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GC MS ANALYSIS, *IN SILICO* & *IN VITRO* ANTIDIABETIC STUDIES OF CHITRAKA TRIPHALADI KASHAYA CHOORNA

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ABSTRACT

Chitraka Triphaladi Kashaya Choorna is a traditional Ayurvedic formulation used for various therapeutic purposes, including the management of diabetes mellitus. In this study, we aimed to investigate the chemical composition of Chitraka Triphala Kashaya Choorna and evaluate its potential antidiabetic activity using *in silico* & *in vitro* methods. A preliminary phytochemical screening and physico-chemical analysis of ethanolic extracts of choorna was done. Antidiabetic study of Chitraka Triphala Kashaya Choorna was done using alpha amylase assay method. A comprehensive chemical profiling of the Chitraka Triphaladi Kashaya Choorna was performed using gas chromatography -mass spectrometry (GC-MS). *In silico* studies were conducted to assess the antidiabetic potential of ligands obtained from GC-MS screening. Molecular docking studies were performed using *AutoDock Vina.V.1.2.7* against various diabetes target proteins, such as insulin receptor, peroxisome proliferator-activated receptor gamma (PPAR-γ), dipeptidyl peptidase IV Enzyme were selected. D-allose ligand showed the best binding affinity with the selected proteins. The docking studies aimed to predict the binding affinity and interactions between bioactive compounds present in the formulation and the target protein. These predictions offer valuable insights into the potential mechanisms of action and therapeutic effects of Chitraka Triphaladi Kashaya Choorna in managing diabetes.

Keywords: Molecular docking, Anti diabetic activity, Chemical profiling, Chitraka Triphaladi Kashaya Choorna, GC-MS

I INTRODUCTION

Herbal treatments are among the oldest forms of medicine and have a long history of being considered among the most effective ways to preserve human health and homeostasis. India has a long history of using traditional herbal medicine. Indian medical pharmacopeia lists triphala as "Choorna," or powder, and it is a well-liked and beneficial medication **[1]**. Choorna is a finely ground powder used as a medicinal agent to cure various illnesses. Infectious diseases are still the greatest cause of death worldwide, and the mortality rate is rising daily **[2, 3]**. Diabetes mellitus (DM) is a major metabolic disease that causes elevated plasma glucose levels as a result of insulin resistance, insufficient insulin, or both, along with disruptions in the metabolism of proteins, carbs, and fats. DM is the leading cause of mortality and morbidity worldwide. Affecting approximately 2.8% of the global population, it is predicted to increase to 5.4% by 2025, making it one of the most common metabolic disorders **[4]**. The complications linked to diabetes mellitus can be broadly classified into two categories: macrovascular complications that impact the heart, brain, and extremities, and microvascular complications that affect the blood vessels of the eye, kidney, nerves, and diabetic retinopathy.

Chitraka Triphaladi Kashaya Choorna is mainly used in treating diabetes. The ingredients of choorna are chitraka (*Plumbago zeylanica*), Triphala, Darvi (*Berberis aristata*) and Kalinga beeja (seeds of *Holarrhena antidysenterica*). The dried fruits of three plant species native to the Indian subcontinent—*Emblica officinalis* (Family: *Euphorbiaceae*), *Terminalia bellerica* (Family: *Combretaceae*), and *Terminalia chebula* (Family *Combretaceae*) are used to make the well-known and highly respected polyherbal medicine known as triphala (Sanskrit: tri = three and phala = fruits). Because it acts gradually and gently,Triphala is known to extend life and rejuvenate those who take it for extended periods without having any negative side effects **[5]**. Chitraka is one of the powerful digestive and carminative herbs of Ayurveda. Gas chromatography (GC) and mass spectroscopy (MS) are effective tools for gaining a deeper understanding of the sample by identifying the different compounds it contains.

There is general agreement in conventional medical systems regarding the use of phytotherapy in the management of various systemic illnesses. Compared to the current medical system, many indigenous and traditional medical systems have stronger protective effects. However, one of the main problems facing conventional medical systems is the absence of empirical data supporting their therapeutic uses. It has been

established that polyherbal formulations yield superior therapeutic outcomes when compared to single herb formulations. This idea is widely documented. The current study focused to find out the antidiabetic potential of ethanolic extract of Chitraka Triphaladi Kashaya Choornam and determination of phytoconstituents through GC-MS screening and performing molecular docking of the identified constituents against various antidiabetic proteins using *AutoDockVina.V[1.2.7]*.

2. MATERIALS AND METHODS

2.1 Collection of Drug

The formulated drug Chitraka Triphaladi Kashaya Choornam from Vaidyaratnam Oushadhasala, Thrissur on 12th of March 2023. Chitraka Triphaladi Kashaya Choornam combines the medicinal properties of two key ingredients: Chitraka (*Plumbago zeylanica*) and Triphala (a combination of three fruits: *Terminalia chebula*, *Terminalia bellirica*, *and Emblica officinalis*).

2.2 Chemicals and Reagents

The chemicals and reagents used in this experiment are procured from local vendors **[4, 5]**.

2.3 Preparation of Extract

The formulation (100 g) was macerated by cold maceration process using ethanol with occasional shaking for 7 days. The extract was decanted, filtered, concentrated, and kept in desiccators for complete removal of solvent. The extract was then packed in an airtight container **[*6*]**.

2.4 Determination of physicochemical parameters

Physiochemical parameters like total ash, acid insoluble ash value, water insoluble ash value, water extractive value, alcohol extractive value and petroleum ether extractive value were performed using standard procedures **[7]**.

2.5 Preliminary phytochemical analysis

The extract were subjected to preliminary phytochemical analysis to determine the presence of alkaloids, carbohydrates, proteins and glycosides **[8]**.

2.6 Alpha Amylase Assay

Different concentration of extract (100,200,300,400 µl) was taken into different test tubes. Made the volume to 0.5ml with phosphate buffer of pH 6.9; Control was prepared by taking 0.5ml of phosphate buffer. The solutions were then treated with 0.5ml of alpha amylase (0.5mg/ml). The solution was incubated at 25ºC for 10 minutes. Added 0.5ml of 1% starch solution in 0.02 M sodium phosphate buffer of pH 6.9 to all the tubes, and then incubate at 25ºC for 10 minutes. The reaction was stopped by adding 1.0 ml of DNS and the reaction mixture was kept in boiling water bath for 5 minutes, cooled to room temperature. The solution was mixed with 8 ml distilled water. Blank was measured by taking 1 ml of phosphate

buffer. Read the absorbance of the solution in colorimeter at 540 nm against blank solution. Standard acarbose was prepared in the same manner at different concentrations and absorbance was measured **[9]**.

2.8 GC MS analysis

The ethanolic extract was filtered through Whatsman No. 1 filter paper and concentrated. The extract obtained was then subjected to GC-MS analysis. Gas chromatography Mass spectroscopy analysis of ethanolic extract was performed using Shimadzu GC-MS Model number: QP2010S equipped with Column - ELITE5MS, 30meter length,0.25mm ID,0.25µm thickness. The oven temperature was programmed from 70.00^{0}C **[10, 11]**.

2.9 Protein and Ligand preparation

The X- ray crystal structure of proteins 1i7i, 1ir3, and 5yp2 **(Figure 1)** is obtained from protein data bank. The water molecules were removed and hydrogen atoms were added before docking to correct the ionisation. Download 3D structure of ligands from Pubchem. SDF files converted to PDBQT using Openbabel GUI and saved in a particular folder. Molecular docking was performed and Docking interactions was identified using pymol **[12]**.

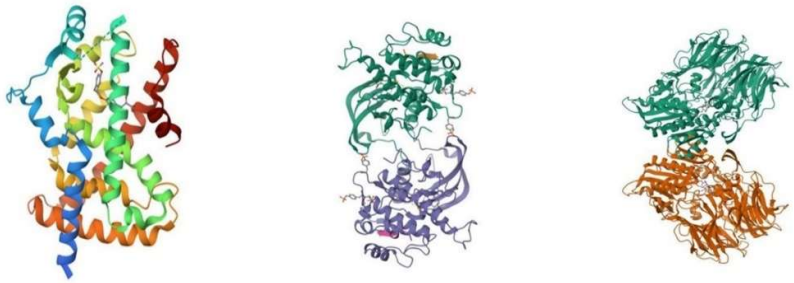


Figure 1: Structures of selected proteins

3. RESULTS AND DISCUSSION

3.1 Physicochemical and Phytochemical Screening

The percentage yield of ethanolic extract was found to be 2%w/w **(Table 1)**. From the report of ash value, choorna contain minimum impurities. The extractive value showed ethanol as best solvent **(Table 2)**. From the preliminary phytochemical screening, the drug extract showed the presence of alkaloids, flavanoids, tannins, phenolic compounds & proteins **(Table 3)**.

3.2 Alpha amylase assay

In-vitro Anti-diabetic activity of Chitraka Triphaladi Kashaya Choornam was measured using Alpha Amylase Assay and the drug extract showed a maximum of 59.25 percentage alpha amylase inhibition **(Table 4, Figure 2)**.

In vitro Anti-diabetic activity of Chitraka Triphaladi Kashaya Choornam was measured using Alpha Amylase Assay and the drug extract shows a maximum of 59.25 percentage alpha amylase inhibition.

3.3 GC- MS Analysis

GC-MS analysis of drug extract showed the presence of Eighteen volatile compounds **(Table 6, Figure 3)**.

Table 1: Yield of drug extract

Extract	Solvent	Weight taken(gm)	Yield(% w/w)
Chitraka Triphaladi Kashaya Choorna	Ethanol	100	2% w/w

Table 2: Physico chemical constituents of the formulations

S. No.	Parameters	%w/w
I	Ash value	
1	Total ash value	6.5
2	Water soluble ash value	2.1
3	Acid insoluble ash value	2.1
II	Extractive value	
1	Water soluble extractive value	15.8
2	Alcohol soluble extractive value	20.2
3	Petroleum ether soluble extractive value	10.2

Table 3: phytochemical screening of extract

S. No.	Constituents	Ethanolic extract
1	Alkaloids	+
2	Glycosides	_
3	Carbohydrates	_
4	Flavanoids	+
5	Tannins	+
6	Phenolic compounds	+
7	Proteins	+
8	Steroids	_
9	Terpenoids	_

Table 4: Percentage inhibition of alpha amylase assay

S. No.	Concentration (%)	Sample (%)	Standard Acarbose (%)
1	100	17.28	44.44
2	200	32.09	54.32
3	300	50.61	62.96
4	400	59.25	72.83

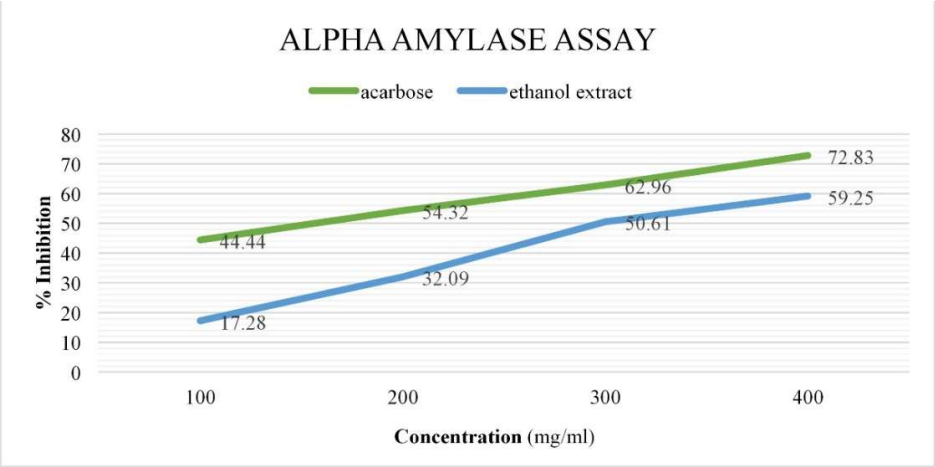


Figure 2: Percentage alpha amylase inhibition of sample and standard

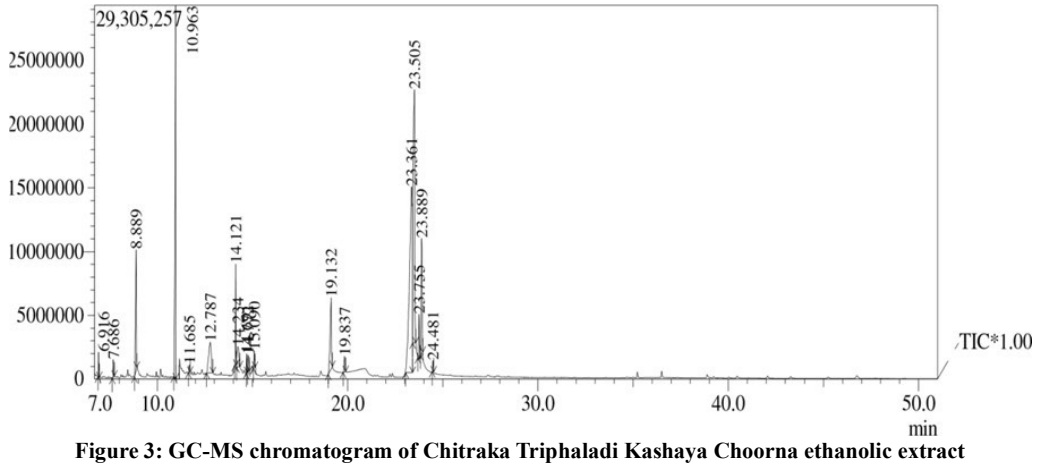


Figure 3: GC-MS chromatogram of Chitraka Triphaladi Kashaya Choorna ethanolic extract

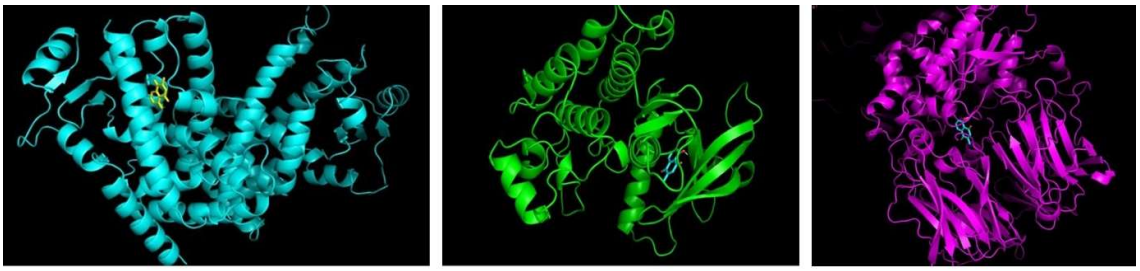
Table 6: Peak Report TIC

Peak	R. Time	Area	Area%	Name
1	6.916	2762548	0.54	1,1,3-TRIETHOXYBUTANE
2	7.686	3021696	0.60	Pyranone
3	8.889	25660769	5.06	5-Hydroxymethylfurfural
4	10.963	73862353	14.55	Pyrogallic acid
5	11.685	1459766	0.29	CINNAMIC ACID
6	12.787	22882271	4.51	D-Allose
7	14.121	14596777	2.88	Plumbagin
8	14.234	11067116	2.18	CHINIC ACID
9	14.691	2180999	0.43	BETA-TURMERONE
10	14.772	4609634	0.91	(1E)-2-Methylcyclohexanone semicarbazone
11	15.090	3300404	0.65	Methyl 3-butyl-4-nitro-4-pentenoate
12	19.132	27017635	5.32	HEXADECANOIC ACID
13	19.837	3745955	0.74	ETHYL PALMITATE
14	23.361	153290895	30.20	Leinoleic acid
15	23.505	116578703	22.97	cis,cis,cis-7,10,13-Hexadecatrienal
16	23.755	10322670	2.03	Ethyl-9,12octadecadienoate
17	23.889	28958680	5.71	11-Dodecynyl acetate
18	24.481	2265934	0.45	ETHYL STEARATE

3.4 Molecular docking and interactions

Table 7: Binding affinity of various ligands against proteins

S. No.	Ligand	Binding affinity against li7i(kcal/mol)	Binding affinity against 1ir3 (kcal/mol)	Binding affinity against 5yp2 (kcal/mol)
1.	1,1,3-Triethoxybutane	- 4.7	- 3.9	- 4.7
2.	Pyranone	- 4.7	- 4.3	- 5.3
3.	5-Hydroxymethylfurfural	- 5.7	- 4.7	- 5.7
4.	Pyrogallic acid	- 6.3	- 5.4	- 6.3
5.	Cinnamic Acid	- 6.0	- 5.2	- 6.3
6.	D-Allose	- 7.2	- 6.9	- 7.8
7.	Plumbagin	- 6.4	- 5.7	- 7.2
8.	Chinic acid	- 7.1	- 6.2	- 6.6
9.	Beta-Turmerone	- 5.8	- 5.6	- 6.7
10.	(1E)-2-Methylcyclohexanone semicarbazone	- 5.9	- 5.1	- 5.9
11.	Methyl3-butyl-4-nitro-4-pentenoate	- 5.4	- 4.8	- 5.3
12.	Hexadecanoic Acid	- 6.6	- 5.6	- 5.7
13.	Ethyl Palmitate	- 5.5	- 5.1	- 4.9
14.	Leinoleic acid	- 6.1	- 5.1	- 5.8
15.	Cis,cis,cis-7,10,13-hexadecatrienal	- 6.9	- 6.1	- 6.4
16.	Ethyl-9,12-octadecadienoate	- 5.1	- 5.2	- 5.7
17.	11-Dodecynyl acetate	- 6.3	- 5.2	- 5.8
18.	Ethyl Stearate	- 6.4	- 5.5	- 5.5



D-Allose with 1i7i D-Allose with 1ir3 D-Allose with 5yp2

Figure 4: Interactions of D-allose with selected proteins

From the docking results, D-Allose was found to be the best ligand with greater binding affinity against the 3 selected antidiabetic proteins.

4. CONCLUSION

From preliminary phytochemical studies, Chitraka Triphaladi Kashaya Churna ethanolic extract showed the presence of alkaloids as major secondary metabolite. Physico-chemical parameters ash value and extractive value proved the presence of minimum impurities and ethanol as the best solvent respectively. From the alpha-amylase assay, the drug extract showed the maximum anti-diabetic activity. From GC-MS analysis the drug contained18 volatile substituents. Molecular docking of obtained volatile substituents were performed using *Auto Dock Vina.V [1.2.7]*. From the results, D-Allose was found to be the best ligand with selected anti-diabetic proteins and can be screened using highly computational method like molecular dynamic simulation for further investigation. This study revealed the antidiabetic potential of Chitraka Triphaladi Kashaya Churna highlighting that D-Allose exhibited the highest binding affinity against target protein. These findings contribute to the understanding of the therapeutic potential of the formulated drug and suggest D-Allose as a promising compound that can screened using highly computational method like molecular dynamic simulation for further investigation.

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