



## GC-MS Analysis and Antioxidant studies of an Ayurvedic drug, Partharishtam

S. Sadhanandham<sup>1</sup>, G.Narayanan<sup>2</sup>, Mudiganti Ram Krishna Rao<sup>3\*</sup>, K.Prabhu<sup>4</sup>, Sumathi Jones<sup>5</sup>, Aparna Ravi<sup>6</sup>, Shruthi Dinakar<sup>7</sup>

<sup>1</sup>Assistant Professor, Dept of Cardiology, Sri Ramachandra Medical College and Hospital, Chennai, India.

<sup>2</sup>Research Scholar, Sree Balaji Medical College & Hospital, Bharath University, Chennai, India.

<sup>3\*</sup>Professor, Department of Industrial Biotechnology, Bharath University, Chennai, India.

<sup>4</sup>Associate Professor, Department of Anatomy, Sree Balaji Medical College & Hospital, Chennai, India.

<sup>5</sup>Professor of Pharmacology, Aasan Dental College, Chennai, India.

<sup>6</sup>PG Student, Department of Pharmacology, Sree Balaji Medical College & Hospital, Chennai, India.

<sup>7</sup>Ayurveda Physician, Kottakkal Arya Vaidya Sala, Chennai, India.

\*Corresponding author's E-mail: [mrkraj1455@gmail.com](mailto:mrkraj1455@gmail.com)

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### ABSTRACT

The present work envisages in understanding the bio molecules present in Partharishtam by GC MS analysis and to find its antioxidant activity. Partharishtam is prepared by processing the bark of *Terminalia arjuna*, flowers of *Madhuca indica* and *Woodfordia fruticosa*, fruits of *Vitis vinefera*, jaggery and water. Partharishtam was subjected to GC MS analysis after suitable preparation. Major bio molecules such as Glycerol tricaprlyate (RT value - 33.104: % Area - 11.44), Piperine (RT Value - 33.900: % Area - 6.22), Pyrazole (RT Value - 32.807: % Area - 5.400) were present among other compounds like 2-(3-chlorophenoxy)- N'- (2,4-dichlorobenzylidene) acethydrazide (RT value - 32.307: % Area - 8.83), Methyl 2 - (4-chlorophenyl)-6-methoxy-7-chlorocinchoninate (RT value - 31.817: % Area - 1.70), 1,3-dipentyl-heptabarbitol (RT value - 35.003: % Area - 4.44), 2-Pyrididone, 3,5-diiodo-n-methyl-1-Dimethyl derivative (RT Value - 31.633: % Area- 1.65), Stanzolol (RT value - 29: % Area - 0.15). The various antioxidant studies of Partharishtam have indicated moderate activities when compared with standards in various methods applied. The medicinal properties of the molecules present in Partharishtam and its antioxidant properties could substantiate well with the claim that Partharishtam is a potent cardio tonic formulation.

**Keywords:** Partharishtam, *Terminalia arjuna*, *Madhuca indica*, *Woodfordia fruticosa*, *Vitis vinefera*, Piperine, Pyrazole.

### INTRODUCTION

Partharishtam or Arjunarishtam is an Ayurvedic liquid formulation, popularly used as herbal heart tonic. Partharishtam contains 6-12 % of self generated alcohol in it. This self generated alcohol and the water present in the product acts as a media to deliver water and alcohol soluble active herbal components to the body. It is also known as Parthadyarishtam.

Partharishtam is useful in the treatment of chest injury, weakness, feeling tired always, chronic respiratory diseases, cough and throat related diseases.<sup>1</sup> It is extensively used as herbal cardiac tonic. It improves strength and helps to cleanse intestines. The Indian subcontinent, which is one of the oldest civilization, is the house of many traditional health care systems like Ayurveda, Siddha, Unani, Naturopathy, Tibetan medicine and Yoga. Ayurveda, one of the most ancient systems of medicine includes various types of medicines including fermented forms namely arishtams and asavas which show high palatability and stability.<sup>2</sup>

The classical Ayurvedic preparations like arishtams are used to treat many ailments typically for digestive and cardiac ailments (Kalaiselvan).<sup>3</sup> One such preparation is Partharishtam or Arjunarishtam which is used for its cardio tonic properties such as nourishing and strengthening heart muscles, promoting cardiac

functioning by regulating blood pressure and cholesterol.<sup>4</sup> The ingredients of Partharishtam are according to Bhaishajya Ratnavali Hrudroga Adhikara 33/75-77 API, AFI, are,

Arjuna tvak – *Terminalia arjuna* (Family – Combrataceae) - Stem bark - 4.8 kg

Mrudvika – *Vitis vinifera* (Family - Vitaceae) - dry grapes - 2.4 kg

Madhuka – *Madhuca indica* (Family – Sapotaceae) – flowers - 960 grams

Dhataki – *Woodfordia fruticosa* (Family - Lythraceae) – Flowers- 960 grams

Guda – Jaggery – 4.8 kg

Water- 49.152 lits.

Arjuna bark, Dry grapes and Madhuka flowers are made to coarse powder and mixed with 49.152 liters of water. This mixture is boiled in slow flame, stirred at regular intervals and made to 12.288 liters, cooled and filtered. Dhataki flowers and jaggery are added and the mixture is allowed to ferment. After the fermentation the contents are filtered and stored in air tight containers. This formulation is manufactured by leading Ayurveda Pharmaceutical companies like, Zandu, Dabur, Baidyanath and Arya Vaidya Sala (Kottakkal).



The constituent plants parts i.e. *Terminalia arjuna* bark, *Vitis vinifera* fruits, *Madhuca indica* flowers and *Woodfordia fruticosa* flowers, are all known for their cardio-protective properties.

*Terminalia arjuna* is one of the most versatile medicinal plants having a wide spectrum of biological activity. The Hypocholesterolaemic effects of *Terminalia arjuna* tree bark was reported by Ram in rabbits.<sup>5</sup> Antioxidant and hypocholesterolaemic effects of *Terminalia arjuna* tree-bark powder was reported by Gupta.<sup>6</sup> The bark of *T. arjuna* is anti-dysenteric, antipyretic, astringent, cardio tonic, litho-triptic, anticoagulant, hypolipidemic, antimicrobial and antiuremic agent.<sup>7-9</sup> Many useful phyto-constituents have been isolated from *T. arjuna* which included triterpenoids for cardiovascular properties, tannins and flavonoids for its anticancer, antimicrobial properties and so on.<sup>10</sup> In studies on mice, its leaves have been shown to have analgesic and anti-inflammatory properties.<sup>11</sup>

Akshatha have reviewed the various medicinal effects of bark of *Madhuca longifolia*. The plant is reported to have activities like antioxidant, wound healing, antimicrobial, antiinflammatory, antipyretic, antidiabetic, antihyperglycemic, anticancer and antiepileptic. The phytochemical studies of leaves of *Madhuca* revealed the presence of beta carotene, palmitic acid, myricetin, O-3-arabinoside, 3-O-rhamnoside, uercetin,3-galactoside, 3b-caproxy acid, Beta- sitosterol, stigmastero, b-sitosterol and B-D glucoside.<sup>12</sup>

The flowers, seeds and seed oil of *Madhuca* have great medicinal value to alleviate pain, skin diseases and used a nasya (sunff) to cure sinusitis, and also used in urinary ailments like burning micturition and dehydration. It is also used for fever and tuberculosis. The phyto chemical screening revealed the presence of secondary metabolites like saponin and flavonoids, which are anti inflammatory, antispasmodic, anti analgesic, antidiuretic and have cardio-protective properties. Annalakshmi have shown the presence of some phyto chemicals in *Madhuca* by GC MS and HPTLC analysis, which are attributed for its various medicinal values.<sup>13</sup>

It was reported by Dubey that the presence of therapeutically potent antimicrobial compounds against MDR bacteria in *Woodfordia fruticosa* and the crude leaf-extract had no host toxicity with human lymphocytes.<sup>14</sup> The n-butanol fraction of the extract was the most suitable bio-active fraction. The terpenes isolated were, phenol, 5-methyl-2-(1-methylethyl)-, phenol, 2-methoxy-4-(2-propenyl)-, 2, 6-octadien-1-ol, 3, 7-dimethyl-(E)-, 2, 6-octadienal, 3, 7-dimethyl-, cyclohexanol, and 2-methylene-5-(1-methylethenyl).

The leaves have sedative properties and the juice of its fresh flowers, when applied on the head, supposed to reduce headache. The curative properties of *Woodfordia* are due to the presence of secondary metabolites like alkaloids, flavonoids, glycosides, phenols, saponins,

sterols etc. Grover and Patni, 2013 have identified 21 compounds in the GC MS analysis of *Woodfordia* leaf extracts with important medicinal properties.<sup>15</sup>

The cardio-protective role of grapes was reported by Dohadwala and Vita, 2009, Leifert and Abeywardaba, 2008, Perez and Saura, 2008 and Folts, 2002.<sup>16-19</sup> The antioxidant properties of the polyphenols such as resveratrol, phenolic acids, anthocyanins and flavonoids present in grapes are attributed to secondarily help to avoid atherosclerosis, platelet aggregation and stenosis. These compounds also possess a range of additional cardio protective and vaso-protective properties including anti-atherosclerotic, anti-arrhythmic, and vaso-relaxation actions.

The combination of these plant parts for preparing Partharishtam itself indicates the genius of the Ayurvedic proponents in developing such combination as a cardio tonic formulation.

The aim of the present study is to find out the active bio molecules present by GC MS analysis and studying the antioxidant properties of Partharishtam. Since the constituent plants are known for their various cardio tonic and other medicinal properties, it is of interest to find out whether the molecules that were present in Partharishtam with cardio tonic properties. This novel approach may lead to a logical conclusion that Partharishtam can be scientifically validated for its claim as a cardio tonic formulation.

## MATERIALS AND METHODS

Partharishtam was procured from a standard local Ayurvedic shop at Chennai.

The medicine which is available in liquid form was subjected to GC MS analysis after necessary procedure.

The metabolites in the samples were identified using a P2010 gas chromatography with thermal desorption system TD20 coupled with mass spectroscopy (Shimadzu).

The ionization voltage 70ev and GC was conducted in the temperature programming mode with a Restek column (0.25mm, 60m, XTI-5). The temperature in the initial column was 80°C for 1 min, and then increased linearly to 70°C to 220°C held for 3 min followed by linear increased temperature 100°C up to 290°C and held for 10min. The injection port temperature was 290°C and the GC/MS interface was maintained at 29°C, the samples were introduced via an all glass injector working in the split mode with helium carrier gas low rate with 1.2 ml per minute.

The identification of metabolites was accomplished by comparison of retention time and fragmentation pattern with mass spectra in the NIST spectral library stored in the computer software (version 1.10 beta, Shimadzu) of the GC-MS. The relative percentage of each extract constituent was expressed with peak area normalization.



### Antioxidant activity (DPPH free radical scavenging activity) determination

The antioxidant activity of the plant extracts was examined on the basis of the scavenging effect on the stable DPPH free radical activity as per the method of Braca.<sup>20</sup>

Ethanol solution of DPPH (0.05 mM) (300 l) was added to 40 l of extract solution with different concentrations (0.02 - 2 mg/ml).

DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7 ml) was added and the mixture was shaken vigorously.

The mixture was left to stand for 5 min and absorbance was measured spectrophotometrically at 517 nm. Ethanol was used to set the absorbance zero.

A blank sample containing the same amount of ethanol and DPPH was also prepared. All readings were performed in triplicate.

The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation (Yen and Duh, 1994).<sup>21</sup>

Percent (%) inhibition of DPPH activity =  $[(AB - AA) / AB] \times 100$ ; where AA and AB are the absorbance values of the test and the blank sample, respectively.

A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and represented as IC50 value for each of the test solutions. The results are mentioned in Table No. 2

### ABTS free radical scavenging assay

The antioxidant capacity of Partharistham extract was measured using 2, 2'-azinobis[3-ethylbenzthiazoline]-6-sulfonic acid (ABTS) assay (Re).<sup>22</sup>

ABTS was dissolved in de-ionized water to 7 mM concentration and potassium persulphate was added to a concentration of 2.45 mM.

The reaction mixture was left to stand at room temperature overnight (12~16 h) in the dark before use. The resultant intensely coloured ABTS•+ radical cation was diluted with 0.01 M PBS (phosphate buffered saline) at pH 7.4 to give an absorbance value of ~0.70 at 734 nm.

The test compound was diluted 100 × with the ABTS solution to a total volume of 1 ml.

Absorbance was measured spectrophotometrically at time intervals of 1 min after addition of each extract.

The assay was performed in triplicate. Controls containing 990 µl of PBS, to replace ABTS, were used to measure absorbance of the extract themselves.

The assay relies on the antioxidant capability of the samples to inhibit the oxidation of ABTS to ABTS•+ radical cation.

The total antioxidant activities were expressed as mM trolox equivalent antioxidant capacity (TEAC). The results are tabulated in Table No.3.

### Hydrogen Peroxide Scavenging Capacity

The ability of Partharistham to scavenge hydrogen peroxide was determined according to the method of Ruch.<sup>23</sup> A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH-7.4). Extracts (100 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both sample and standard compounds were calculated and tabulated as mentioned in Table No. 4.

### Total Antioxidant Activity-Ferric Thiocyanate Method

The antioxidant activity of Partharistham and standards was determined according to the ferric thiocyanate method in linoleic acid emulsion (Mitsuda).<sup>24</sup> With this method peroxide formation occurred during the oxidation of linoleic acid oxidation. These compounds oxidized Fe<sup>2+</sup> to Fe<sup>3+</sup>. The latter ions form a complex with thiocyanate and this complex has a maximum absorbance at 500 nm. The results are shown in Table No 5.

### Hydroxyl radical scavenging assay

Hydroxyl radicals were generated by a Fenton reaction (Fe<sup>3+</sup>-ascorbate-EDTA-H<sub>2</sub>O<sub>2</sub> system), and the scavenging capacity towards the hydroxyl radicals was measured by using deoxyribose method [Halliwell].<sup>25</sup>

The reaction mixture contained 2-deoxy-2-ribose (2.8 mM), phosphate buffer (0.1 mM, pH 7.4), ferric chloride (20 µM), EDTA (100 µM), hydrogen peroxide (500 µM), ascorbic acid (100 µM) and various concentrations (10-1000 µg/ml) of the test sample in a final volume of 1 ml.

The mixture was incubated for 1 h at 37 °C.

After the incubation an aliquot of the reaction mixture (0.8 ml) was added to 2.8% TCA solution (1.5 ml), followed by TBA solution (1% in 50 mM sodium hydroxide, 1 ml) and sodium dodecyl sulphate (0.2ml). The mixture was then heated (20 min at 90 °C) to develop the colour.

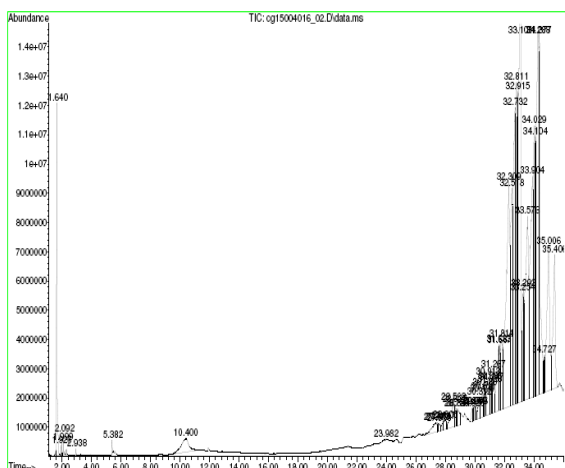
After cooling, the absorbance was measured at 532 nm against an appropriate blank solution.

All experiments were performed in triplicates. The results are tabulated in Table No. 6.



**RESULTS AND DISCUSSION**

The GC MS pattern is represented in Figure 1.



**Figure 1:** The GC-MS pattern of Partharistham

The various chemicals present in the GC MS profile of Partharistham is tabulated in Table 1.

The GC MS patterns and the peaks indicated important bioactive molecules like Glycerol tricaprilate (RT value - 33.104: % Area - 11.44), 2-(3-chlorophenoxy)- N'- (2,4-dichlorobenzylidene) acethydrazide (RT value - 32.307: % Area - 8.83), Propanoic acid, 3, 3'-thiobis-didodecyl ester (RT Value – 33.307: % Area – 6.43), Piperine (RT Value - 33.900: % Area - 6.22), Pyrazole (RT Value - 32.807: % Area - 5.400), 1, 3-Dipentyl-heptabarbital (RT Value – 35.03: % Area – 4.440), Isopropylphosphonic acid, fluoro anhydride-, decyl ester (RT Value – 32.920; % Area – 2.10), Ethyl alcohol (RT value – 1.638; % Area – 2.01), Methyl 2-(4-Chlorophenyl)-6-methoxy-7-chlorocinchonate ( RT value – 3.817; % Area – 1.70), 2-Pyrididone, 3, 5-diiodo-n-methyl-1-Dimethyl derivative ( RT Value - 31.633: % Area- 1.65), Diisopropyl sulfide (RT Value – 10.401: % Area – 1.21) among some minor compounds like etc. among other compounds like Stanzolol (RT value – 29; % Area - 0.15), 1,3-Dipentyl-heptabarbital (RT Value – 31.664: % Area – 0.56), 1,3-Dipentyl-heptabarbital (RT Value - 31.664: % Area – 0.45) etc.

**Table 1:** GC MS Profile of Partharistham (Reference Library-NIST05a.L)

Sl. No.	Retention Time (RT) (Min)	Phyto-component	% Peak area
1.	1.638	Ethyl alcohol	2.01
2.	1.924	Pentane, 2-methyl-	0.05
3.	1.996	Pentane, 3-methyl-	0.05
4.	2.088	Hexane	0.08
5.	2.935	Acetic acid	0.04
6.	5.386	2,3-Butanediol	0.08
7.	10.401	Diisopropyl sulfide	1.21
8.	23.984	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.01
9.	27.354	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.53
10.	27.476	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.08
11.	27.579	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.16
12.	25.844	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.15
13.	28.007	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.21
14.	28.559	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.92
15.	28.630	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.32
16.	28.763	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.19
17.	28.845	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.42
18.	29.815	Stanzolol,n,o-bis (trimethylsilyl) derivative	0.15
19.	29.989	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.22
20.	30.060	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.18
21.	30.316	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.40
22.	30.499	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.60
23.	30.612	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.25
24.	30.683	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.41
25.	30.908	Propanoic acid, 3,3'-thiobis-didodecyl ester	1.05
26.	31.020	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.18
27.	31.061	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.36
28.	31.143	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.19
29.	31.265	Propanoic acid, 3,3'-thiobis-didodecyl ester	0.85



30.	31.633	2-Pyridone,3,5-diiodo-N-methyl-1-dimethyl(phenyl)silyloxyhexadecane	1.65
31.	31.664	1,3-Dipentyl-heptabarbital	0.56
32.	31.817	Methyl 2-(4-Chlorophenyl)-6-methoxy-7-Chlorocinchonate	1.70
33.	32.307	2-(3-Chlorophenoxy)-N'-(2,4-dichlorobenzylidene)acethydrazide	8.83
34.	32.521	Glycerol Tricaprylate	4.91
35.	32.736	Glycerol Tricaprylate	6.96
36.	32.807	Pyrazole,1-methyl-4-nitro-	5.40
37.	32.920	Isopropylphosphonic acid, fluoro anhydride-,decyl ester	2.10
38.	32.104	Glycerol Tricaprylate	11.44
39.	33.257	Propanoic acid, 3,3'-thiobis-didodecyl ester	1.36
40.	33.287	Propanoic acid, 3,3'-thiobis-didodecyl ester	1.11
41.	33.573	Propanoic acid, 3,3'-thiobis-didodecyl ester	6.43
42.	33.900	Piperine	6.22
43.	34.033	Glycerol Tricaprylate	4.79
44.	34.104	Glycerol Tricaprylate	1.62
45.	34.288	Glycerol Tricaprylate	9.82
46.	34.380	Glycerol Tricaprylate	5.23
47.	34.727	Dichloroacetic acid, heptadecyl ester	0.45
48.	35.033	1,3-Dipentyl-heptabarbital	4.44
49.	35.401	Glycerol Tricaprylate	3.48

**Table 2:** *In vitro* antioxidant activity by DPPH Scavenging Activity

Sl. No.	% of Inhibition		
	Concentration ( $\mu\text{g/ml}$ )	Partharishtam	Ascorbic Acid
1	20	12.2 $\pm$ 4.36	38 $\pm$ 3.6
2	40	23.1 $\pm$ 3.12	51.6 $\pm$ 3.4
3	60	45.6 $\pm$ 2.46	66.31 $\pm$ 1.72
4	80	58.46 $\pm$ 1.06	81.62 $\pm$ 2.46
5	100	63.42 $\pm$ 4.37	94.6 $\pm$ 1.63

From Table No. 2 it is indicated that maximum antioxidant activity of Partharishtam was observed at 60  $\mu\text{g/ml}$  concentration of the sample.

**Table 3:** *In vitro* antioxidant activity by ABTS Scavenging Activity

Sl. No.	% of Inhibition		
	Concentration( $\mu\text{g/ml}$ )	Partharishtam	BHT
1	20	5.2 $\pm$ 3.86	30 $\pm$ 3.6
2	40	11.3 $\pm$ 5.16	45.6 $\pm$ 1.4
3	60	24.2 $\pm$ 1.21	60.31 $\pm$ 1.42
4	80	32 $\pm$ 3.16	82.2 $\pm$ 3.46
5	100	40 $\pm$ 4.64	96.4 $\pm$ 3.13

From Table No.3 it was observed that the maximum antioxidant activity of Partharishtam was observed at 20  $\mu\text{g/ml}$  concentration.

**Table 4:** *In vitro* antioxidant activity by Hydrogen peroxide Scavenging Activity

Sl. No.	% of Inhibition		
	Concentration ( $\mu\text{g/ml}$ )	Partharishtam	BHT
1	20	8.43 $\pm$ 2.86	40 $\pm$ 2.6
2	40	13.9 $\pm$ 4.12	65.6 $\pm$ 1.4
3	60	33.2 $\pm$ 2.57	80.21 $\pm$ 3.42
4	80	43 $\pm$ 3.26	91 $\pm$ 1.62
5	100	51.72 $\pm$ 4.63	98.2 $\pm$ 6.13



Table 4 indicates maximum antioxidant activity at a concentration of 20 µg/ml of Partharishtam.

**Table 5:** *In vitro* antioxidant activity by Hydroxyl radical Scavenging Activity

Sl. No.	% of Inhibition		
	Concentration (µg/ml)	Partharishtam	Ascorbic acid
1	20	5.9±1.86	12±6.6
2	40	10±3.12	25.6±4.2
3	60	18±2.71	45.31±2.12
4	80	26±4.06	68.2±4.26
5	100	43±3.37	84.4±2.61

Table No. 5 shows that maximum inhibition was seen at a concentration of 20 µg/ml of partharishtam.

**Table 6:** *In vitro* Total antioxidant activity by ferric reducing activity

Sl. No.	% of Inhibition		
	Concentration (µg/ml)	Partharishtam	Ascorbic acid
1	20	7.2 ±3.86	14 ±8.6
2	40	16.3±5.16	25.6±6.2
3	60	28.2±1.21	46.31±3.7
4	80	36±3.16	88.2±3.26
5	100	48 ±4.64	94.4±5.61

The antioxidant activity was maximum at a concentration of 20 µg/ml of Partharishtam as shown in Table No.6.

Majority of the present day medicines have their origin from phyto chemicals.

Extracting active bio molecules with medicinal properties from plants material is time consuming, costly and also may affect the biodiversity as a whole.

Therefore, these molecules are being synthesized in bulk for the manufacture of medicines in large scale.

Ayurveda and Sidhha medical practice, however, still follow the traditional practice of manufacturing the various formulations from plants, natural salts or the combination of both.

Each of the Ayurvedic formulation has many important medicinal ingredients and they are believed to function in a synergistic way.

It is also believed that since they are more biocompatible to human beings, the chances of their causing side effects become less as compared to the modern pharmaceutical products.

The present work envisages finding out the various bio active molecules present in one Ayurvedic formulation, Partharishtam, which is a known cardio tonic medicine. The GC MS analysis indicated the presence of certain major molecules like, Glycerol tricaprylate, Piperine, Pyrazoles along with some minor ingredients.

It is quite interesting to find that all the three major components have many medicinal properties among which their role as cardio tonic is major one.

Glycerol tricaprylate is a known food supplement for children and patients. It has antihistaminic and skin conditioning properties.

2-(3-Chlorophenoxy)-N'-(2,4-dichlorobenzylidene) acetylhydrazide molecules are well known for their biological activity as antibacterial, antifungal, anticancer and antiviral.<sup>26,27</sup>

Piperine has diverse biological and supportive therapeutic activities. It helps in the absorption of selenium, vitamin B and Beta carotene as well as other nutrients. Manoharan have demonstrated the chemo-preventive role of piperine.<sup>28</sup> Selvendiran have shown the anticancer role of Piperine.<sup>29-31</sup> The antiulcer role of Piperine was demonstrated by Bai and Xu, 2000 in rat model.<sup>32</sup> Piperine is known to have radio protective, immune modulatory, anti tumor activities, antidepressant, anticonvulsant, antinociceptive, and anti-arthritis.<sup>33-41</sup> Faas have reported the role of piperine in pigmentation in rat models.<sup>42</sup> Among the various properties of piperine, the most important is that it facilitates the bioavailability of medicines by depressing the activity of drug metabolizing enzymes.<sup>43</sup> In Partharishtam the role of Piperine could be its anti-inflammatory, antioxidant and as facilitator of bio-availability of other medicines.

Pyrazole moiety and its various derivatives which is found in Partharishtam was found to possess various pharmacological potentials. Pyrazoles and its derivatives are a class of nitrogen heterocyclic compounds which occupy an important position in medicinal and pesticide chemistry. Pyrazoles are known to have many medicinal roles like antibacterial, anticancer, anti-inflammatory, antidepressant, anti-hyperglycemic, anti-pyretic, antimicrobial, antifungal, CNS regulant and enzyme



inhibitory activity. Its role as antihypertensive is reported by Demirayak.<sup>44</sup> In Partharishtam the role of pyrazole could be antihypertensive and also enzyme inhibitory.<sup>45</sup>

The derivatives of Methyl 2 - (4-chlorophenyl) - 6-methoxy-7-chlorocinchoninate is medically used to reduce fever, pain and functions as Non-Steroidal Anti-Inflammatory Drug (NSAID). Similarly the derivatives of 2-Pyrididone, 3, 5-diiodo-n-methyl-1-Dimethyl are used as analgesics.

Although present in small amount, the presence of Stanozolol in Partharishtam is of significance. Stanozolol has been used in both animal and human patients for a number of conditions. For weak animals this drug is administered for muscle growth, RBC production, for increasing bone density and for increasing the appetite. In human beings this is an anabolic steroid used for performance enhancing in athletes.

Some of the molecules like Pyrazoles and Piperine directly contribute as cardio tonic or cardio protective while other molecules present in Partharishtam have various other activities like analgesic, antipyretic, anti inflammatory etc. Further work is going on to prove their role as cardio tonic or cardio protective by understanding the mechanism of action of these components. Since they are found in Partharishtam this study is all the more valid. It is also possible that these molecules could have some synergistic role. Thus Partharishtam can facilitate all these conditions in patients.

The constituent plants of Partharishtam are known for their antioxidant activities. The study of this activity in Partharishtam was to see whether antioxidant capacity of the individual component plants is reflected in Partharishtam. The results have shown moderate positive antioxidant activity when compared with the standards. It was observed that Except for DPPH test all the other tests indicated that at 20 µg/ml concentration the effect was maximum although when compared to the standards it was less. In DPPH antioxidant study the results showed a maximum activity at 60 µg/ml. The lower activities of Partharishtam when compared to the standards might indicate the slow rate of action of this medicine. This can also lead to the surmise that since this medicine is a mixture of number of plants, the lower antioxidant activity could be due to the synergy among the various molecules which could lead to slow activity. Further work is in progress to understand these aspects.

## CONCLUSION

From the discussion it may be concluded that the Ayurvedic proponents have chosen only those plants and plant parts for the preparation of Partharishtam which have cardio-tonic properties. And Partharishtam, as a medicine, reflects the similar medicinal properties. Once the constituents were mixed in proper proportion and processed in a particular methodology, the medicine, i. e. Partharishtam, could give better results as compared to its constituents due to the synergy among the new bio

molecules which were synthesized during the preparation of the formulation. This could be one of the reasons for the low antioxidant activity of Partharishtam when compared with the corresponding standards used during the study. The present study is a preliminary report and further work is in progress to establish the scientific validity of this Ayurvedic formulation.

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