IN-SILICO AND IN-VITRO ANTI-DIABETIC ACTIVITY OF *CASSIA AURICULATA, GYMNBEMA SYLVESTRE* AND *TINOSPORA CORDIFOLIA*

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Abstract

This research was performed to study the selected medicinal plants *Cassia auriculata, Gymnema sylvestre*, and *Tinospora cordifolia* against *invitro* and *in silico* antidiabetic activity. These plants were collected and extracted with different solvents. The alcohol extracts of these plants were used in the *in vitro* study. The major active constituents were identified from the selected medicinal plants, and 3D structures of the active constituents were retrieved from pubchem chemical databases or chemsketch software. Docking studies were carried out on selected phytochemicals against alpha amylase using Molegro virtual docker. Inhibition of alpha-amylase enzyme *in an in vitro* study was performed by using the standard protocol against plant extracts. *In-silico* docking results show the ability of the phytoconstituents to bind with the targets, as measured by the moldock score. The moldock score was used as the parameter for analyzing the docking results, by which the phytoconstituents were ranked. The ligand possessing the highest molecular dock score showed a strong affinity towards its target. Ranking based on moldock score and H-bonding of phytoconstituents from *Cassia auriculata*, *Gymnema sylvestre*, and *Tinospora cordifolia* on alpha amylase (PDB ID: 3OLD). *In-vitro* antidiabetic activity was performed by the alpha-amylase inhibition method. Inhibition of the alpha amylase enzyme would delay the degradation of carbohydrates, which would in turn cause a decrease in the absorption of glucose and a reduction in postprandial blood glucose levels. Based upon the experimental evidences, it was found that *Gymnema sylvestre* (92%) possesses higher antidiabetic activity when compared to *Cassia auriculata* (79%) and *Tinospora cordifolia* (64%). The *in-silico* and *in-vitro* studies reveal that *Gymnema sylvestre* possesses the potential diabetic control activity against the alpha amylase.

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Keywords: *in-silico; in-vitro*; anti-diabetic; *Cassia auriculata; Gymnema sylvestre; Tinospora cordifolia.*
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INTRODUCTION

Diabetes mellitus is a disease caused by inadequate control of blood glucose levels. As a result of widespread social pressure and shifting lifestyles, it has become a major issue across the country. In India, diabetes was once only seen in the upper class, but this perception has changed throughout the years. Many of the synthetic drugs used in treatment also have undesirable side effects. Herbal medicine is a major component of traditional medicine and a common element in ayurvedic, homeopathic, naturopathic, traditional oriental, and native American Indian medicine. However, the plant sources cause fewer side effects when compared with synthetic medicines. Recently, there has been a shift in the universal trend from synthetic to herbal medicine, which we can call "a return to nature. The aims and objectives of this study are comparative antidiabetic activity of some selected medicinal plants by *in-silico* and *in-vitro* methods. The literature overview of decided on medicinal flowers *Cassia auriculata*, *Gymnema sylvestre*, and*Tinospora cordifolia* changed into substantially surveyed, and these three medicinal florae have been used in this gift have a look at; they belong to the households fabaceae, asclepiadaceae, and menispermaceae, respectively. Various parts of these plants, such as leaves, flowers, barks, roots, stems, and seeds, possess a wide range of pharmacological actions, of which anti-diabetic activity is a significant one. There are many studies that report various therapeutic actions of these herbs, like anti-fungal,[1] anti-anthelmintic,[2] anti-oxidant,[3][4][5] anti-microbial,[6][7][8][9] anti-tumor,[10][11] hepatoprotective, laxative, and diuretic activity. Among these, the anti-diabetic activity of these plants has also been studied using various experimental methods.[12][13][14] However, there is no scientific publication that has compared these three plant species by an alpha amylase inhibition method using *in-silico* and *in-vitro* techniques. Hence, this research work was undertaken .

RESOURCES AND TECHNIQUES:

The process of making plant extracts (maceration)

Tinospora cordifolia, *Cassia auriculata*, and *Gymnema sylvestre* were the three plants that were chosen. *Tinospora cordifolia stems*, *Gymnema sylvestre leaves*, and *Cassia auriculata* flowers were obtained and crushed to form a powder. A closed jar was filled with the powder, and the necessary amount of ethanol was then added. It was occasionally shook and left to stand for 72 hours. In order to acquire the plant extract, the liquid was strained out. As much of the solid residue as possible has been recovered, and it has been purified by subsidence or filtration, followed by rotary evaporation and concentration.

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Preliminary qualitative physiochemical and phytochemical investigation

The ethanolic plant extracts were standardized using various physicochemical parameters by following the standard procedures, and the results are reported in table 1.[15] Standard procedures were followed to test the ethanolic extract of each plant species for identifying the active constituents present in it by phytochemical investigation. The tests for major active constituents were performed, such as those for proteins, alkaloids, glycosides, tannins, saponins, carbohydrates, flavonoids, steroids, etc. The results of the phytochemical investigation are given in table 2.

MOLECULAR DOCKING STUDIES:

Creating the ligand

From the chosen medicinal plants, *Cassia auriculata*,*Gymnema sylvestre*, and *Tinospora cordifolia*, the main active ingredients were discovered. The 3D structures of the active ingredients (gymnemic acid, berberine, aloe-emodin, hexadecanoic acid, gymnemagenin, and gypenosides) are either retrieved from pubchem chemical databases [16] or created using chemsketch software [17] and saved in .mol format. The anti-diabetic benefits of syringin have long been asserted, The workspace served as the final preparation step for the imported ligands. comparing the docking scores of the active component to those of the reference drugs (acarbose and voglibose).[18]

Making of an enzyme

Alpha amylase was chosen as one of the targets for docking investigations. In order to perform a docking analysis, one must first choose the disease's target and then obtain the protein data bank's 3D model of alpha amylase (3old) in pdb format. [19,20] It was commonly known that the pdb file format was incapable of storing bond order information and that explicit hydrogen assignments were frequently incorrect or omitted from pdb files. As a result, the mvd was used to assign the appropriate bonds, bond orders, hybridization, and charges. Mvd's internal cavity detection technique was used to determine the possible binding locations of both targets. A subset region of 25.0 angstroms around the centre of the search space used in the docking studies was examined. The water molecules also taken into consideration and the replaceable water molecules were given a score of 0.50.

Molegro Virtual Docker’s docking search algorithms and scoring functions

The recently released and popular among medicinal chemists, Molegro Virtual Docker (MVD) was used to conduct ligand docking investigations. The most likely conformation of a ligand binding to a macromolecule is provided by the quick and adaptable docking

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tool known as MVD. The Mol Dock programme is built using a novel heuristic search approach that combines cavity prediction with differential evolution.[21] It uses an interactive optimization method called Evolutionary Algorithms (EA), which draws inspiration from Darwinian evolution theory. In this method, a population of individuals is subjected to competitive selection, which weeds out subpar solutions. New solutions were produced through mutation and recombination. The piecewise linear potential serves as the foundation for MolDock's scoring function (PLP), which is a condensed potential whose parameters are fitted to binding data scoring function and protein-ligand structures.[22] GEMDOCK (Generic Evolutionary Method for molecular DOCK)[23] added a new hydrogen bonding term and charge schemes, significantly extending this.

Optimizer for MolDock

For the guided differential evolution algorithm in MVD, the following parameters were used: population size = 50, number of runs = 5 (by checking the confine poses to cavity option), maximum interactions = 2000, cross over rate = 0.9, and scaling factor = 0.5. Instead of using root mean square deviation (RMSD), a variance-based termination approach was chosen. Pose clustering was used to generate numerous binding modes, ensuring the best binding mode in the binding cavity.

PARAMETERS FOR SCORING FUNCTIONS:

MolDock score

To disregard atoms far from the binding site, use the ignore-distant-atoms option. In addition, it was claimed that hydrogen bond directionality was used to determine whether or not probable donors and acceptors could form hydrogen bonds. The binding site on the protein was identified as having a 25 angstrom radius and extending in the X, Y, and Z directions around the chosen cavity. [24]

In-vitro anti-diabetic activity

Inhibition of the alpha-amylase enzyme

Stirring 0.1 g of potato starch in 100 ml of 16 mM sodium acetate buffer produced a starch solution (0.1 % w/v). Alpha-amylase in the amount of 27.5 mg was combined with 100 ml of distilled water to create the enzyme solution. The 3,5-dinitrosalicylic acid solution and sodium potassium tartrate solution are combined to create the colorimetric reagent. The alkaline conditions of an alpha-amylase solution at 25 °C were applied to a starch solution, and both control and plant extracts were added. Three minutes were used to measure the response. By reducing 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid, the production of maltose was measured. As according Malik and Singh (1980), the response can be observed at 540 nm [25]

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Fifty percentage inhibitory concentration calculation (IC50)

The percentage scavenging activities at five different concentrations of the extract were used to determine the concentration of the plant extracts needed to scavenge 50% of the radicals (IC50). The percentage of inhibition (I%) was determined using I%=AC-AS/AC 100

RESULTS AND DISCUSSION

Herbal products or plant products are rich in flavonoids, phenolic compounds, coumarins, terpenoids, and other constituents that help reduce blood glucose levels. In this aspect, we designed the present *in-silico* and *in-vitro* comparative studies between three medicinal plants (*Cassia auriculata*,*Gymnema sylvestre*, and *Tinospora cordifolia*). Physiochemical analysis was performed for the three medicinal plants, and phytochemical screening was performed and found to have: flavonoids, alkaloids, steroids, saponins, glycosides, proteins, and carbohydrate. *In-silico* docking results show the ability of the phytoconstituents to bind with the targets and are given in terms of the MolDock Score. The MolDock Score was used as the parameter for analyzing the docking results. The phytoconstituents were ranked according to their MolDock Score. The ligand possessing the highest moldock score shows a strong affinity towards its target. *In-silico* docking analysis of phytoconstituents from *Cassia auriculata*, *Gymnema sylvestre*, and *Tinospora cordifolia*, on alpha amylase (PDB ID: 3OLD) ranking based on MolDock Score was represented in table 3 and H-Bond was represented in table 4. The figures 1–9 correspond to the docking pose evaluated and captured by the ligand energy inspector tool in the Molegro virtual docker. *In-vitro* antidiabetic activity was performed for *Cassia auriculata*, *Gymnema sylvestre*, and *Tinospora cordifolia* by α-amylase method. The results illustrated that *Gymnema sylvestre* possesses higher activity when compared to that of *Cassia auriculata* and *Tinospora cordifolia*. Inhibition of the alpha amylase enzyme would delay the degradation of carbohydrates, which would in turn cause a decrease in the absorption of glucose, resulting in the reduction of postprandial blood glucose levels. Based upon the experimental evidence, it was found that *Gymnema sylvestre* possesses higher antidiabetic activity when compared to *Cassia auriculata* and *Tinospora cordifolia.* The percentage of inhibitory concentration of antidiabetic activity depends on the concentration of extracts. The table 5 shows the percentage of inhibitory concentrations that inhibit the alpha-amylase enzyme.

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Table 1.Physicochemical Parameters of *Cassia auriculata, Gymnema sylvestre&Tinospora cordifolia.*

S.No	Physicochemical parameters	Observations (percentage w/w)		
		Cassia auriculata	***Gymnema sylvestre***	***Tinospora coridoflia***
1.	Ash value			
	a. Total ash	9.16	8.3	17.6
	b. Water soluble ash	4.56	4.12	6.35
	c. Acid insoluble ash	0.5	0.9	1.06
	d. Sulfated ash	11.36±1.1	15.3	12.47±1.36
2.	Extractive value			
	a. Alcohol soluble extract	81.24	20.8	35.76
	b. Water soluble extract	89.57	30.56	27.84
3	Fiber extract	28.31	14.05	22.41
4	Loss on drying	0.468±0.01	5.6	3.31

Note: It shows that ash value is higher in *Tinospora cordifolia* and extractive value was higher in *Cassia auriculata,* and fiber extract is higher in *Cassia auriculata*.

Table 2: Phytochemical screening results of *Cassia auriculata, Gymnema sylvestre&Tinospora cordifolia.*

S. No	Chemical test	*Cassia auriculata*	*Gymnema sylvestre*	*Tinospora coridoflia*
1	Alkaloids	+	+	+
2	Steroids	+	+	+
3	Phenolic/tannins	-	-	+
4	Saponins	+	+	-
5	Proteins	+	+	+
6	Carbohydrates	+	+	+
7	Glycosides	+	+	+
8	Flavonoids	+	-	-

(+) presence, (-) absence

Note: All the three plants containing similar active constituents like Alkaloids, Steroids, saponins, Proteins, Carbohydrates Glycosides, Flavonoids and Tannins.

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Table 3: *In-silico* docking analysis of phytoconstituents from *Cassia auriculata, Gymnema sylvestre&Tinospora cordifolia.* [based on MolDock Score]

Alpha amylase (PDB ID: 3OLD) ranking based on MolDock Score				
Name	**Ligand**	**MolDock Score**	**Rerank Score**	**H Bond**
[01]Acarbose	Acarbose	-160.975	-122.538	-18.6195
[00]Syringin	Syringin	-121.049	-87.3002	-15.7107
[00]Gymnemic acid	Gymnemic acid	-116.499	-59.9086	-12.9317
[00]Berberine	Berberine	-100.289	-82.0917	-4.1822
[00]Voglibose	Voglibose	-97.6005	-78.5413	-12.2219
[00]Aloe-emodin	Aloe-emodin	-85.2878	-74.5445	-8.519
[00]Hexadecanoic acid	Hexadecanoic acid	-75.5086	-58.5492	0
[00]Gymnemagenin	Gymnemagenin	11.0564	36.0831	-8.60944
[00]Gypenosides	Gypenosides	8503.14	565.527	-18.539

Table 4: *In-silico* docking analysis of phytoconstituents from *Cassia auriculata, Gymnema sylvestre& Tinospora cordifolia* [based on H bond]

Alpha Amylase (PDB ID: 3OLD) ranking based on H Bond				
Name	**Ligand**	**MolDock Score**	**Rerank Score**	**HBond**
[01]Acarbose	Acarbose	-160.975	-122.538	-18.6195
[00]Gypenosides	Gypenosides	8503.14	565.527	-18.539
[00]Syringin	Syringin	-121.049	-87.3002	-15.7107
[00]Gymnemic acid	Gymnemic acid	-116.499	-59.9086	-12.9317
[00]Voglibose	Voglibose	-97.6005	-78.5413	-12.2219
[00]Gymnemagenin	Gymnemagenin	11.0564	36.0831	-8.60944
[00]Aloe-emodin	Aloe-emodin	-85.2878	-74.5445	-8.519
[00]Berberine	Berberine	-100.289	-82.0917	-4.1822
[00]Hexadecanoic acid	Hexadecanoic acid	-75.5086	-58.5492	0

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Table 5: *In-vitro* antidiabetic activity result of *Cassia auriculata, Gymnema sylvestre&Tinospora cordifolia.*

S. No	Concentration of the Sample (ml)	Percentage of Inhibitory Concentration		
		Cassia auriculata	*Gymnema sylvestre*	*Tinospora cordifolia*
1.	0.2	42	68	24
2.	0.4	57	72	32
3.	0.6	66	77	40
4.	0.8	71	83	53
5.	1.0	79	92	64

Figures

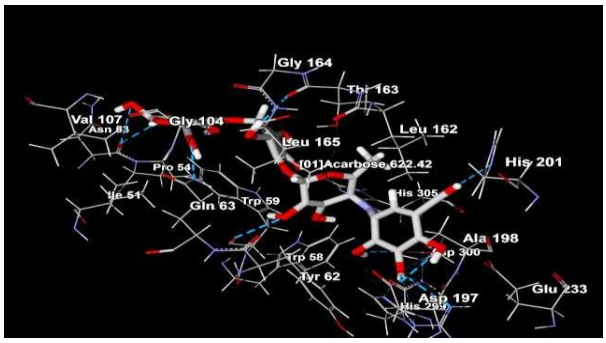

Figure 1: Docking complex of Alpha Amylase (PDB ID: 3OLD) with Acarbose.

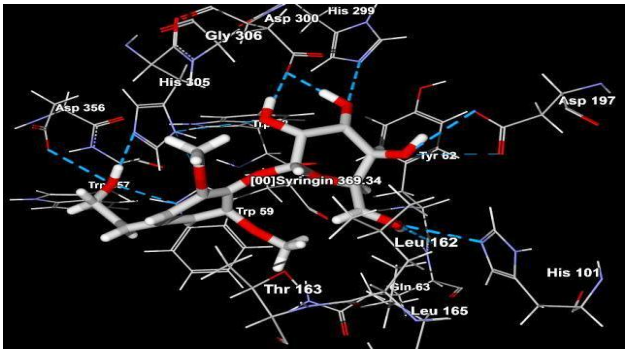

Figure 2: Docking complex of Alpha Amylase (PDB ID: 3OLD) with Syringin.

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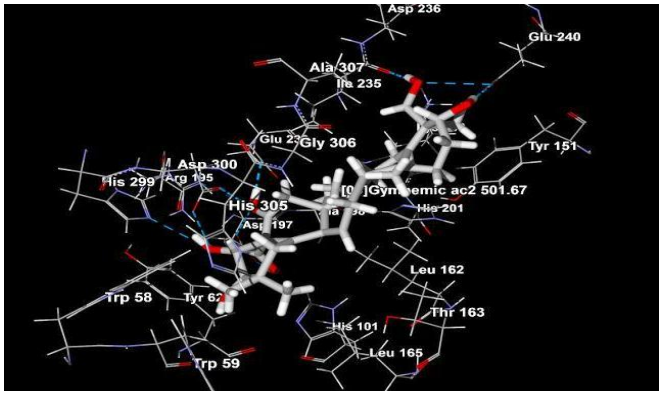

Figure 3: Docking complex of Alpha Amylase (PDB ID: 3OLD) with Gymnemic acid.

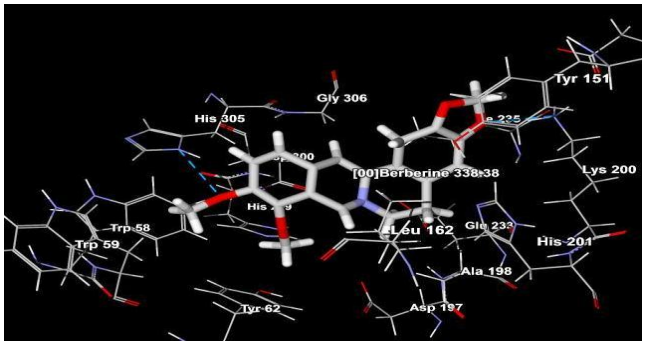

Figure 4: Docking complex of Alpha Amylase (PDB ID: 3OLD) with Berberine.

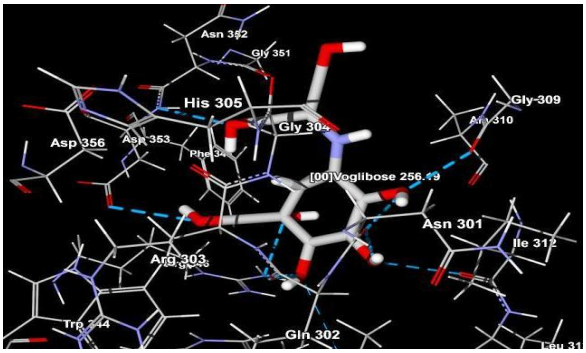

Figure 5: Docking complex of Alpha Amylase (PDB ID: 3OLD) with Voglibose.

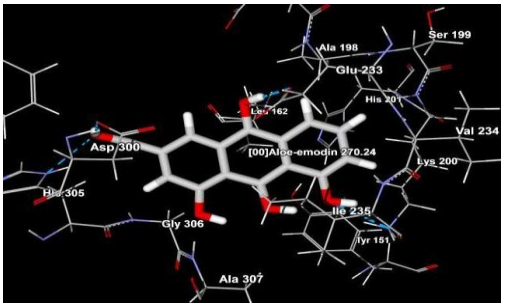

Figure 6: Docking complex of Alpha Amylase (PDB ID: 3OLD) with Aloe-emodin.

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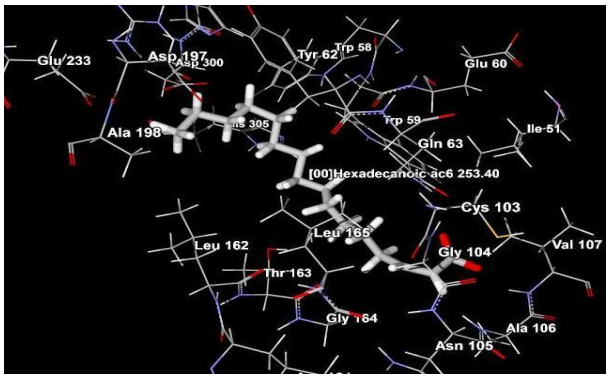

Figure 7: Docking complex of Alpha Amylase (PDB ID: 3OLD) with Hexaddecanoic acid.

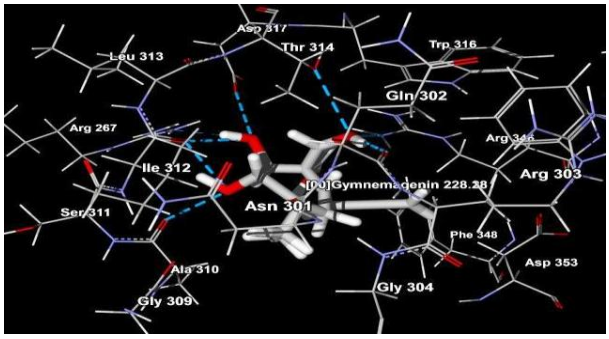

Figure 8: Docking complex of Alpha Amylase (PDB ID: 3OLD) with Gymnemagenin.

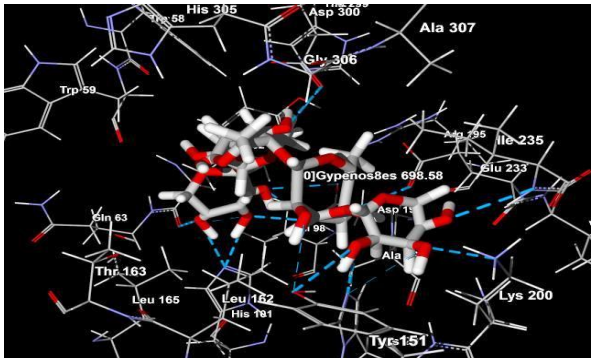

Figure 9: Docking complex of Alpha Amylase (PDB ID: 3OLD) with Gypenosides.

CONCLUSION:

Herbal plants are widely used in traditional systems of medicine. [26] India has often been referred to as the "Medicinal Garden of the World." Diabetes mellitus is one of the major causes of global public health concern, imposing a heavy global burden on public health worldwide and socio-economic development [27]. Currently, there is growing interest in herbal remedies due to the side effects associated with oral hypoglycaemic agents for the treatment of diabetes mellitus. Hence, traditional herbal medicine, mainly obtained from plants, was used in the management of diabetes mellitus [28]. In the

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recent years, herbal medicines have started to gain importance as a source of hypoglycaemic agents. It was estimated that more than 1000 plant species have been used as folk medicines for diabetes mellitus. The plant *Gymbnema sylvestre* has a high potency of antidiabetic activity when compared to *Cassia auriculata* and *Tinospora cordifolia*. Further studies have to explore the mechanisms underlying the antidiabetic activity of the phytoconstituents of the plant *Gymnema sylvestre*.

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Conflict of Interest:

We are declaring that there is no conflict of interest in this study.

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