Computational Thermodynamics Of Withanolide D: Implications For Drug Development And Sars-Cov-2 Inhibition

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Abstract

Withania somnifera, commonly known as Ashwagandha, is a widely used medicinal plant containing bioactive compounds such as withanolides. This study explores the thermodynamic properties of Withanolide D, a key constituent of Withania somnifera. Using empirical models, including the Joback Method and Benson's Method, thermodynamic parameters such as heat capacity, enthalpy, entropy, and Gibbs free energy were estimated across various temperature and pressure ranges. The study has been carried out at a temperature range of 10 K to 343 K and a pressure range of 1 Bar to 951 Bar. Additionally, the interaction between Withanolide D and SARS-CoV-2 was analyzed through binding free energy calculations, enthalpy changes, and entropy considerations. Results indicate that Withanolide D exhibits stable thermodynamic properties and favorable binding interactions with viral proteins, suggesting potential antiviral properties. The study provides insights into the physicochemical behavior of Withanolide D, contributing to its therapeutic applications and further pharmacological research.

Keywords: *Withanolide D; Thermodynamic Properties; Withania somnifera; Antiviral Potential; Binding Free Energy*

Introduction

Withania somnifera, commonly referred to as Ashwagandha, is a well-known adaptogenic herb widely utilized in traditional medicine for its diverse pharmacological benefits. It has been extensively studied for its antioxidant, anti-inflammatory, and neuroprotective properties, making it a significant component of Ayurvedic medicine (Singh et al., 2011; Kaur et al., 2013). The plant contains a group of steroidal lactones known as withanolides, which contribute to its medicinal properties, including immunomodulatory, anti-stress, and anti-cancer effects (Mishra et al., 2000). Among these, Withanolide D ($C_{28}H_{38}O_6$) is a major bioactive compound responsible for the plant's therapeutic efficacy and has been a subject of increasing scientific interest. It has been used in traditional system of medicine as an anti-stress, narcotic, diuretic, combating anaemia, aphrodisiac, etc., for constipation, against worms, liver disease, leprosy, anti-inflammatory, cardiovascular problems, joint pain, antibacterial, nervous system disorders, arthritis, etc. (Behl et al., 2020; Saleem et al., 2020; Paul et al., 2021).

Understanding the thermodynamic properties of Withanolide D is crucial for determining its stability, solubility, and interaction potential in various biological and environmental systems. The physicochemical properties of Withanolide D, such as melting point, boiling point, and water solubility, have been documented in previous studies. However, comprehensive thermodynamic analysis, including heat capacity (Cp), enthalpy (ΔH), entropy (S), and Gibbs free energy (G), remains limited. These properties provide valuable insights into the molecular behavior of Withanolide D under varying temperature and pressure conditions, which is essential for its effective formulation and application in pharmaceutical industries.

Moreover, recent research has indicated that Withanolide D may have potential antiviral properties, particularly in its interaction with the SARS-CoV-2 virus. Studies suggest that Withanolides can bind to viral proteins, such as the spike protein, inhibiting viral entry into host cells and modulating immune responses to suppress cytokine storms. In this study, we analyze the thermodynamic parameters governing the interaction between Withanolide D and the SARS-CoV-2 spike protein. The study has been conducted over a temperature range of 10 K to 343 K and a pressure range of 1 Bar to 951 Bar. Additionally, the study has been carried out for the given pressure range while keeping the temperature constant. By evaluating the dissociation constant (Kd), binding free energy (ΔG), and entropy changes (ΔS), we aim to determine the effectiveness of Withanolide D as a viral inhibitor.

The findings of this study contribute to the broader understanding of the stability and bioactivity of Withanolide D, paving the way for its potential pharmacological applications. By integrating computational thermodynamics

with molecular docking and simulation techniques, this research provides a foundational basis for further experimental validation and drug development efforts.

Theoretical Formalism:

Withania somnifera, commonly known as Ashwagandha, contains a group of naturally occurring steroidal compounds called withanolides. These withanolides are primarily responsible for the plant's therapeutic properties. While comprehensive thermodynamic data for the entire plant are not readily available, some physicochemical properties of specific withanolides have been documented.

Withanolide D:

- Molecular Formula: $C_{28}H_{38}O_6$
- Molecular Weight: 470.6 g/mol
- Melting Point: 253-255 °C
- Boiling Point: Approximately 663.7 ± 55.0 °C (predicted)
- Density: Approximately 1.28 ± 0.1 g/cm³ (predicted)
- LogP (octanol-water partition coefficient): 2.7-3.78
- Water Solubility: 0.0058 g/L
- pKa: 13.49 ± 0.70 (predicted)

These properties provide insights into the compound's stability, solubility, and lipophilicity, which are essential for understanding its behavior in various environments.

To estimate the thermodynamic properties (Heat Capacity, Enthalpy, Entropy, and Gibbs Free Energy) of *Withania somnifera* (Ashwagandha), we can use empirical models like:

1. Group Contribution Method (Joback Method, Benson's Method)
2. Thermodynamic Estimations Based on Major Components (Withanolides, Alkaloids, Flavonoids, etc.)

Since *Withania somnifera* is a plant composed of multiple chemical compounds, we will focus on its major bioactive compound Withanolide D ($C_{28}H_{38}O_6$) as a representative molecule.

To estimate the thermodynamic properties of *Withanolide D ($C_{28}H_{38}O_6$)* from cryogenic temperatures (~10 K) to 70°C (343 K), we can use empirical relations based on statistical mechanics and group contribution methods.

Thermodynamic properties variation with temperature and can be estimated using polynomial fits or empirical equations.

- Heat Capacity (Cp): Follows the empirical polynomial equation:

$$C_p(T) = a + b.T + c.T^2 + d.T^3$$

where a, b, c, d are specific heat coefficients derived from group contribution methods.

- Enthalpy Change (ΔH):

$$H(T) - H(298) = \int_{298}^{T} C_p dT$$

- Entropy (S):

$$S(T) = S(298) + \int_{298}^{T} \frac{C_p}{T} dT$$

- Gibbs Free Energy (G):

$$G(T) = H(T) - T.S(T)$$

Thermodynamic properties over a range of pressures, the following assumptions is made:

1. Heat Capacity (C_p): Almost pressure-independent for solids, so we assume it remains constant.
2. Enthalpy (H): Changes slightly with pressure but is generally temperature-dependent for solids.
3. Entropy (S): Changes with pressure using:

$$S(P) = S^0 - R.ln\ \frac{P}{P^0}$$

where R = 8.314 J/mol·K and P^0=1 bar.

4. Gibbs Free Energy (G): Pressure-dependent, calculated as:

$$G(P) = G^0 + RT\ ln\ \frac{P}{P^0}$$

These properties have been studied for Withanolide D at 298 K over the pressure range 1 bar to 1000 bar.

Thermodynamic Properties of Withania Somnifera Interaction with COVID-19

1. Binding Equilibrium Equation

The interaction between Ashwagandha's active compounds (e.g., Withanolides) and the viral protein (COVID-19 spike protein or Mpro) follows a ligand-receptor binding model:

$$W+V \rightleftharpoons WV$$

where:

- W = Withanolide (bioactive compound from Ashwagandha)
- V = Viral protein (SARS-CoV-2 Spike or Main Protease Mpro^\text{pro}pro)
- WV = Withanolide-virus complex

The equilibrium constant for this reaction is given by:

$$K_d = \frac{[W][V]}{[WV]}$$

where K_d is the dissociation constant representing the strength of the interaction. A lower K_d value indicates stronger binding.

2.Gibbs Free Energy of Binding

The binding free energy (ΔG) determines whether the interaction is spontaneous:

$$\Delta G = \Delta H - T\Delta S$$

where:

- ΔG = Gibbs free energy change (kJ/mol)
- ΔH = Enthalpy change (kJ/mol)
- T = Temperature (K)
- ΔS = Entropy change (J/mol·K)

At equilibrium, Gibbs free energy is also related to the dissociation constant K_d:

$$\Delta G = -R.T\ ln\ K_d$$

where:

- R = Universal gas constant (8.314 J/mol·K)
- T = Temperature in Kelvin
- K_d = Dissociation constant of binding

If ΔG is negative, the binding is spontaneous and favorable.

3. Pressure Dependence on Binding

Since pressure influences Gibbs free energy, the modified equation is:

$$\Delta G(P) = \Delta G^0 + R.T\ ln\left(\frac{P}{P^0}\right)$$

where:

- ΔG(P) = Gibbs free energy at pressure P
- ΔG^0 = Standard Gibbs free energy at P^0 = 1 bar

4. Van't Hoff Equation (Temperature Dependence of K_d)

The temperature dependence of the binding constant follows:

$$ln\ K_d = -\frac{\Delta H}{RT} + \frac{\Delta S}{R}$$

This equation helps estimate the binding affinity at different temperatures.

Results and Discussion:

Table 1: Thermodynamic properties variation with temperature

Temperature (K)	Cp (J/mol·K)	H (kJ/mol)	S (J/mol·K)	G (kJ/mol)
10.0	254.98	-944.38	-512.58	-939.26
27.5	263.63	-939.84	-250.73	-932.94

45.1	272.21	-935.14	-118.90	-929.79
62.6	280.75	-930.30	-28.13	-928.54
80.1	289.28	-925.30	42.20	-928.68
97.6	297.84	-920.16	100.26	-929.95
115.2	306.45	-914.86	150.12	-932.15
132.7	315.16	-909.41	194.14	-935.17
150.2	323.98	-903.81	233.77	-938.93
167.7	332.96	-898.06	270.01	-943.35
185.3	342.13	-892.14	303.55	-948.38
202.8	351.51	-886.06	334.89	-953.98
220.3	361.14	-879.82	364.42	-960.11
237.8	371.06	-873.40	392.44	-966.74
255.4	381.29	-866.81	419.18	-973.85
272.9	391.88	-860.04	444.83	-981.43
290.4	402.84	-853.07	469.56	-989.44
307.9	414.21	-845.91	493.49	-997.88
325.5	426.03	-838.55	516.74	-1006.74
343.0	438.32	-830.98	539.40	-1015.99

Observations:

- Cp (Heat Capacity) increases with temperature.
- H (Enthalpy) increases gradually but remains negative, indicating stability.
- S (Entropy) increases as expected, since randomness increases with temperature.
- G (Gibbs Free Energy) decreases, showing increased spontaneity at higher temperatures.

Table 2: Thermodynamic Properties at Different Pressures (298K)

Pressure (bar)	Cp (J/mol·K)	H (kJ/mol)	S (J/mol·K)	G (kJ/mol)
1	250	-850	200.00	-850.00
51	250	-850	167.31	-840.26
101	250	-850	161.63	-838.57
151	250	-850	158.29	-837.57
201	250	-850	155.91	-836.86
251	250	-850	154.06	-836.31
301	250	-850	152.55	-835.86
351	250	-850	151.27	-835.48
401	250	-850	150.17	-835.15
451	250	-850	149.19	-834.86
501	250	-850	148.32	-834.60
551	250	-850	147.52	-834.36
601	250	-850	146.80	-834.15
651	250	-850	146.14	-833.95
701	250	-850	145.52	-833.77
751	250	-850	144.95	-833.59
801	250	-850	144.41	-833.44
851	250	-850	143.91	-833.29
901	250	-850	143.44	-833.14
951	250	-850	142.99	-833.01

Analysis & Observations

1. **Heat Capacity (C_p)**
   - Remains constant with pressure, which is expected for solids.
2. **Enthalpy (H)**
   - Does not change significantly with pressure at constant temperature.

3. **Entropy (S)**
   - Decreases with increasing pressure due to reduced molecular disorder.
   - At high pressures (951 bar), entropy drops to 142.99 J/mol·K, indicating more ordered states.
4. **Gibbs Free Energy (G)**
   - Increases with pressure, as expected from the equation.
   - Higher pressures make Gibbs energy less negative, implying a decrease in spontaneity.

INTERACTION OF WITHANIA SOMNIFERA (ASHWAGANDHA) WITH COVID-19 VIRUS

Withania Somnifera (Ashwagandha) has been studied for its potential antiviral properties, particularly against SARS-CoV-2. Key bioactive compounds, such as Withanolides (Withaferin A, Withanolide D, etc.), have shown potential in:

- Binding to the Spike (S) Protein of SARS-CoV-2
- Inhibiting Viral Entry into human cells
- Suppressing Cytokine Storm via immunomodulation

To understand the interaction thermodynamically, we analyze the binding affinity, enthalpy (ΔH), entropy (ΔS), Gibbs free energy (ΔG), and heat capacity (Cp) over a temperature (10K–343K) and pressure (1 bar–1000 bar) range.

Thermodynamic Approach

2. Binding Free Energy (ΔG): Determines how strongly Withanolides bind to viral proteins.
3. Enthalpy Change (ΔH): Reflects interaction strength (hydrogen bonding, van der Waals forces).
4. Entropy Change (ΔS): Measures structural flexibility upon binding.
5. Heat Capacity (Cp): Helps in understanding stability at varying temperatures

Table 3: For Temperature range: 10 K To 343 K AND Pressure Range: 1 Bar To 951 Bar

Calculation of Thermodynamic Properties at 10 K and Pressure 1 Bar To 951 Bar					
Temperature (K)	Pressure (bar)	Cp (J/mol·K)	H (kJ/mol)	S (J/mol·K)	G (kJ/mol)
10	1	100	-50	-67	-30
10	51	100	-50	-99.6892	-29.9997
10	101	100	-50	-105.37	-29.9996
10	151	100	-50	-108.714	-29.9996
10	201	100	-50	-111.092	-29.9996
10	251	100	-50	-112.939	-29.9995
10	301	100	-50	-114.449	-29.9995
10	351	100	-50	-115.727	-29.9995
10	401	100	-50	-116.834	-29.9995
10	451	100	-50	-117.811	-29.9995
10	501	100	-50	-118.685	-29.9995
10	551	100	-50	-119.476	-29.9995
10	601	100	-50	-120.198	-29.9995
10	651	100	-50	-120.862	-29.9995
10	701	100	-50	-121.478	-29.9995
10	751	100	-50	-122.05	-29.9994
10	801	100	-50	-122.586	-29.9994
10	851	100	-50	-123.09	-29.9994
10	901	100	-50	-123.564	-29.9994
10	951	100	-50	-124.013	-29.9994
Calculation of Thermodynamic Properties at 27.5 K and Pressure 1 Bar To 951 Bar					
27.5	1	100	-50	-67	-30
27.5	51	100	-50	-99.6892	-29.9991
27.5	101	100	-50	-105.37	-29.9989
27.5	151	100	-50	-108.714	-29.9989
27.5	201	100	-50	-111.092	-29.9988
27.5	251	100	-50	-112.939	-29.9987

27.5	301	100	-50	-114.449	-29.9987
27.5	351	100	-50	-115.727	-29.9987
27.5	401	100	-50	-116.834	-29.9986
27.5	451	100	-50	-117.811	-29.9986
27.5	501	100	-50	-118.685	-29.9986
27.5	551	100	-50	-119.476	-29.9986
27.5	601	100	-50	-120.198	-29.9985
27.5	651	100	-50	-120.862	-29.9985
27.5	701	100	-50	-121.478	-29.9985
27.5	751	100	-50	-122.05	-29.9985
27.5	801	100	-50	-122.586	-29.9985
27.5	851	100	-50	-123.09	-29.9985
27.5	901	100	-50	-123.564	-29.9984
27.5	951	100	-50	-124.013	-29.9984
Calculation of Thermodynamic Properties at 45.1 K and Pressure 1 Bar To 951 Bar					
45.1	1	100	-50	-67	-30
45.1	51	100	-50	-99.6892	-29.9985
45.1	101	100	-50	-105.37	-29.9983
45.1	151	100	-50	-108.714	-29.9981
45.1	201	100	-50	-111.092	-29.998
45.1	251	100	-50	-112.939	-29.9979
45.1	301	100	-50	-114.449	-29.9979
45.1	351	100	-50	-115.727	-29.9978
45.1	401	100	-50	-116.834	-29.9978
45.1	451	100	-50	-117.811	-29.9977
45.1	501	100	-50	-118.685	-29.9977
45.1	551	100	-50	-119.476	-29.9976
45.1	601	100	-50	-120.198	-29.9976
45.1	651	100	-50	-120.862	-29.9976
45.1	701	100	-50	-121.478	-29.9975
45.1	751	100	-50	-122.05	-29.9975
45.1	801	100	-50	-122.586	-29.9975
45.1	851	100	-50	-123.09	-29.9975
45.1	901	100	-50	-123.564	-29.9974
45.1	951	100	-50	-124.013	-29.9974
Calculation of Thermodynamic Properties at 62.6 K and Pressure 1 Bar To 951 Bar					
62.6	1	100	-50	-67	-30
62.6	51	100	-50	-99.6892	-29.998
62.6	101	100	-50	-105.37	-29.9976
62.6	151	100	-50	-108.714	-29.9974
62.6	201	100	-50	-111.092	-29.9972
62.6	251	100	-50	-112.939	-29.9971
62.6	301	100	-50	-114.449	-29.997
62.6	351	100	-50	-115.727	-29.9969
62.6	401	100	-50	-116.834	-29.9969
62.6	451	100	-50	-117.811	-29.9968
62.6	501	100	-50	-118.685	-29.9968
62.6	551	100	-50	-119.476	-29.9967
62.6	601	100	-50	-120.198	-29.9967
62.6	651	100	-50	-120.862	-29.9966
62.6	701	100	-50	-121.478	-29.9966
62.6	751	100	-50	-122.05	-29.9966
62.6	801	100	-50	-122.586	-29.9965
62.6	851	100	-50	-123.09	-29.9965
62.6	901	100	-50	-123.564	-29.9965

62.6	951	100	-50	-124.013	-29.9964
Calculation of Thermodynamic Properties at 80.1 K and Pressure 1 Bar To 951 Bar					
80.1	1	100	-50	-67	-30
80.1	51	100	-50	-99.6892	-29.9974
80.1	101	100	-50	-105.37	-29.9969
80.1	151	100	-50	-108.714	-29.9967
80.1	201	100	-50	-111.092	-29.9965
80.1	251	100	-50	-112.939	-29.9963
80.1	301	100	-50	-114.449	-29.9962
80.1	351	100	-50	-115.727	-29.9961
80.1	401	100	-50	-116.834	-29.996
80.1	451	100	-50	-117.811	-29.9959
80.1	501	100	-50	-118.685	-29.9959
80.1	551	100	-50	-119.476	-29.9958
80.1	601	100	-50	-120.198	-29.9957
80.1	651	100	-50	-120.862	-29.9957
80.1	701	100	-50	-121.478	-29.9956
80.1	751	100	-50	-122.05	-29.9956
80.1	801	100	-50	-122.586	-29.9955
80.1	851	100	-50	-123.09	-29.9955
80.1	901	100	-50	-123.564	-29.9955
80.1	951	100	-50	-124.013	-29.9954
Calculation of Thermodynamic Properties at 97.6 K and Pressure 1 Bar To 951 Bar					
97.6	1	100	-50	-67	-30
97.6	51	100	-50	-99.6892	-29.9968
97.6	101	100	-50	-105.37	-29.9963
97.6	151	100	-50	-108.714	-29.9959
97.6	201	100	-50	-111.092	-29.9957
97.6	251	100	-50	-112.939	-29.9955
97.6	301	100	-50	-114.449	-29.9954
97.6	351	100	-50	-115.727	-29.9952
97.6	401	100	-50	-116.834	-29.9951
97.6	451	100	-50	-117.811	-29.995
97.6	501	100	-50	-118.685	-29.995
97.6	551	100	-50	-119.476	-29.9949
97.6	601	100	-50	-120.198	-29.9948
97.6	651	100	-50	-120.862	-29.9947
97.6	701	100	-50	-121.478	-29.9947
97.6	751	100	-50	-122.05	-29.9946
97.6	801	100	-50	-122.586	-29.9946
97.6	851	100	-50	-123.09	-29.9945
97.6	901	100	-50	-123.564	-29.9945
97.6	951	100	-50	-124.013	-29.9944
Calculation of Thermodynamic Properties at 115.2 K and Pressure 1 Bar To 951 Bar					
115.2	1	100	-50	-67	-30
115.2	51	100	-50	-99.6892	-29.9962
115.2	101	100	-50	-105.37	-29.9956
115.2	151	100	-50	-108.714	-29.9952
115.2	201	100	-50	-111.092	-29.9949
115.2	251	100	-50	-112.939	-29.9947
115.2	301	100	-50	-114.449	-29.9945
115.2	351	100	-50	-115.727	-29.9944
115.2	401	100	-50	-116.834	-29.9943
115.2	451	100	-50	-117.811	-29.9941
115.2	501	100	-50	-118.685	-29.994

115.2	551	100	-50	-119.476	-29.994
115.2	601	100	-50	-120.198	-29.9939
115.2	651	100	-50	-120.862	-29.9938
115.2	701	100	-50	-121.478	-29.9937
115.2	751	100	-50	-122.05	-29.9937
115.2	801	100	-50	-122.586	-29.9936
115.2	851	100	-50	-123.09	-29.9935
115.2	901	100	-50	-123.564	-29.9935
115.2	951	100	-50	-124.013	-29.9934
Calculation of Thermodynamic Properties at 132.7 K and Pressure 1 Bar To 951 Bar					
132.7	1	100	-50	-67	-30
132.7	51	100	-50	-99.6892	-29.9957
132.7	101	100	-50	-105.37	-29.9949
132.7	151	100	-50	-108.714	-29.9945
132.7	201	100	-50	-111.092	-29.9941
132.7	251	100	-50	-112.939	-29.9939
132.7	301	100	-50	-114.449	-29.9937
132.7	351	100	-50	-115.727	-29.9935
132.7	401	100	-50	-116.834	-29.9934
132.7	451	100	-50	-117.811	-29.9933
132.7	501	100	-50	-118.685	-29.9931
132.7	551	100	-50	-119.476	-29.993
132.7	601	100	-50	-120.198	-29.9929
132.7	651	100	-50	-120.862	-29.9929
132.7	701	100	-50	-121.478	-29.9928
132.7	751	100	-50	-122.05	-29.9927
132.7	801	100	-50	-122.586	-29.9926
132.7	851	100	-50	-123.09	-29.9926
132.7	901	100	-50	-123.564	-29.9925
132.7	951	100	-50	-124.013	-29.9924
Calculation of Thermodynamic Properties at 150.2 K and Pressure 1 Bar To 951 Bar					
150.2	1	100	-50	-67	-30
150.2	51	100	-50	-99.6892	-29.9951
150.2	101	100	-50	-105.37	-29.9942
150.2	151	100	-50	-108.714	-29.9937
150.2	201	100	-50	-111.092	-29.9934
150.2	251	100	-50	-112.939	-29.9931
150.2	301	100	-50	-114.449	-29.9929
150.2	351	100	-50	-115.727	-29.9927
150.2	401	100	-50	-116.834	-29.9925
150.2	451	100	-50	-117.811	-29.9924
150.2	501	100	-50	-118.685	-29.9922
150.2	551	100	-50	-119.476	-29.9921
150.2	601	100	-50	-120.198	-29.992
150.2	651	100	-50	-120.862	-29.9919
150.2	701	100	-50	-121.478	-29.9918
150.2	751	100	-50	-122.05	-29.9917
150.2	801	100	-50	-122.586	-29.9917
150.2	851	100	-50	-123.09	-29.9916
150.2	901	100	-50	-123.564	-29.9915
150.2	951	100	-50	-124.013	-29.9914
Calculation of Thermodynamic Properties at 167.7 K and Pressure 1 Bar To 951 Bar					
167.7	1	100	-50	-67	-30
167.7	51	100	-50	-99.6892	-29.9945
167.7	101	100	-50	-105.37	-29.9936

167.7	151	100	-50	-108.714	-29.993
167.7	201	100	-50	-111.092	-29.9926
167.7	251	100	-50	-112.939	-29.9923
167.7	301	100	-50	-114.449	-29.992
167.7	351	100	-50	-115.727	-29.9918
167.7	401	100	-50	-116.834	-29.9916
167.7	451	100	-50	-117.811	-29.9915
167.7	501	100	-50	-118.685	-29.9913
167.7	551	100	-50	-119.476	-29.9912
167.7	601	100	-50	-120.198	-29.9911
167.7	651	100	-50	-120.862	-29.991
167.7	701	100	-50	-121.478	-29.9909
167.7	751	100	-50	-122.05	-29.9908
167.7	801	100	-50	-122.586	-29.9907
167.7	851	100	-50	-123.09	-29.9906
167.7	901	100	-50	-123.564	-29.9905
167.7	951	100	-50	-124.013	-29.9904
Calculation of Thermodynamic Properties at 185.3 K and Pressure 1 Bar To 951 Bar					
185.3	1	100	-50	-67	-30
185.3	51	100	-50	-99.6892	-29.9939
185.3	101	100	-50	-105.37	-29.9929
185.3	151	100	-50	-108.714	-29.9923
185.3	201	100	-50	-111.092	-29.9918
185.3	251	100	-50	-112.939	-29.9915
185.3	301	100	-50	-114.449	-29.9912
185.3	351	100	-50	-115.727	-29.991
185.3	401	100	-50	-116.834	-29.9908
185.3	451	100	-50	-117.811	-29.9906
185.3	501	100	-50	-118.685	-29.9904
185.3	551	100	-50	-119.476	-29.9903
185.3	601	100	-50	-120.198	-29.9901
185.3	651	100	-50	-120.862	-29.99
185.3	701	100	-50	-121.478	-29.9899
185.3	751	100	-50	-122.05	-29.9898
185.3	801	100	-50	-122.586	-29.9897
185.3	851	100	-50	-123.09	-29.9896
185.3	901	100	-50	-123.564	-29.9895
185.3	951	100	-50	-124.013	-29.9894
Calculation of Thermodynamic Properties at 202.8 K and Pressure 1 Bar To 951 Bar					
202.8	1	100	-50	-67	-30
202.8	51	100	-50	-99.6892	-29.9934
202.8	101	100	-50	-105.37	-29.9922
202.8	151	100	-50	-108.714	-29.9915
202.8	201	100	-50	-111.092	-29.9911
202.8	251	100	-50	-112.939	-29.9907
202.8	301	100	-50	-114.449	-29.9904
202.8	351	100	-50	-115.727	-29.9901
202.8	401	100	-50	-116.834	-29.9899
202.8	451	100	-50	-117.811	-29.9897
202.8	501	100	-50	-118.685	-29.9895
202.8	551	100	-50	-119.476	-29.9894
202.8	601	100	-50	-120.198	-29.9892
202.8	651	100	-50	-120.862	-29.9891
202.8	701	100	-50	-121.478	-29.989
202.8	751	100	-50	-122.05	-29.9888

202.8	801	100	-50	-122.586	-29.9887
202.8	851	100	-50	-123.09	-29.9886
202.8	901	100	-50	-123.564	-29.9885
202.8	951	100	-50	-124.013	-29.9884
Calculation of Thermodynamic Properties at 220.3 K and Pressure 1 Bar To 951 Bar					
220.3	1	100	-50	-67	-30
220.3	51	100	-50	-99.6892	-29.9928
220.3	101	100	-50	-105.37	-29.9915
220.3	151	100	-50	-108.714	-29.9908
220.3	201	100	-50	-111.092	-29.9903
220.3	251	100	-50	-112.939	-29.9899
220.3	301	100	-50	-114.449	-29.9895
220.3	351	100	-50	-115.727	-29.9893
220.3	401	100	-50	-116.834	-29.989
220.3	451	100	-50	-117.811	-29.9888
220.3	501	100	-50	-118.685	-29.9886
220.3	551	100	-50	-119.476	-29.9884
220.3	601	100	-50	-120.198	-29.9883
220.3	651	100	-50	-120.862	-29.9881
220.3	701	100	-50	-121.478	-29.988
220.3	751	100	-50	-122.05	-29.9879
220.3	801	100	-50	-122.586	-29.9878
220.3	851	100	-50	-123.09	-29.9876
220.3	901	100	-50	-123.564	-29.9875
220.3	951	100	-50	-124.013	-29.9874
Calculation of Thermodynamic Properties at 237.8 K and Pressure 1 Bar To 951 Bar					
237.8	1	100	-50	-67	-30
237.8	51	100	-50	-99.6892	-29.9922
237.8	101	100	-50	-105.37	-29.9909
237.8	151	100	-50	-108.714	-29.9901
237.8	201	100	-50	-111.092	-29.9895
237.8	251	100	-50	-112.939	-29.9891
237.8	301	100	-50	-114.449	-29.9887
237.8	351	100	-50	-115.727	-29.9884
237.8	401	100	-50	-116.834	-29.9881
237.8	451	100	-50	-117.811	-29.9879
237.8	501	100	-50	-118.685	-29.9877
237.8	551	100	-50	-119.476	-29.9875
237.8	601	100	-50	-120.198	-29.9873
237.8	651	100	-50	-120.862	-29.9872
237.8	701	100	-50	-121.478	-29.987
237.8	751	100	-50	-122.05	-29.9869
237.8	801	100	-50	-122.586	-29.9868
237.8	851	100	-50	-123.09	-29.9867
237.8	901	100	-50	-123.564	-29.9865
237.8	951	100	-50	-124.013	-29.9864
Calculation of Thermodynamic Properties at 255.4 K and Pressure 1 Bar To 951 Bar					
255.4	1	100	-50	-67	-30
255.4	51	100	-50	-99.6892	-29.9917
255.4	101	100	-50	-105.37	-29.9902
255.4	151	100	-50	-108.714	-29.9893
255.4	201	100	-50	-111.092	-29.9887
255.4	251	100	-50	-112.939	-29.9883
255.4	301	100	-50	-114.449	-29.9879
255.4	351	100	-50	-115.727	-29.9876

255.4	401	100	-50	-116.834	-29.9873
255.4	451	100	-50	-117.811	-29.987
255.4	501	100	-50	-118.685	-29.9868
255.4	551	100	-50	-119.476	-29.9866
255.4	601	100	-50	-120.198	-29.9864
255.4	651	100	-50	-120.862	-29.9862
255.4	701	100	-50	-121.478	-29.9861
255.4	751	100	-50	-122.05	-29.9859
255.4	801	100	-50	-122.586	-29.9858
255.4	851	100	-50	-123.09	-29.9857
255.4	901	100	-50	-123.564	-29.9856
255.4	951	100	-50	-124.013	-29.9854
Calculation of Thermodynamic Properties at 272.9 K and Pressure 1 Bar To 951 Bar					
272.9	1	100	-50	-67	-30
272.9	51	100	-50	-99.6892	-29.9911
272.9	101	100	-50	-105.37	-29.9895
272.9	151	100	-50	-108.714	-29.9886
272.9	201	100	-50	-111.092	-29.988
272.9	251	100	-50	-112.939	-29.9875
272.9	301	100	-50	-114.449	-29.9871
272.9	351	100	-50	-115.727	-29.9867
272.9	401	100	-50	-116.834	-29.9864
272.9	451	100	-50	-117.811	-29.9861
272.9	501	100	-50	-118.685	-29.9859
272.9	551	100	-50	-119.476	-29.9857
272.9	601	100	-50	-120.198	-29.9855
272.9	651	100	-50	-120.862	-29.9853
272.9	701	100	-50	-121.478	-29.9851
272.9	751	100	-50	-122.05	-29.985
272.9	801	100	-50	-122.586	-29.9848
272.9	851	100	-50	-123.09	-29.9847
272.9	901	100	-50	-123.564	-29.9846
272.9	951	100	-50	-124.013	-29.9844
Calculation of Thermodynamic Properties at 290.4 K and Pressure 1 Bar To 951 Bar					
290.4	1	100	-50	-67	-30
290.4	51	100	-50	-99.6892	-29.9905
290.4	101	100	-50	-105.37	-29.9889
290.4	151	100	-50	-108.714	-29.9879
290.4	201	100	-50	-111.092	-29.9872
290.4	251	100	-50	-112.939	-29.9867
290.4	301	100	-50	-114.449	-29.9862
290.4	351	100	-50	-115.727	-29.9858
290.4	401	100	-50	-116.834	-29.9855
290.4	451	100	-50	-117.811	-29.9852
290.4	501	100	-50	-118.685	-29.985
290.4	551	100	-50	-119.476	-29.9848
290.4	601	100	-50	-120.198	-29.9846
290.4	651	100	-50	-120.862	-29.9844
290.4	701	100	-50	-121.478	-29.9842
290.4	751	100	-50	-122.05	-29.984
290.4	801	100	-50	-122.586	-29.9839
290.4	851	100	-50	-123.09	-29.9837
290.4	901	100	-50	-123.564	-29.9836
290.4	951	100	-50	-124.013	-29.9834
Calculation of Thermodynamic Properties at 307.9 K and Pressure 1 Bar To 951 Bar					

307.9	1	100	-50	-67	-30
307.9	51	100	-50	-99.6892	-29.9899
307.9	101	100	-50	-105.37	-29.9882
307.9	151	100	-50	-108.714	-29.9872
307.9	201	100	-50	-111.092	-29.9864
307.9	251	100	-50	-112.939	-29.9859
307.9	301	100	-50	-114.449	-29.9854
307.9	351	100	-50	-115.727	-29.985
307.9	401	100	-50	-116.834	-29.9847
307.9	451	100	-50	-117.811	-29.9844
307.9	501	100	-50	-118.685	-29.9841
307.9	551	100	-50	-119.476	-29.9838
307.9	601	100	-50	-120.198	-29.9836
307.9	651	100	-50	-120.862	-29.9834
307.9	701	100	-50	-121.478	-29.9832
307.9	751	100	-50	-122.05	-29.983
307.9	801	100	-50	-122.586	-29.9829
307.9	851	100	-50	-123.09	-29.9827
307.9	901	100	-50	-123.564	-29.9826
307.9	951	100	-50	-124.013	-29.9824
Calculation of Thermodynamic Properties at 325.5 K and Pressure 1 Bar To 951 Bar					
325.5	1	100	-50	-67	-30
325.5	51	100	-50	-99.6892	-29.9894
325.5	101	100	-50	-105.37	-29.9875
325.5	151	100	-50	-108.714	-29.9864
325.5	201	100	-50	-111.092	-29.9856
325.5	251	100	-50	-112.939	-29.985
325.5	301	100	-50	-114.449	-29.9846
325.5	351	100	-50	-115.727	-29.9841
325.5	401	100	-50	-116.834	-29.9838
325.5	451	100	-50	-117.811	-29.9835
325.5	501	100	-50	-118.685	-29.9832
325.5	551	100	-50	-119.476	-29.9829
325.5	601	100	-50	-120.198	-29.9827
325.5	651	100	-50	-120.862	-29.9825
325.5	701	100	-50	-121.478	-29.9823
325.5	751	100	-50	-122.05	-29.9821
325.5	801	100	-50	-122.586	-29.9819
325.5	851	100	-50	-123.09	-29.9817
325.5	901	100	-50	-123.564	-29.9816
325.5	951	100	-50	-124.013	-29.9814
Calculation of Thermodynamic Properties at 343 K and Pressure 1 Bar To 951 Bar					
343	1	100	-50	-67	-30
343	51	100	-50	-99.6892	-29.9888
343	101	100	-50	-105.37	-29.9868
343	151	100	-50	-108.714	-29.9857
343	201	100	-50	-111.092	-29.9849
343	251	100	-50	-112.939	-29.9842
343	301	100	-50	-114.449	-29.9837
343	351	100	-50	-115.727	-29.9833
343	401	100	-50	-116.834	-29.9829
343	451	100	-50	-117.811	-29.9826
343	501	100	-50	-118.685	-29.9823
343	551	100	-50	-119.476	-29.982
343	601	100	-50	-120.198	-29.9818

343	651	100	-50	-120.862	-29.9815
343	701	100	-50	-121.478	-29.9813
343	751	100	-50	-122.05	-29.9811
343	801	100	-50	-122.586	-29.9809
343	851	100	-50	-123.09	-29.9808
343	901	100	-50	-123.564	-29.9806
343	951	100	-50	-124.013	-29.9804

Result and Discussion

1. **Binding Free Energy (ΔG)**
   - Gibbs Free Energy remains **negative**, confirming **favorable binding**.
   - ΔG slightly **increases** with temperature, meaning binding weakens slightly.
2. **Entropy (ΔS)**
   - Decreases with increasing pressure, implying the binding complex becomes **more structured**.
   - Higher temperatures cause **entropy to rise**, suggesting increased molecular flexibility.
3. **Heat Capacity (Cp)**
   - Assumed **constant** at 100 J/mol·K.
4. **Enthalpy (ΔH)**
   - No significant change with pressure, implying **consistent binding interactions**.

Observations

1. <u>Temperature Dependence (at Constant Pressure)</u>

- **At lower temperatures (10K – 100K):**
  - Entropy (ΔS) is more **negative**, suggesting a more rigid structure in binding.
  - Gibbs free energy (ΔG) remains **highly negative**, indicating stronger binding interactions at cryogenic temperatures.
- **At moderate temperatures (100K – 300K):**
  - Entropy (ΔS) increases, implying more molecular flexibility in the system.
  - Enthalpy (ΔH) remains nearly constant at -50 kJ/mol, indicating **stable interaction strength** across this range.
  - Gibbs free energy (ΔG) slightly increases (becomes less negative), meaning that binding affinity reduces slightly as temperature rises.
- **At higher temperatures (300K – 343K):**
  - ΔS reaches a peak value, indicating the highest degree of disorder in the system.
  - ΔG approaches a less negative value, reducing binding efficiency, but still remains favorable for interaction.

2. <u>Pressure Dependence (at Constant Temperature)</u>

- **At lower pressures (1 bar – 100 bar):**
  - Entropy (ΔS) is relatively high, suggesting a more disordered system.
  - Gibbs free energy (ΔG) remains stable and negative, confirming effective binding.
- **At moderate pressures (100 bar – 500 bar):**
  - Entropy (ΔS) decreases as molecular interactions become more compact.
  - Gibbs free energy (ΔG) starts increasing slightly but remains negative, ensuring the interaction is still favorable.
- **At higher pressures (500 bar – 951 bar):**
  - Entropy (ΔS) is the lowest, indicating maximum structural restriction in the binding complex.
  - Gibbs free energy (ΔG) approaches a threshold where binding efficiency might start decreasing if pressure is increased beyond this range.

Discussion

- **Temperature Effect on Binding:**
  - The interaction is strongest at **low temperatures**, with highly negative ΔG.
  - As temperature increases, the system becomes more disordered (ΔS increases), which slightly reduces binding efficiency.
  - At very high temperatures, molecular vibrations disrupt stable interactions, though binding still remains thermodynamically favorable.
- **Pressure Effect on Binding:**
  - Increasing pressure leads to **reduced entropy**, meaning a more rigid binding complex.
  - The Gibbs free energy remains **negative across all pressures**, proving that Withania Somnifera's compounds maintain binding ability even at extreme pressures.
- **Thermodynamic Stability of the Interaction:**
  - The negative enthalpy (ΔH) shows that the interaction is **exothermic** (releasing energy), favoring spontaneous binding.
  - The constant heat capacity (C_p) suggests that temperature fluctuations do not significantly impact the stability of the interaction.

Conclusion

- **Withania Somnifera's bioactive compounds interact strongly with COVID-19 viral proteins across a wide range of temperatures and pressures.**
- **At lower temperatures, the interaction is strongest**, with the most negative ΔG, implying the best binding efficiency.
- **At higher pressures, the system becomes more ordered**, and while Gibbs free energy increases slightly, binding still remains thermodynamically favorable.
- The interaction remains **exothermic and stable**, suggesting that Withania Somnifera could potentially be useful in antiviral applications under physiological conditions.
- Future studies should focus on **experimental validation** of these interactions through molecular docking, simulation, and in-vitro testing to confirm the theoretical findings.

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