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PREPARATION AND PHYTOCHEMICAL ANALYSIS OF ARJUNA KSHEERAPAKA WITH SPECIAL REFERENCE TO GCMS

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ABSTRACT

Ayurveda is the science of life. Ayurvedic medicines are used since time immemorial. The preparation of various medicines, there indications are clearly explained in the classics. The explanations for same are also documented. The science and technology has developed to its maximum now and hence it is mandatory to go for the standardization and quality control parameters using the modern techniques and the action of the medicines can also be concluded on the basis of the phyto-constituents present in them.

Arjuna ksheerapaka is an ayurvedic formulation containing Arjuna (*Terminalia arjuna*), milk and water. It is prepared as per the classical guidelines. One part of coarse powder of drug is added with eight parts of milk and thirtytwo parts of water and boiled and reduced to quantity of milk, filtered. This is subjected to organoleptic, physicochemical, phytochemical analysis and fingerprinting and GCMS for standardization.

In Physico-chemical analysis P^H is 6.01, L.O.D is 89.65%, Acid insoluble ash is 0.007%, Total ash is 0.58%, Alcohol soluble extractive is 13.57%, Water soluble extractive is 22.07%. Phytochemical screening showed the presence of Alkaloids, glycosides, Flavanoids, Triterpenoids, Tannins,

Carbohydrate, Essential Fatty oil and also Ca, Mg, K, Na, Fe, PO_4, Cl are detected. Various components were detected in HPTLC finger printing. Detailed chemical profile of the sample was obtained from GCMS.

The values and results obtained from the classically prepared sample meets the standard of API, hence the pharmaceutical preparation is authenticated. The phytoconstituents and the components from chemical profiling adds to the action of the Arjuna Ksheerapaka.

Keywords: Arjuna ksheerapaka, Physico-chemical, Phytoconstiuents, HPTLC finger printing, GCMS

INTRODUCTION

Ayurveda is the science of life. It is a holistic science which lie close to the nature. Ayurveda medicines include pure herbal preparations and herbo-mineral preparations. Main types of Ayurveda formulations include Swarasa (juice extracts of the drug), Kalka (Paste of the drugs), Kwatha (Decoctions), Hima (Cold infusions) and Phanta (Hot infusions) **[1]**. Ksheerapaka is an ayurvedic formulation which is a modification of Kashaya kalpana. This is a palatable preparation where ksheera(milk) is a main ingredient. Here the drugs are processed with milk, water and the respective drug. In this preparation apart from the water extractive principle the fat extractive components of the drug also get extracted. Also the medicine becomes more palatable, nutritive value increases and the tikshnata of the drug is reduced. The use of ksheerpaka in treatment can be traced from the Bruhatrayis (classical text books of Ayurveda- Charaka Samhita, Sushrutha Samhita and Ashtanga Hrudaya.

There are many methods for the preparation of Ksheerapaka as per Ayurveda classics. They are the ratio of drug:water:milk in ratio 1:8:32 **[2]**, 1:15:15 **[3]**, preparation of decoction with the drug and later this decoction is processed with milk.

For the study, the general method of preparation of ksheerapaka as per Sharangadhara is taken into consideration. Here one part of coarse powder of dried Arjuna bark is added with eight parts of milk and thirtytwo parts of water and is reduced to the quantity of milk. Arjuna (Terminalia arjuna) is the drug of choice for Hridroga and Arjuna Ksheerapaka is specifically indicated for Hridroga.

The drug has to be identified and authenticated for the preparation. There are a variety of tests available that can be utilized for this. Before moving to a clinical trial all other steps are to be covered. The physico-chemical parameters, pharmaceutical evaluations, HPTLC and GCMS is evaluated here. The analysis of the phytoconstituents with respect to its

concentration is very important and is the main core of the study.

MATERIALS AND METHODS

1. Collection and authentication

All the drugs of Arjuna Ksheerapaka as per API were obtained from local market, Kerala. Cow's milk is purchased from farm. The drugs were powdered separately, and they were subjected to Pharmacognostical evaluation at Care Keralam, Kerala.

2. Pharmaceutical Study

The Arjuna Ksheerapaka is prepared at Dept of Rasasastra and Bhaishajya Kalpana Laboratory of Santhigiri Ayurveda Medical College, Palakkad.

The bark of Arjuna was collected. It was coarsely powdered. The preparation of Arjuna ksheerapaka was carried out as per the opinion of Sharangadara Acharya. One part of the coarsely powdered drug is added with 8 parts of milk and 32 times of water and is boiled to the quantity of milk. This is then filtered and the filtrate is ksheerapaka. Here, Coarsely powdered Arjuna bark (20g) is added with 8 times of milk(160ml) and 32 times of water(640ml) and is boiled in mild fire and is reduced to the quantity of milk. Then it is filtered and the filtrate is used.

3. Analytical Study

The Analytical studies, Phytochemical studies, HPTLC and GCMS of milk and Arjuna ksheerapaka was conducted at CARe KERALAM LTD (CKL), Koratty, Kerala.

Table 1: Organoleptic Characters

Parameters
Color
Odour
Consistency

Table 2: Physico- chemical analysis- Wet Analysis

Parameters	Test Method
Foreign matter	API Part 1 , Vol 1
Total ash	IP 2018
Alcohol soluble extractive	1P 2018
Water soluble extractive	IP 2018
Acid insoluble ash	IP 2018
P^H	API Part 1, Vol 1
Loss on drying	API part 1, Vol 1

Table 3: Phyto chemical Screening

Parameters	Test Method
Alkaloids	Dragendroff's reagent test
Glycosides	Picric acid test
Flavanoids	Shinoda test
Saponins	Foam test
Triterpenoids	Salkowski reaction test
Tannins	Ferric chloride test
Proteins	Biurette test
Steroids	Salkowski reaction test
Carbohydrate	Benedict Solution test
Essential Oil	Solubility test
Fatty Oil	Solubility test
Carbonates	Experimental chemistry

Table 4: Inorganic Chemical Constituents

Parameters	Test Method
Calcium	AOAC 21ST Edition 2019
Magnesiun	CKL/ANL/FP-019
Potassium	AOAC 21ST Edition 2019
Sodium	AOAC 21ST Edition 2019
Iron	AOAC 21ST Edition 2019
Sulphate	IS: 3025 Part 24
Phosphate	IS: 1797 Page 7
Chloride	IS: 1797 Page 7
Nitrate	IS: 3025 Part 34

High Performance Thin Layer Chromatography

HPTLC was done as per CKL/ANL/HPTLC-001 method.

HPTLC is the technique that is used in separation of compounds from a mixture. This is the major technique that is used for analysis of herbal medicine. A finger print of the formulation is obtained **[4, 5]**.

Table 2: Equipment and material specification for HPTLC

Instrument	CAMAG Linomat V Automatic Sample Spotter (CamagMuttenz, Switzerland); the syringe, 100μL (from Hamilton)
Development mode	Ascending
Chamber type	CAMAG glass twin trough chamber (5x10 cm)
Absorbant	Silica gel 60 F254 TLC plates(E.Merk) 0.2mm thickness
Solvent System	Isopropanol: Dichlormethane: Water(5:2.5:1)
Scanning Wave length	366nm
Lamp used	Deuterium,Tungsten, Mercury

3gram sample was extracted in methanol and TLC was carried out. Apply extract on plate and dried. Observe plate under 366nm.Record Rf value and colour of resolved bands, After visualization and scanning, spray the plate with isaldehyde sulphuric acid reagent and heat at 105ºC till the colour of bands appear. Record Rf value and colour of bands.

Observe the plate under UV light at 366nm.Record the Rf value and colour of the resolved bands. After visualization and scanning, spray the plate with an isaldehyde sulphuric acid reagent and heat at 105^0C till the colour of the bands appear. Record the Rf value and colour of the bands.

Fatty Acid Profile – GCMS

Two drops of sample was mixed with 1ml hexane, shaken for 10 seconds. 200µL of 2N methanolic NaOH was added vortexed, then 200µL of 2N methanolic HCl was added and vortexed. Supernatent soln was taken through a syringe filter (Nylon 13mm 0.2um) and injected to GCMS.

Analysis

Instrument model – 7890 A GC with 5975C with triple axis detector

Column – DB 5MS 30mx0.250mm diameter x 0.25 mm thickness

Analysis was performed by injecting 1µL of the sample with a split ratio of 100:1. Helium gas (99.995%) was used as the

carrier gas at a flow rate of 1ml/min. The analysis was performed in the EI (electron impact) mode with 70 EV of ionization energy. The injector temperature was maintained at 280ºC(constant).

Table 6

Oven	RateºC/min	ValueºC/min	Hold time
Initial		80	5
R amp 1	4	230	5

RESULTS:

Pharmaceutical part:

Arjuna twak weighing 20g were taken and 160ml of milk was added and to this 640ml of water was added and reduced to quantity of milk. Final product obtained was 160ml.

Analytical Part:

The results of the analytical studies are enlisted in the **Tables 07 To 14**. The results of the HPTLC and GCMS are also enlisted.

Table 7: Observations during the preparation of Arjuna Ksheerapaka

Amount of Arjuna bark coarse powder(g)	Quantity of milk Ml	Quantity of water (ml)	Max temp Deg celcius	Total yield Ml
20g	160ml	640ml	90-95^{0}c	160ml

Table 8: Organoleptic Charactors

Parameters	Result
Color	Brown
Odour	Choclate
Consistency	Liquid

Table 9: Physico- chemical analysis- Wet Analysis

Parameters	Result	Test Method
Foreign matter	Nil	API Part 1 , Vol 1
Total ash	0.58	IP 2018
Alcohol soluble extractive	13.57	1P 2018
Water soluble extractive	22.07	IP 2018
Acid insoluble ash	0.007	IP 2018
P^H	6.01	API Part 1, Vol 1
Loss on drying	89.65	API part 1 , Vol 1

Table 10: Phyto chemical Screening

Parameters	Result	Test Method
Alkaloids	Present	Dragendroff's reagent test
Glycosides	Present	Picric acid test
Flavanoids	Present	Shinoda test
Saponins	Absent	Foam test
Triterpenoids	Present	Salkowski reaction test
Tannins	Present	Ferric chloride test
Proteins	Absent	Biurette test
Steroids	Absent	Salkowski reaction test
Carbohydrate	Present	Benedict Solution test
Essential Oil	Absent	Solubility test
Fatty Oil	Present	Solubility test
Carbonates	Absent	Experimental chemistry

Table 11: Inorganic Chemical Constituents

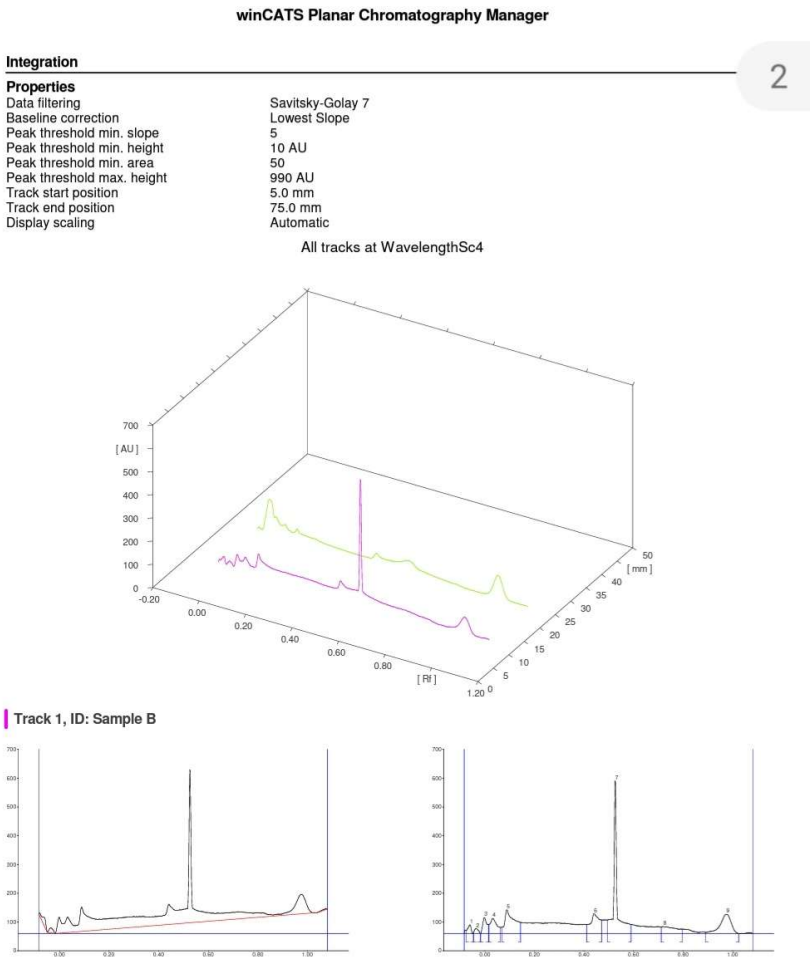
Parameters	Result	Test Method
Calcium	0.013%	AOAC 21ST Edition 2019
Magnesiun	0.02%	CKL/ANL/FP-019
Potassium	0.105%	AOAC 21ST Edition 2019
Sodium	0.126%	AOAC 21ST Edition 2019
Iron	0.001%	AOAC 21ST Edition 2019
Sulphate	0.055%	IS: 3025 Part 24
Phosphate	0.0017%	IS: 1797 Page 7
Chloride	0.20%	IS: 1797 Page 7
Nitrate	Not detected	IS: 3025 Part 34

Table 12: HPTLC of Arjuna Ksheerapaka

winCATS Planar Chromatography Manager

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.08	11.8	-0.06	30.9	3.30	-0.05	2.0	291.0	2.03	unknown *
2	-0.05	5.2	-0.04	17.8	1.90	-0.02	0.0	186.2	1.30	unknown *
3	-0.02	0.6	-0.00	55.8	5.97	0.01	31.5	673.6	4.70	unknown *
4	0.02	31.9	0.03	53.6	5.72	0.06	22.0	1039.1	7.24	unknown *
5	0.07	22.0	0.09	83.7	8.95	0.14	38.7	2300.0	16.04	unknown *
6	0.41	30.9	0.44	70.1	7.49	0.47	47.2	1787.1	12.46	unknown *
7	0.50	47.5	0.53	531.6	56.79	0.59	31.2	4957.6	34.56	unknown *
8	0.71	23.7	0.72	24.9	2.66	0.80	15.3	1031.0	7.19	unknown *
9	0.89	4.3	0.98	67.6	7.23	1.03	0.5	2077.8	14.49	unknown *

Graph 1: HPTLC of Arjuna Ksheerapaka



GCMS

GCMS of milk and Arjuna Ksheerapaka was carried out.The compounds were identified after comparing the spectral configrations obtained with that of available mass spectral data base(NIST-08 SPECTRAL DATA.

Table 13: Identified Com[ounds of Arjuna Ksheerapaka- GCMS

Parameters	Unit	Milk	Arjuna Ksheerapaka	Test method
Caprylic Acid(C8:0)	%	0.79	0.72	CKL/ANL/GC-003
Capric Acid(C10:0)	%	1.96	1.84	CKL/ANL/GC-003
Lauric Acid(C12:0)	%	3.11	2.94	CKL/ANL/GC-003
Myristic Acid(C14:0)	%	11.89	11.47	CKL/ANL/GC-003
Palmitic Acid(C16:0)	%	36.59	39.79	CKL/ANL/GC-003
Stearic Acid (C18:0)	%	3.96	1.62	CKL/ANL/GC-003
Oleic Acid(C18:1)	%	36.59	39.79	CKL/ANL/GC-003
Linoleic Acid (C18:2)	%	1.21	1.26	CKL/ANL/GC-003

Graph 2: GCMS-Milk Chromatogram

File :D:\GCMSD\2022\DECEMBER\27.12.2022\T1919.D
Operator :
Acquired : 27 Dec 2022 14:28 using AcqMethod FATTY ACID STD.M
Instrument : GCMS
Sample Name: SAMPLE A
Misc Info :
Vial Number: 3

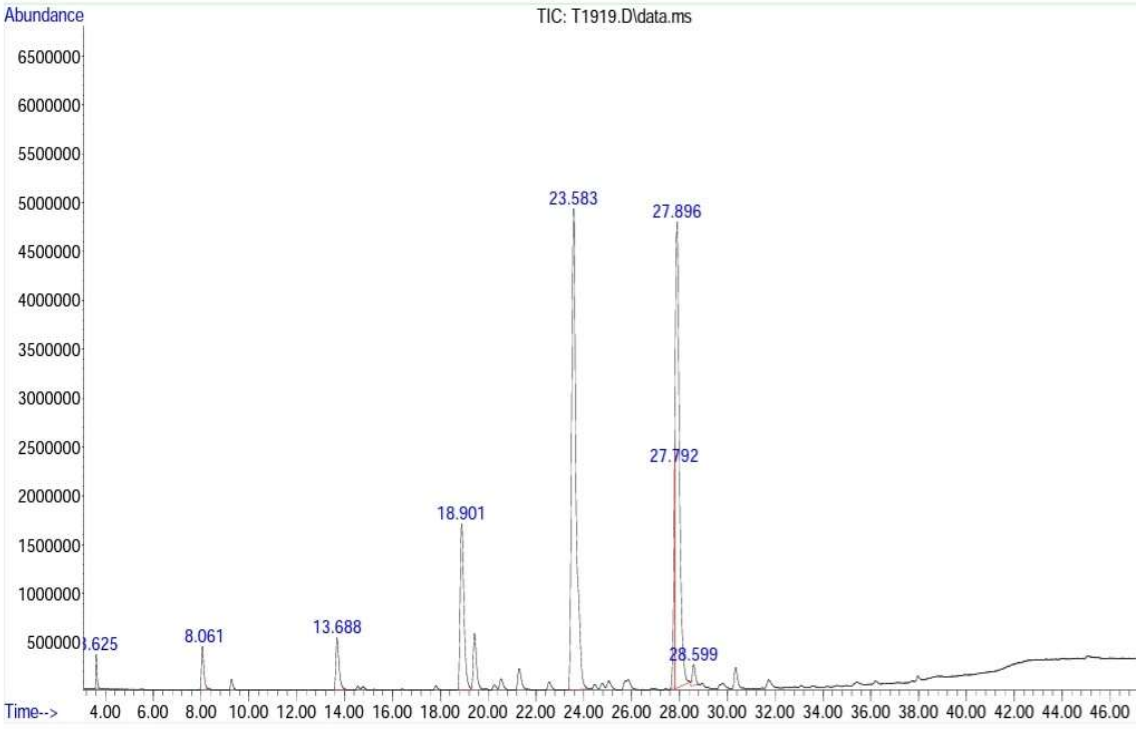


Table 14: GCMS- Milk%

Area Percent Report

Data Path : D:\GCMSD\2022\DECEMBER\27.12.2022\
Data File : T1919.D
Acq On : 27 Dec 2022 14:28
Sample : SAMPLE A

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.625	55	63	79	BB 2	347031	12429522	1.95%	0.789%
2	8.061	565	578	610	BB 2	441836	30940977	4.85%	1.964%
3	13.688	1214	1232	1273	BB 2	532641	48981735	7.68%	3.108%
4	18.901	1816	1838	1883	BV 4	1697760	187329257	29.36%	11.888%
5	23.583	2352	2383	2463	BB 2	4922740	638002176	100.00%	40.489%
6	27.792	2852	2872	2873	M3	2261056	62408295	9.78%	3.961%
7	27.896	2873	2884	2946	M5	4773969	576556704	90.37%	36.590%
8	28.599	2952	2966	2993	VB 7	213623	19089505	2.99%	1.211%

Sum of corrected areas: 1575738172

Graph 3: GCMS Arjuna Ksheerapaka chromatogram

File :D:\GCMSD\2022\DECEMBER\27.12.2022\T1920.D
Operator :
Acquired : 27 Dec 2022 16:10 using AcqMethod FATTY ACID STD.M
Instrument : GCMS
Sample Name: SAMPLE B
Misc Info :
Vial Number: 4

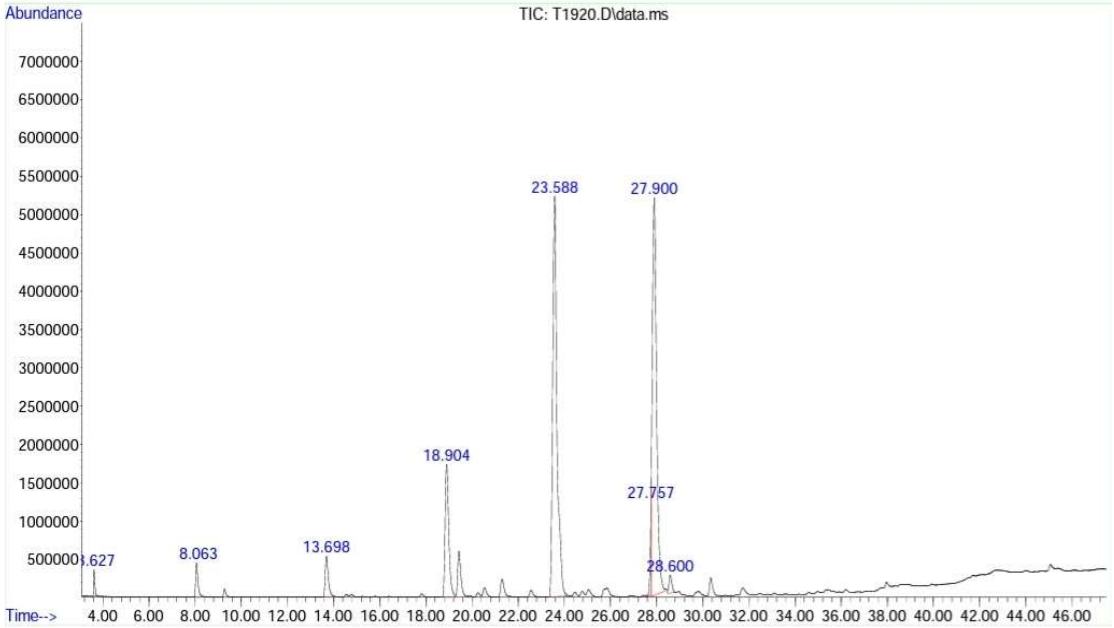


Table 15: GCMS –Arjuna ksheerapaka %

Area Percent Report

Data Path : D:\GCMSD\2022\DECEMBER\27.12.2022\
Data File : T1920.D
Acq On : 27 Dec 2022 16:10
Sample : SAMPLE B

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.627	55	63	78	BB	342392	11933397	1.78%	0.718%
2	8.063	563	578	610	BB 3	440466	30571699	4.56%	1.840%
3	13.698	1215	1234	1272	BB 2	526057	48867810	7.29%	2.941%
4	18.904	1817	1839	1883	BV 2	1721179	190572083	28.41%	11.468%
5	23.588	2354	2383	2460	BB 2	5204382	670770036	100.00%	40.366%
6	27.757	2844	2868	2869	M	1232360	26884358	4.01%	1.618%
7	27.900	2869	2885	2938	M5	5191860	661145373	98.57%	39.787%
8	28.600	2950	2966	2989	VB 8	229878	20974134	3.13%	1.262%

Sum of corrected areas: 1661718890

DISCUSSION:

The main objective of the present study was to prepare the Arjuna Ksheerapaka as per classical parameters and to perform the basic physico-chemical analysis, Phytochemical screening, HPTLC and GCMS of the formulation. The results of the analytical studies give the data of their phytoconstituents, protency and properties. Arjuna Ksheerapaka was prepared according to the general method of preparation explained in Sharangadhara Samhita. One part of drug is boiled with eight parts of milk and thirtytwo times of water reduced to the quantity of milk. Here, in this preparation both the water and fat soluble extracts are obtained. Along with this the attributes of the milk are also added. Results of the organoleptic charactors, physico-chemical analysis indicates the genuinity of the drugs used. Phytochemical screening revealed the presence of alkaloids, glycosides, favanoids, triterpenoids, tannins, carbohydrates and fatty oil. Inorganic chemical constituents detected are Calcium, magnesium, potassium, sodium, iron, sulphate, phosphate and chloride. In HPTLC seven phytoconstituents were detected.

Fatty acid profiling of the sample showed the presence of Caprylic acid, Capric acid, Lauric acid, Myristic acid, Palmitic acid, Stearic acid, Oleic acid and Linoleic acid in varying percentage.

CONCLUSION:

The Analytical studies of Arjuna Ksheerapaka shows the presence of Alkaloids, they are known for their anaesthetics, cardioprotective and anti-inflammatory effects **[6]**. Glycosides are found to be good for cardiovascular and neuro-degenarative illness. It also has anti-bacterial, anti-cancerous and anti-

inflammatory effect. **[7]**. Flavanoids are attributed to their anti-inflammatory, anti-mutagenic, anti-carcinogenic activities. Researches have proved their effectiveness in preventing CHD **[8]**. In researches, it is found Triterpenoid consumption reduced the occurrence of coronary heart disease and is cardioprotective **[9]**. Tannins are reported to have effect in diseases like Coronary artery disease, restrictive cardiomyopathy, dilated cardiomyopathy, endothelial dysfunction and cardiac hypertrophy **[10]**.

Calcium ions play vital role in cardiac contraction. Calcium regulates contractility **[11]**. Magnesium plays major role in modulating neuronal excitation, intra cardiac conduction and myocardial contraction by regulating a number of ion transporters including potassium and calcium channels **[12]**.

Potassium helps in the transmission of electrical impulses in the heart and regulates the heartbeat **[13]**. Iron is essential for oxygen transport and storage, mitochondrial function and enzyme activity **[14]**. Studies have proved that depletion of phosphate can lead to ventricular arrhythmias and elimination of ATP synthesis resulting in reveversible Myocardial dysfunction **[15]**. Chloride is the key electrolyte or renin-angiotensin-aldosterone system in kidney and the electrolyte regulating body fluid distribution **[16]**.

Caprylic acid improves Lipid metabolism **[17]**. Lauric acid is a fatty acid unlike other. It is known to increase high-density lipoprotein (HDL) ie: the good cholesterol. Also, as a medium-chain triglyceride (MCT), it gets fully metabolized by the liver. So, unlike other fatty acids it does not get stored as fat and is thus good for weight management. Oleic acid helps against cardiovascular insulin resistance and in atherosclerosis **[18-20]**.

The presence of these constituents in ksheerapaka is responsible for their action in hridroga as per the references. The GC-MS led to the identification of several compounds. The results are interpreted using the database of National Institute of Standards and Technology Library.

LIMITATIONS AND SCOPE OF STUDY:

This study has analysed the phytoconstituents, responsible for the mode of action of the drug. The formulation can be more logically planned according the condition of the patient.

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