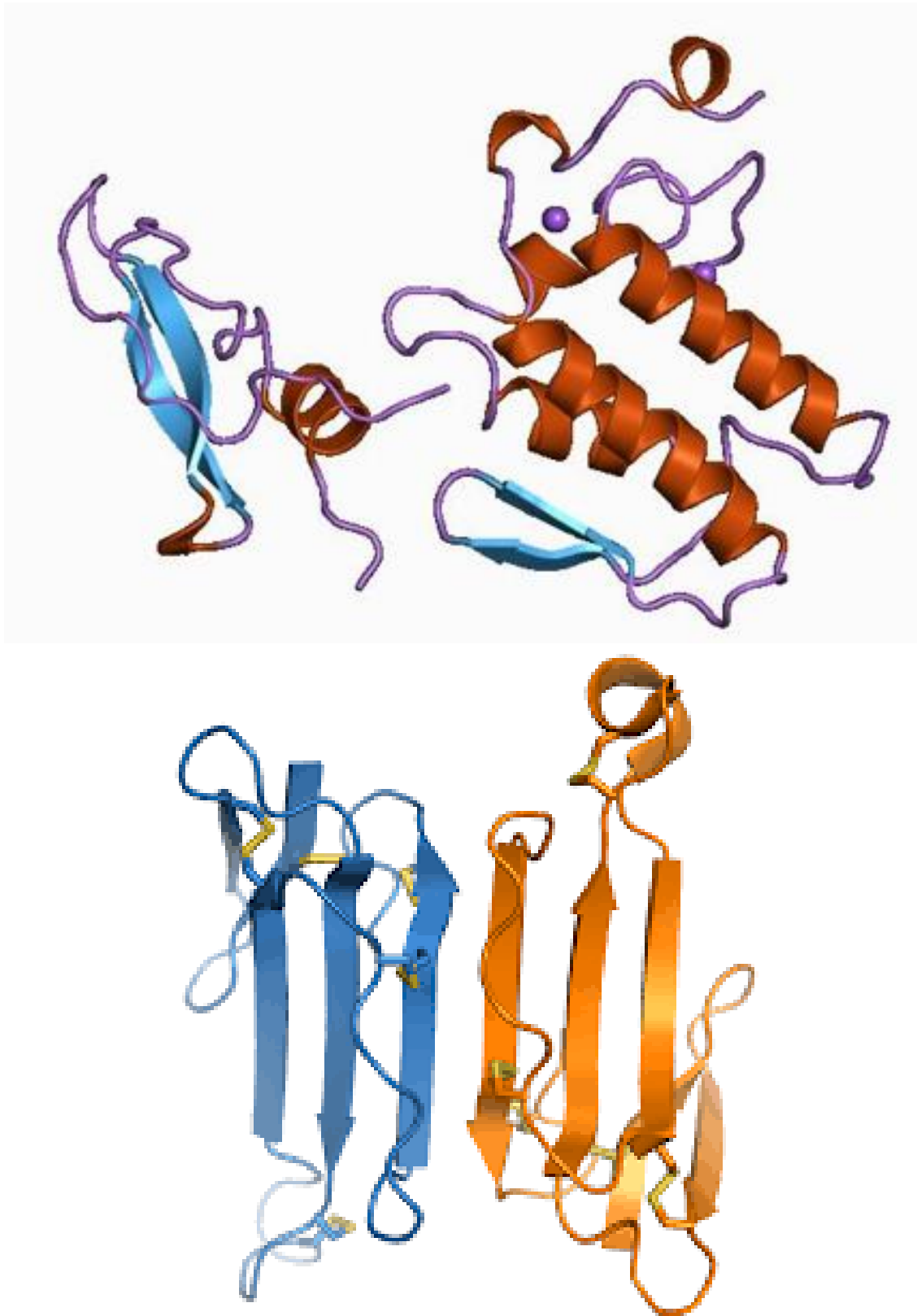
Alpha-Bungarotoxin, Beta-Bungarotoxin and Kappa-Bungarotoxin are tropical weed belonging to Toxins family. Amongst Toxins, Alpha-Bungarotoxin, Beta-Bungarotoxin and Kappa-Bungarotoxin contain anti-fungal activity, acetylcholinesterase inhibitory activity, anti-oxidant activity [48-64], mast cell stabilization and membrane protection activity [65-92], anti-bacterial activity [93-127] and anti-cancer activity [128-145], anti-hyperglycemic an anti-hyperlipidemic effects [146-156] and anti-arthritic activity, immunomodulatory activity [157-173] and anti-diabetic activity [174-188]. Our earlier report stated that the Alpha-Bungarotoxin, Beta-Bungarotoxin and Kappa-Bungarotoxin have high antioxidant activity [189-201]. The observations show that Alpha-Bungarotoxin, Beta-Bungarotoxin and Kappa-Bungarotoxin can be used for pharmaceutical applications. In this view, Alpha-Bungarotoxin, Beta-Bungarotoxin and Kappa-Bungarotoxin were taken and examined for its phytochemical and active principles in vitro antioxidant models and in silico approach for antihistamine activity. Free radicals are atoms with unpaired electrons which can cause various diseases. Intake of vitamin E can reduce the problems associated with free radicals in the body [202-210]. The unpaired electrons of free-radical accumulation cause oxidative stress in the body. Oxidative stress causes cell damage leading to various health issues such as chronic disease, cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular diseases, neurodegenerative diseases, respiratory disorders [211-219] and also the induced oxidative stress causes bronchial contraction by the release of cyclooxygenase and lipooxygenase in the airway that leads to bronchial asthma in human [220-226]. Asthma is a chronic inflammatory lung disease that happens due to the respiratory infection triggered by the inhalation of allergens like tobacco smoke, air pollutants, genetic and environment factors [227-233] which leads to the release of histamine and leukotrienes from the mast cell in the lung. The high release of histamine due to allergic reactions is regulated by histamine H1 receptor [234-326]. Histamine affects the immune response and related functions in human through H1, H2, H3 and H4 receptors activation with their intracellular signals [237-240]. The present research work demonstrates the chemotaxonomy of such valuable plant, from the genus of Alpha-Bungarotoxin, Beta-Bungarotoxin and Kappa-Bungarotoxin. In addition, pharmaceutical applications such as in vitro antioxidant and in silico anti-histamine activity of their active principles as natural remedy was examined.

### Details of Calculations

All calculations of molecular orbital in the base of ab are performed by Gaussian 09. In calculation process, the structure of Alpha-Bungarotoxin, Beta-Bungarotoxin and Kappa-Bungarotoxin molecule (Figure 1) is optimized and FT-IR and Raman wavenumbers are calculated using HF/6-31G\*, HF/6-31++G\*\*, MP2/6-31G, MP2/6-31++G\*\*, BLYP/6-31G, BLYP/6-31++G\*\*, B3LYP/6-31G and B3LYP6-31-HEG\*\* base [241]. All optimized structures are adjusted with minimum energy. Harmonic vibrational wavenumbers are calculated using second degree of derivation to adjust convergence on potential surface as good as possible and to evaluate vibrational energies at zero point. In optimized structures considered in the current study, virtual frequency modes are not observed which indicates that the minimum potential energy surface is correctly chosen. The optimized geometry is calculated by minimizing the energy relative to all geometrical quantities without forcing any constraint on molecular symmetry. Calculations were performed by Gaussian 09. The current calculation

is aimed to maximize structural optimization using density functional theory. The calculations of density functional theory are performed by HF/6-31G\*, HF/6-31++G\*\*, MP2/6-31G, MP2/6-31++G\*\*, BLYP/6-31G, BLYP/6-31++G\*\*, B3LYP/6-31G and B3LYP6-31-HEG\*\* function in which non-focal functions of Becke and correlation functions of Lee-Yang-Parr beyond the Franck-Condon approximation are used. After completion of optimization process, the second order derivation of energy is calculated as a function of core coordination and is investigated to evaluate whether the structure is accurately minimized. Vibrational frequencies used to simulate spectrums presented in the current study are derived from these second order derivatives. All calculations are performed for room temperature of 474 (K).





**Figure 1:** Different sections of the Alpha-Bungarotoxin (upper), Beta-Bungarotoxin (middle) and Kappa-Bungarotoxin (lower) [43-93].

## Vibration Analysis

Analysis of vibrational spectrum of Alpha-Bungarotoxin, Beta-Bungarotoxin and Kappa-Bungarotoxin is performed based on theoretical simulation and FT-IR empirical spectrum and Raman empirical spectrum using density functional theory in levels of HF/6-31G\*, HF/6-31++G\*\*, MP2/6-31G, MP2/6-31++G\*\*, BLYP/6-31G, BLYP/6-31++G\*\*, B3LYP/6-31G and B3LYP6-31-HEG\*\*. Vibration modes of methylene, carboxyl acid and phenyl cycle are separately investigated.

C-H stretching vibrations in single replacement of benzene cycles are usually seen in band range of 3201-3451  $\text{cm}^{-1}$ . Weak Raman bands are at 3190  $\text{cm}^{-1}$  and 3203  $\text{cm}^{-1}$ . C-C stretching mode is a strong Raman mode at 1200  $\text{cm}^{-1}$ . Raman weak band is seen at 1674  $\text{cm}^{-1}$ , too. Bending mode of C-H is emerged as a weak mode at 1399  $\text{cm}^{-1}$  and 1198  $\text{cm}^{-1}$  and a strong band at 1282  $\text{cm}^{-1}$  in Raman spectrum. Raman is considerably active in the range of 1201-1451  $\text{cm}^{-1}$  which 1194  $\text{cm}^{-1}$  indicates this issue.

C-H skew-symmetric stretching mode of methylene group is expected at 3186  $\text{cm}^{-1}$  and its symmetric mode is expected at 3000  $\text{cm}^{-1}$ . Skew-symmetric stretching mode of  $\text{CH}_2$  in Alpha-Bungarotoxin, Beta-Bungarotoxin and Kappa-Bungarotoxin has a mode in mid-range of Raman spectrum at 3101-3221  $\text{cm}^{-1}$ . When this mode is symmetric, it is at 3096  $\text{cm}^{-1}$  and is sharp. The calculated wavenumbers of higher modes are at 3064  $\text{cm}^{-1}$  and 3094  $\text{cm}^{-1}$  for symmetric and skew-symmetric stretching mode of methylene, respectively.

Scissoring vibrations of  $\text{CH}_2$  are usually seen at the range of 1528-1582  $\text{cm}^{-1}$  which often includes mid-range bands. Weak bands at 1541  $\text{cm}^{-1}$  are scissoring modes of  $\text{CH}_2$  in Raman spectrum. Moving vibrations of methylene are usually seen at 1470  $\text{cm}^{-1}$ . For the investigated chemical in the current study, these vibrations are at 1340  $\text{cm}^{-1}$  were calculated using density functional theory. Twisting and rocking vibrations of  $\text{CH}_2$  are seen in Raman spectrum at 916  $\text{cm}^{-1}$  and 1190  $\text{cm}^{-1}$ , respectively, which are in good accordance with the results at 900  $\text{cm}^{-1}$  and 1165  $\text{cm}^{-1}$ , respectively.

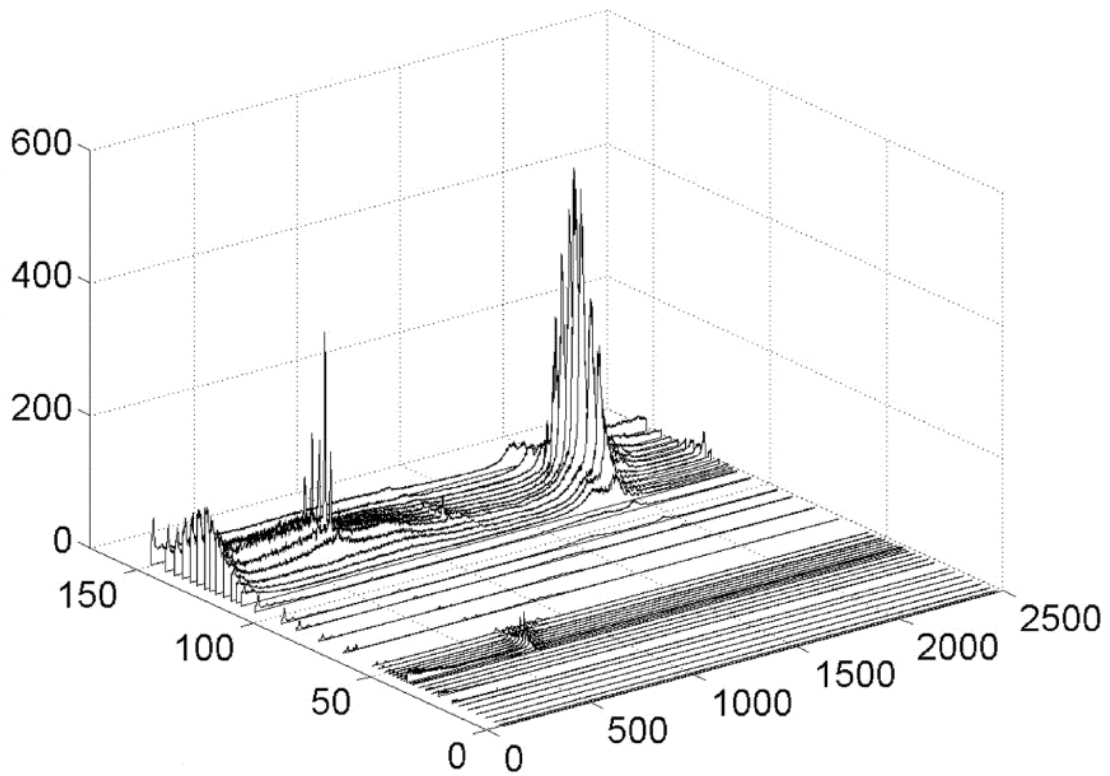
In a non-ionized carboxyl group ( $\text{COOH}$ ), stretching vibrations of carbonyl [ $\text{C=O}$ ] are mainly observed at the range of 1841-1889  $\text{cm}^{-1}$ . If dimer is considered as an intact constituent, two stretching vibrations of carbonyl for symmetric stretching are at 1741-1786  $\text{cm}^{-1}$  in Raman spectrum. In the current paper, stretching vibration of carbonyl mode is at 1798  $\text{cm}^{-1}$  which is a mid-range value.

Stretching and bending bands of hydroxyl can be identified by width and band intensity which in turn is dependent on bond length of Hydrogen. In dimer form of Hydrogen bond, stretching band of O-H is of a strong Raman peak at 1368  $\text{cm}^{-1}$  which is due to in-plane metamorphosis mode. Out-of-plane mode of O-H group is a very strong mode of peak at 1050  $\text{cm}^{-1}$  of Raman spectrum. The stretching mode of C-O (H) emerges as a mid-band of Raman spectrum at 1248  $\text{cm}^{-1}$ .

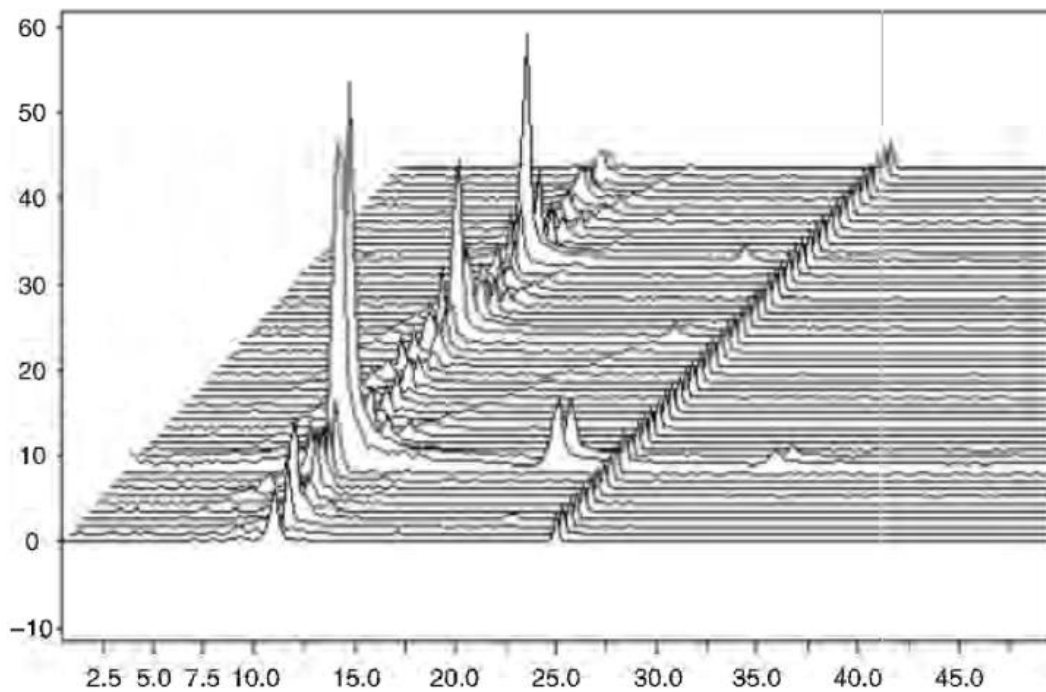
Lattice vibrations are usually seen at the range of 0-600  $\text{cm}^{-1}$ . These modes are induced by rotary and transferring vibrations of molecules and vibrations and are including Hydrogen bond. Bands with low wavenumbers of Hydrogen bond vibrations in FT-IR and Raman spectrum (Figure 2) are frequently weak, width and unsymmetrical. Rotary lattice vibrations are frequently stronger than transferring ones. Intra-molecular vibrations with low wavenumbers involving two-bands O-H ...O dimer at 89  $\text{cm}^{-1}$ , 194  $\text{cm}^{-1}$  and 250  $\text{cm}^{-1}$  are



attributed to a rotary moving of two molecules involving in-plane rotation of molecules against each other.



(a)



(b)

**Figure 2:** 3D Simulation of (a) FT-IR spectrum and (b) Raman spectrum of Alpha-Bungarotoxin.

## Conclusion and Summary

Calculations of density functional theory using HF/6-31G\*, HF/6-31++G\*\*, MP2/6-31G, MP2/6-31++G\*\*, BLYP/6-31G, BLYP/6-31++G\*\*, B3LYP/6-31G and B3LYP6-31-HEG\*\* levels were used to obtain vibrational wavenumbers and intensities in single crystal of Alpha-Bungarotoxin, Beta-Bungarotoxin and Kappa-Bungarotoxin. Investigation and consideration of vibrational spectrum confirm the formation of dimer cycles in the investigated crystal with carboxyl groups from each Hydrogen molecule of acid protected from adjacent molecules. The calculated vibrational spectrum which obtains from calculations of density functional theory is in good accordance with recorded empirical values which indicates successful simulation of the problem. The obtained results indicate that the results obtained from theoretical calculations are valid through comparing with empirical recorded results.

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