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SHORT COMMUNICATION

Emblica officinalis Gaertn. (*Amalaki*): A Natural Herbal Remedy to Enhance Cardiorespiratory Fitness in *Ayurveda* - An *In Silico* Molecular Docking Approach

Amal S. Chandran[1*], Ashok Patil[1], Sanjiv Kumar[2], Krishika Muralidharan[3] and Sreeja V. Kaimal[4]

[1]Department of Swasthavritta, KLE Academy of Higher Education and Research's Shri B. M. Kankanawadi *Ayurveda* Mahavidyalaya, Belagavi – 590003, Karnataka, India; dr.amal1409@gmail.com

[2]Department of Neurophysiotherapy, KLE Academy of Higher Education and Research's Institute of Physiotherapy, Belagavi – 590010, Karnataka, India

[3]Department of Rasayana Evam Vajeekarana, KLE Academy of Higher Education and Research's Shri B. M. Kankanawadi *Ayurveda* Mahavidyalaya, Belagavi – 590003, Karnataka, India

[4]Department of Rasashastra and Bhaishajya Kalpana, KLE Academy of Higher Education and Research's Shri B. M. Kankanawadi *Ayurveda* Mahavidyalaya, Belagavi – 590003, Karnataka, India

Abstract

Background: Cardiorespiratory fitness is the capacity of the circulatory and respiratory systems to transport oxygen to the skeletal muscle mitochondria for energy production during physical activity. Cardiorespiratory fitness has been recognized as a vital health biomarker and is very crucial for sports persons. Physical activities, aerobic exercise, yoga, meditation, nutritional supplements, and ergogenic aids are the ways to enhance cardiorespiratory fitness. *Emblica officinalis* Gaertn. is one of the widely used drugs in *Ayurveda* traditional medicine to enhance Cardiorespiratory Fitness (CRF). However, there is a dearth of clear information regarding how *Emblica officinalis* can improve CRF. **Objective:** The current *in silico* molecular docking study was planned to identify the phytochemicals, and targets of endurance and predict the probable mode of action of the drug and thereby substantiate the ability of *Emblica officinalis* as a natural and ethical way of enhancing cardiorespiratory fitness. **Methods:** The phytochemicals and targets are collected from reliable sources, and the effectiveness of these gene targets was validated using network pharmacology ligand-target interaction methods. The Protein Data Bank and PubChem were used to find the ligands and targets, and PyRx was used to do docking. **Conclusion:** *Emblica officinalis* is found to have a positive influence on the 12 metabolic pathways that act in enhancing the cardio-respiratory endurance in the human being. PRKCA was analyzed and concluded as the highly modulated gene target with the lowest binding energy. Thus, *Emblica officinalis* was found to have an action in enhancing cardio-respiratory endurance.

Keywords: Aerobic Fitness, Endurance, Molecular Docking, Network Pharmacology, Stamina, Strength

1. Introduction

Cardiorespiratory fitness is the capacity of the circulatory and respiratory systems to transport oxygen to the skeletal muscle mitochondria for energy production during physical activity. It is also referred to as cardiovascular endurance, cardiorespiratory endurance, aerobic capacity, and aerobic fitness. Cardiorespiratory Fitness (CRF) has

*Author for correspondence

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been recognized as a vital health biomarker and reduced CRF is a powerful predictor of adult mortality. When it comes to health indicators for young people, CRF is a good predictor of academic performance, mental health, early CVD, and cardiometabolic health. Athletes must have a good CRF to perform at their best in sports, therefore it is crucial for them as well. The ways to enhance CRF include physical activities, aerobic exercise, Yoga, meditation, nutritional supplements, ergogenic aids and others[1-4]. Dietary supplements are used by athletes for a variety of benefits, such as speeding up recovery, preserving their health, enhancing performance, bolstering their immune systems, adjusting body composition, and making up for bad dietary choices. However, the quality of these products cannot be assured as these supplements may contain illegal compounds without the knowledge of athletes or manufacturers. Studies also showed that these nutritional supplements contain prohibited substances which may result in athletes failing doping tests. The unethical usage of performance-enhancing drugs can lead to serious consequences for the health of athletes[5]. So, a natural, efficacious, safe, affordable, convenient and ethical method of increasing CRF without having any health consequences is the need of the hour.

Ayurveda, the traditional Indian system, has a diverse spectrum of medications that can improve CRF. *Ayurveda* medications like *Chyavanaprash*, *Amalaki*, *Shatavari*, *Ashwagandha*, and *Arjuna* are used for enhancing CRF. *Ayurveda* preparations like *Chyavanaprash*, *Amalaki rasayana*, and *Ratnaprash* contain *Amalaki* as the key ingredient and are proven to improve performance, stamina and cardiorespiratory endurance[6-9]. Several studies have been conducted on the effect of *Amalaki* and its combination in sports medicine especially as a natural performance enhancer. However, there is a lack of conclusive evidence related to how *Amalaki* helps improve cardiorespiratory endurance. So, the current *in silico* molecular docking study is planned to identify and substantiate the ability of *Amalaki* as a natural and ethical way of enhancing cardiorespiratory fitness.

2. Materials and Methods

The phytochemicals in *Emblica officinalis* are derived from the GCMS data, Dr. Duke's Phytochemical and Ethnobotanical database and the IMPPAT database[10,11]. The drug-likeness properties of the phytochemicals were evaluated with the help of Molsoft software[12]. Lipinski's rule fulfilled phytochemicals were selected and the structural data were derived from the PubChem software and the Swiss ADME tool[13,14]. The respective targets of each phytochemical were found using Swiss target prediction software[15]. The targets directly or indirectly enhancing CRF endurance are taken from relevant e-resources[16-23]. Both the CRF gene targets and phytochemical targets are evaluated for similar targets.

The pathways directly or indirectly enhance the CRF endurance that can be modulated by the phytochemicals detected by the STRING biological database[24]. The network was constructed in Cytoscape 3.7.2 software by using highly modulated pathways, phytochemicals and targets (protein molecules). A scale of colours and node sizes was used to understand the network, which is based on the number of edges and the sizes of the nodes. The target protein data and the phytochemical's three-dimensional structure were collected from the RCMB protein data bank and PubChem databases, respectively. Discovery Studio Visualizer was used to remove the water molecules and the protein molecule's heteroatoms[25]. The binding affinity between the phytochemicals and the receptors was determined using PyRx software[26]. The Discovery Studio visualizer is used to depict the interaction between the ligand and protein of the molecule with the lowest binding energy. The detailed methodology of the study is illustrated in Figure 1.

3. Discussion of Results

A total of 193 phytochemicals of *Emblica officinalis* are retrieved from GCMS data and Dr. Duke's Phytochemical and Ethnobotanical database (Table 1).

The phytochemicals are evaluated in Molsoft software and phytochemicals which satisfy Lipinski's rule are selected. The structural data were derived from the PubChem software and the Swiss ADME tool. The phytochemical target of all 65 phytochemicals was taken from Swiss target prediction software (Table 2).

81 targets that are related to CRF are taken from relevant e-literature sources. 27 Phytochemicals were

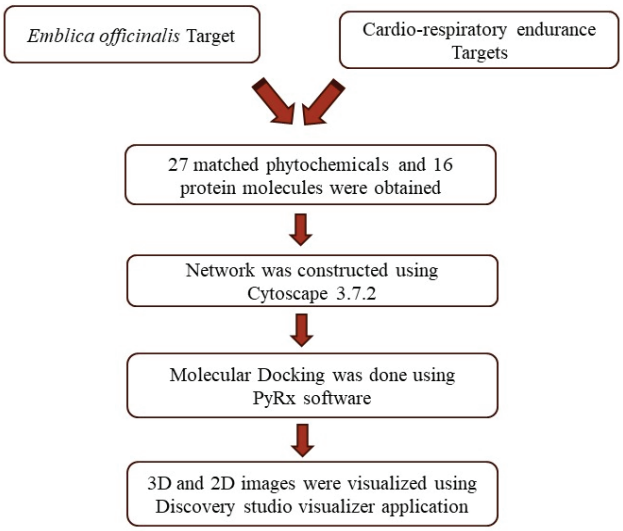

Figure 1. Methodology of the study.

Table 1. List of phytochemicals in *Emblica officinalis*

Sl. No.	Source of Data	Phytochemicals
1	GCMS[10]	2,4-Dimethylfuran
2		4-(2-Hydroxyethyl)-3-methyl-2-pyrazolin-5-one
3		Trans-2,3-Epoxyoctane
4		2-Furancarboxylic acid, 2-ethylhexyl ester
5		Heptanoic acid, 3-hydroxy-, methyl ester
6		2-(3-Methylguanidino) ethanol
7		d-Glycero-d-ido-heptose
8		Paromomycin
9		Octadecanoic acid
10		9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione
11		n-Propyl nonyl ether
12		Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester
13		n-Hexadecanoic acid
14		Z-(13,14-Epoxy) tetradec-11 -en-1-ol acetate
15		6,9,12,15-Docosatetraenoic acid, methyl ester
16		Cyclopentaneundecanoic acid
17		1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
18	Dr. Duke's Phytochemical and Ethnobotanical database	3-6-di-O-galloyl-glucose
19		Alanine
20		Amlaic-acid
21		Arginine

Table 1. to be Continued…

Sl. No.	Source of Data	Phytochemicals
22		Ascorbic-acid
23		Aspartic-acid
24		Astragalin
25		Beta-carotene
26		Beta-sitosterol
27		Boron
28		Calcium
29		Carbohydrates
30		Chebulagic-acid
31		Chebulaginic-acid
32		Chebulic-acid
33		Chebulininic-acid
34		Chibulinic-acid
35		Chloride
36		Copper
37		Corilagic-acid
38		Corilagin
39		Cystine
40		D-fructose
41		D-glucose
42		Ellagic-acid
43		Emblicol
44		Ethyl-gallate
45		Fat
46		Fiber
47		Gallic-acid
48		Gallic-acid-ethyl-ester
49		Gallo-tannin
50		Gibberellin-A-1
51		Gibberellin-A-3
52		Gibberellin-A-4
53		Gibberellin-A-7
54		Gibberellin-A-9
55		Glucogallin
56		Glucose
57		Glutamic-acid
58		Glycine
59		Histidine
60		Iron
61		Isoleucine
62		Kaempferol

Table 1. to be Continued…

Sl. No.	Source of Data	Phytochemicals
63		Kaempferol-3-O-glucoside
64		Kaempferol-3-O-glucoside
65		Kilocalories
66		Leucine
67		Leucodelphinidin
68		Linoleic-acid
69		Linolenic-acid
70		Lupenone
71		Lupeol
72		Lysine
73		Magnesium
74		Manganese
75		Methionine
76		Myo-inositol
77		Myristic-acid
78		Niacin
79		Nitrogen
80		Oleic-acid
81		Palmitic-acid
82		Pectin
83		Phenylalanine
84		Phosphorus
85		Phyllantidine
86		Phyllantine
87		Phyllemblic-acid
88		Phyllemblin
89		Phyllemblinic-acid
90		Polysaccharide
91		Potassium
92		Proline
93		Protein
94		Quercetin
95		Riboflavin
96		Rutin
97		Selenium
98		Serine
99		Silica
100		Sodium
101		Starch
102		Stearic-acid
103		Sucrose
104		Sulfur

Table 1. to be Continued…

Sl. No.	Source of Data	Phytochemicals
105		Tannin
106		Terchebin
107		Thiamin
108		Threonine
109		Trigalloyl-glucose
110		Tryptophan
111		Tyrosine
112		Valine
113		Water
114		Zeatin
115		Zeatin-Nucleotide
116		Zeatin-Riboside
117		Zinc
118	IMPPAT Database	Procyanidin
119		Proanthocyanidin
120		Tannic acid
121		Leucodelphidin
122		Lupeol
123		Pyrogallol
124		1,3,6-tri-O-galloyl-beta-D-glucose
125		Riboflavin
126		Furosin
127		Terchebin
128		Phloroglucinol
129		trans-Zeatin
130		Chebulagic acid
131		Catechol
132		Quercetin
133		Ellagic acid
134		Methyl gallate
135		Ascorbic acid
136		Kzeyiyxacmutrm-uhfffaoysa-
137		Phyllantidine
138		Ethyl gallate
139		beta-Glucogallin
140		Galactaric acid
141		Chebulinic acid
142		Corilagin
143		Chebulic acid
144		Geraniin
145		Tannic acid

Table 1. to be Continued…

Sl. No.	Source of Data	Phytochemicals
146		Gallic acid
147		Trigalloylglucose
148		Tryptase
149		1,3,6-tri-O-galloyl-beta-D-glucose
150		Terchebin
151		Kaempferol
152		Chebulagic acid
153		Ellagic acid
154		Phyllantidine
155		Galactaric acid
156		Chebulinic acid
157		Corilagin
158		Chebulic acid
159		Gallic acid
160		Lupeol
161		Astragalin
162		beta-Sitosterol
163		Oleanolic aldehyde
164		Ellagic acid
165		Epigallocatechin gallate
166		Oleanolic acid
167		Lupeol
168		Myristic acid
169		Riboflavin
170		Stearic acid
171		Palmitic acid
172		Nicotinic acid
173		Galactaric acid
174		alpha-carotene
175		Oleic acid
176		D-Glucose
177		D-Galacturonic Acid
178		Eriodictyol-7-O-glucoside
179		Lupeol
180		Pyrogallol
181		Procyanidin
182		Proanthocyanidin
183		3-6-Di-o-galloyl-glucose
184		Chebulagic acid
185		2-Methoxycarbonyl-6-oxo-3-[2,3,4-trihydroxy-6-[4-hydroxy-6-(hydroxymethyl)-2-(3,4,5-trihydroxybenzoyl) oxyoxan-3-yl] oxycarbonylphenyl] oxane-4-carboxylic acid

Table 1. to be Continued…

Sl. No.	Source of Data	Phytochemicals
186		Nicotinic acid
187		Inositol
188		Corilagin
189		Geraniin
190		Leucodelphidin
191		Gallic acid
192		Astragalin
193		D-Galacturonic Acid

Table 2. Phytochemicals and canonical SMILES

Sl. No.	Phytochemicals	Canonical SMILES
1	2,4-Dimethylfuran	CC1=CC(=CO1)C
2	4-(2-Hydroxyethyl)-3-methyl-2-pyrazolin-5-one	CC1=NNC(=O)C1CCO
3	Trans-2,3-Epoxyoctane	CCCCCC1C(O1)C
4	2-Furancarboxylic acid, 2-ethylhexyl ester	CCCCC(CC)COC(=O)C1=CC=CO1
5	Heptanoic acid, 3-hydroxy-, methyl ester	CCCCC(CC(=O)OC)O
6	2-(3-Methylguanidino) ethanol	CN=C(N)NCCO
7	9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione	COC1(C2CCCC1C(=O)CC2=O)OC
8	n-Propyl nonyl ether	CCCCCCCCCOCCC
9	Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	CCCCCCCCCCCCCCCCCC(=O)OCCOCCO
10	Z-(13,14-Epoxy) tetradec-11 -en-1-ol acetate	CC(=O)OCCCCCCCCCCC=CC1CO1
11	Cyclopentaneundecanoic acid	C1CCC(C1)CCCCCCCCCCC(=O)O
12	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	CCCCC(CC)COC(=O)C1=CC(=CC=C1)C(=O)OCC(CC)CCCC
13	Alanine	CC(C(=O)O)N
14	Arginine	C(CC(C(=O)O)N)CN=C(N)N
15	Ascorbic-Acid	C(C(C1C(=C(C(=O)O1)O)O)O)O
16	Aspartic-Acid	C(C(C(=O)O)N)C(=O)O
17	Beta-Sitosterol	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C
18	Cystine	C(C(C(=O)O)N)SSCC(C(=O)O)N
19	D-Fructose	C1C(C(C(C(O1)(CO)O)O)O)O
20	D-Glucose	C(C1C(C(C(C(O1)O)O)O)O)O
21	Ellagic-Acid	C1=C2C3=C(C(=C1O)O)OC(=O)C4=CC(=C(C(=C43)OC2=O)O)O
22	Ethyl-Gallate	CCOC(=O)C1=CC(=C(C(=C1)O)O)O
23	Gallic-Acid	C1=C(C=C(C(=C1O)O)O)C(=O)O
24	Gallic-Acid-Ethyl-Ester	CCOC(=O)C1=CC(=C(C(=C1)O)O)O
25	Gibberellin-A-1	CC12C(CCC3(C1C(C45C3CCC(C4)(C(=C)C5)O)C(=O)O)OC2=O)O
26	Glutamic-Acid	C(CC(=O)O)C(C(=O)O)N
27	Glycine	C(C(=O)O)N
28	Histidine	C1=C(NC=N1)CC(C(=O)O)N

Table 2. to be Continued…

Sl. No.	Phytochemicals	Canonical SMILES
29	Isoleucine	CCC(C)C(C(=O)O)N
30	Kaempferol	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O
31	Leucine	CC(C)CC(C(=O)O)N
32	Lysine	C(CCN)CC(C(=O)O)N
33	Methionine	CSCCC(C(=O)O)N
34	Myo-Inositol	C1(C(C(C(C(C1O)O)O)O)O)O
35	Myristic-Acid	CCCCCCCCCCCCCC(=O)O
36	Niacin	C1=CC(=CN=C1)C(=O)O
37	Palmitic-Acid	CCCCCCCCCCCCCCCC(=O)O
38	Pectin	C1(C(C(OC(C1O)O)C(=O)O)O)O
39	Phenylalanine	C1=CC=C(C=C1)CC(C(=O)O)N
40	Phyllantidine	C1CCN2C(C1)C34CC(O2)C=CC3=CC(=O)O4
41	Phyllemblin	CCOC(=O)C1=CC(=C(C(=C1)O)O)O
42	Proline	C1CC(NC1)C(=O)O
43	Quercetin	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O
44	Riboflavin	CC1=CC2=C(C=C1C)N(C3=NC(=O)NC(=O)C3=N2)CC(C(C(CO)O)O)O
45	Serine	C(C(C(=O)O)N)O
46	Stearic-Acid	CCCCCCCCCCCCCCCCCC(=O)O
47	Thiamin	CC1=C(SC=[N+]1CC2=CN=C(N=C2N)C)CCO
48	Threonine	CC(C(C(=O)O)N)O
49	Tryptophan	C1=CC=C2C(=C1)C(=CN2)CC(C(=O)O)N
50	Tyrosine	C1=CC(=CC=C1CC(C(=O)O)N)O
51	Valine	CC(C)C(C(=O)O)N
52	Zeatin	CC(=CCNC1=NC=NC2=C1NC=N2)CO
53	Zeatin-Riboside	CC(=CCNC1=C2C(=NC=N1)N(C=N2)C3C(C(C(O3)CO)O)O)CO
54	Pyrogallol	C1=CC(=C(C(=C1)O)O)O
55	Phloroglucinol	C1=C(C=C(C=C1O)O)O
56	trans-Zeatin	CC(=CCNC1=NC=NC2=C1NC=N2)CO
57	Catechol	C1=CC=C(C(=C1)O)O
58	Ellagic acid	C1=C2C3=C(C(=C1O)O)OC(=O)C4=CC(=C(C(=C43)OC2=O)O)O
59	Methyl gallate	COC(=O)C1=CC(=C(C(=C1)O)O)O
60	Ascorbic acid	C(C(C1C(=C(C(=O)O1)O)O)O)O
61	Ethyl gallate	CCOC(=O)C1=CC(=C(C(=C1)O)O)O
62	Gallic acid	C1=C(C=C(C(=C1O)O)O)C(=O)O
63	Myristic acid	CCCCCCCCCCCCCC(=O)O
64	Nicotinic acid	C1=CC(=CN=C1)C(=O)O
65	D-Galacturonic Acid	C1(C(C(OC(C1O)O)C(=O)O)O)O

found to have similar targets with the targets of exercise endurance (Table 3).

STRING biological database was utilized to obtain the pathways related to CRF. A total of 12 out of 16 pathways that have their action directly on the CRF were selected for network construction (Table 4).

The network that connects 27 phytochemicals and 16 protein molecules was made (Figure 2). The size of

Table 3. List of phytochemicals and the matching targets

Sl. No.	Phytochemicals	Name of Targets	Number of Successful Targets
1	1,3-Benzenedicarboxylic Acid, Bis(2-Ethylhexyl) Ester	PRKCA	1
2	2-(3-Methylguanidino) Ethanol	ACE, PPARG, NOS3	3
3	2-Furancarboxylic Acid, 2-Ethylhexyl Ester	SLC6A4	1
4	4-(2-Hydroxyethyl)-3-Methyl-2-Pyrazolin-5-One	NOS3	1
5	9,9-Dimethoxybicyclo [3.3.1] Nona-2,4-Dione	PRKCA, PPARA	2
6	Ascorbic-Acid	PRKCA, PPARG, EGLN1	3
7	Aspartic-Acid	EGLN1	1
8	Beta-Sitosterol	PPARA, SLC6A4	2
9	Cyclopentaneundecanoic Acid	RARG, PPARA, PPARG	3
10	D-Fructose	PRKCA, ACE, ADRA1A, VEGFA	4
11	D-Galacturonic Acid	PRKCA, ADRA1A, VEGFA	3
12	D-Glucose	PRKCA, ACE, ADRA1A, VEGFA	4
13	Gallic-Acid	RARG, ADRB2, ADRA1A, FTO	4
14	Gibberellin-A-1	PRKCA, ACE, SLC6A4	3
15	Glutamic-Acid	PPARA, NOS3, EGLN1	3
16	Methyl Gallate	PRKCA, RARG, ADRB2, ADRA1A, PPARG, SLC6A4	6
17	Myo-Inositol	PPARA	1
18	Myristic-Acid	RARG, PPARA, PPARG, SLC6A4	4
19	Nicotinic Acid	NOS3, EGLN3	2
20	N-Propyl Nonyl Ether	ADRA1A, SLC6A4	2
21	Octadecanoic Acid, 2-(2-Hydroxyethoxy) Ethyl Ester	PRKCA, ADRB2, ADRA1A	3
22	Palmitic-Acid	RARG, PPARA, PPARG	3
23	Pectin	PRKCA, ADRA1A, VEGFA	3
24	Phyllantidine	ADRA1A, SLC6A4	2
25	Stearic-Acid	RARG, PPARA, ACE, PPARG	4
26	Trans-2,3-Epoxyoctane	ADRA1A, SLC6A4	2
27	Z-(13,14-Epoxy) Tetradec-11 -En-1-Ol Acetate	PPARG, HIF1A	2

nodes and edge count were the basis for the preparation of the network. The interpretation of the network was done by node size scale and colour. The network that connects 12 pathways and 16 protein molecules was made (Figure 3).

PubChem database was utilized to obtain the 3D structure of 27 phytochemicals. RCSB protein data bank was utilized to obtain the protein data of highly modulated three 3 target molecules[39]. Discovery Studio Visualizer was used to remove the water molecules and the protein molecule's heteroatoms. The binding affinity between the phytochemicals and the receptors was determined using PyRx software. The Discovery Studio visualizer is used to depict the interaction between the ligand and protein of the molecule with the lowest binding energy (Figure 4). The binding energy of highly modulated Phytochemicals and Targets is given in the tabular column (Table 5).

As is the case with other tissues, regulatory DNA sequences, particularly enhancers, play a significant role in controlling gene expression in muscle. Enhancers are non-coding regions in the genome that

Table 4. List of pathways and the mode of action

Sl. No.	Pathways	Action on Cardiorespiratory Fitness
1	HIF-1 signalling pathway	This pathway serves as the principal controller of oxygen homeostasis[27].
2	Calcium signalling pathway	Ca^{2+} ions are used in calcium signalling, which is frequently a stage in signal transduction, to communicate and power intracellular processes[28].
3	VEGF signalling pathway	During vasculogenesis and angiogenesis, VEGF signalling via VEGF-R1/R2 controls the activity of many kinases and, as a result, directs cell proliferation, migration, survival, and vascular permeability. The cells that make up the tip and stalk of endothelial cells are at the forefront of vascular proliferation[29].
4	Salivary secretion	Saliva analysis during exercise can reveal important details on training stress, adaptability, and exercise performance. The changes in salivary composition that occur before, during, and after exercise reflect the advantages of exercise, any potential hazards, and the saliva's potential to be a health indicator[30].
5	AGE-RAGE signalling pathway in diabetic complications	This results in the reduction of plaque deposition in the vascular structures and helps in enhancing cardiac endurance[31].
6	Relaxin signalling pathway	This pathway has its action on vasodilation and has actions like anti-fibrotic, angiogenic, anti-apoptotic or anti-inflammatory and thus promotes cardiac endurance[32].
7	Adrenergic signalling in cardiomyocytes	This pathway decreases glycogenolysis, increases insulin-mediated glucose uptake, and activates adenylyl cyclase. G proteins that are both stimulating and inhibitory can bind to three receptors alternately. They promote the oxidation of fat and the usage of energy. They also help in the relaxation of the muscles and dilate blood vessels and bronchioles[33].
8	cGMP-PKG signalling pathway	This pathway regulates a variety of functions, including the control of vascular smooth muscle cell relaxation and contraction, prevention of cardiac hypertrophy, prevention of atherosclerosis, and prevention of vascular damage and restenosis[34].
9	Renin secretion	The physiological reaction and adaptation of fluid-electrolyte balance, as well as cardiovascular function to exercise in humans, are primarily regulated by the renin-angiotensin-aldosterone system[35].
10	EGFR tyrosine kinase inhibitor resistance	The capacity for dysregulated proliferation, survival, invasion, and angiogenesis is increased in cells expressing EGFR[36].
11	PPAR signalling pathway	Manages the energy storage, stimulates fatty acid metabolism without affecting either type of muscle fibre or mitochondrial content, preserves systemic glucose levels, acts to delay the onset of hypoglycaemia and extends running time used to treat metabolic disease, dystrophies and unavoidably, the enhancement of athletic performance[37].
12	PI3K-Akt signalling pathway	By activating downstream matching effector molecules, which play a significant role in the cell cycle, growth, and proliferation, this pathway is involved in the control of numerous cellular physiological functions[38].

work in conjunction with their target genes' promoters to activate the expression of distant target genes[40]. Any treatment, medication or supplement that alters and enhances the endurance capacity of an individual can be considered rejuvenation therapy[41]. *Amalaki* is one of the best rejuvenating and anti-ageing drugs in *Ayurveda*. Telomere length has a favourable correlation with VO2 max[42]. Endurance athletes have been shown to have high telomerase activity and a lower rate of telomere degradation[43]. *Amalaki Rasayana* has been shown to promote telomerase activity and telomere length maintenance suggesting its potential to improve

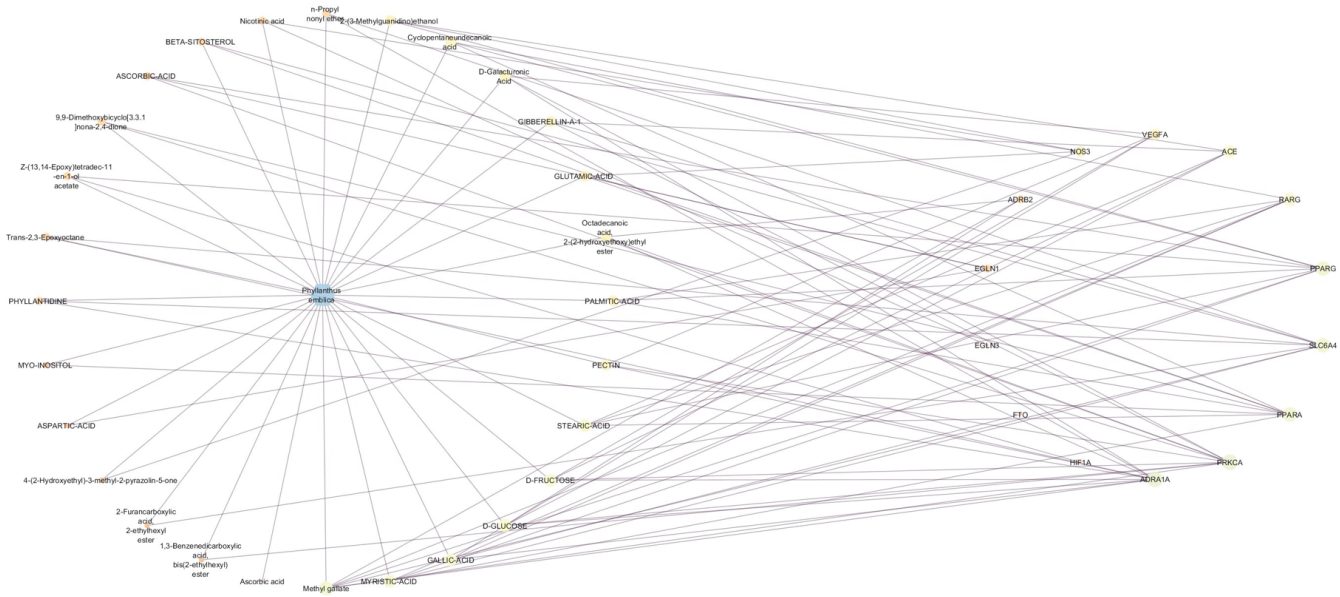

Figure 2. Network connecting the phytochemicals and protein molecules of *Amalaki.*

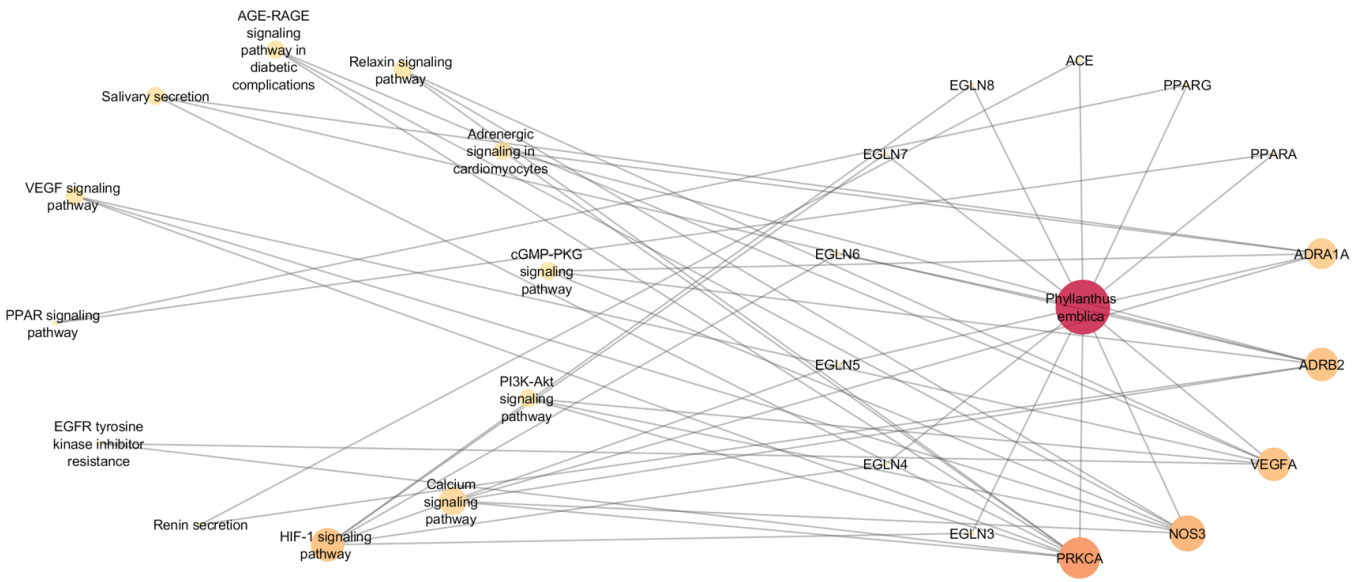

Figure 3. Networks that connect pathways and protein.

CRF[44]. *Amalaki rasayana* has also been found to lengthen fatigue time, indicating that it improves exercise tolerance[8]. *Amalaki*, the prime ingredient in *Ratnaprash*, improved the ability of mice to swim under resistance, demonstrated increased anti-fatigue activity, decreased blood lactate levels, and elevated tissue ATP levels suggesting a potential role in boosting strength and stamina and contributing anti-fatigue activity[9].

The targets of *Amalaki* and the targets which may positively alter the cardio-respiratory endurance are taken from the respective databases. The selected targets and the pathways by which they may act are evaluated and the network was constructed by using the obtained relevant data. Among the targets, highly modulated gene targets such as PRKCA, NOS3, and VEGFA are selected for molecular docking. Among these PRKCA is found to have the lowest binding energy. PRKCA

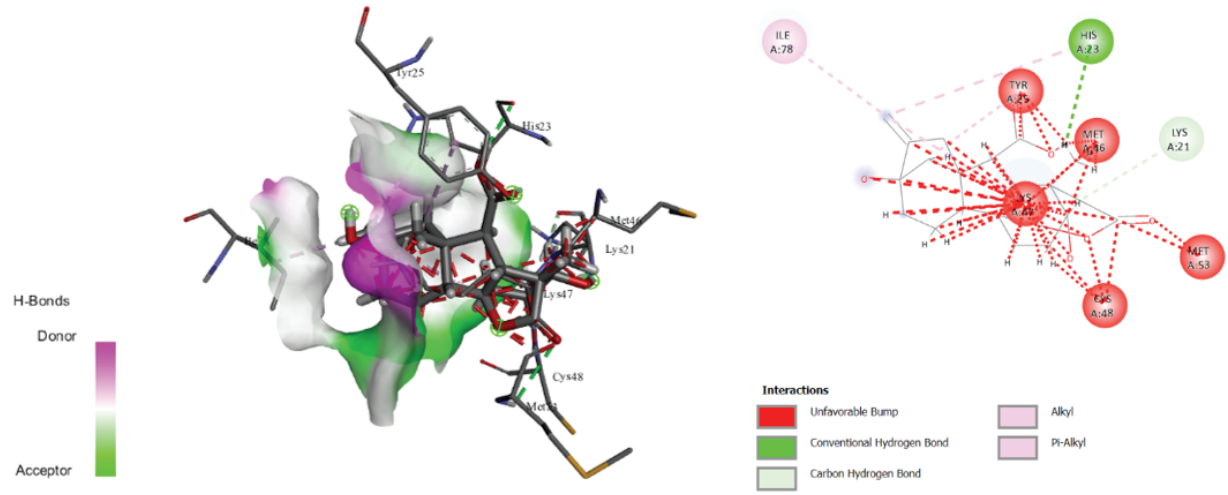

Figure 4. Interaction of PRKCA receptor with D-Glucose (2 Dimensional and 3 Dimensional).

Table 5. List of the binding energy of the highly modulated phytochemicals and targets

Sl. No.	Target	Phytochemicals	Binding Energy (kcal/mol)
1.	PRKCA	1,3-Benzenedicarboxylic Acid, Bis(2-Ethylhexyl) Ester	-5
		9,9-Dimethoxybicyclo [3.3.1] Nona-2,4-Dione	-5
		Ascorbic Acid	-4.9
		D-Fructose	-4.9
		Galacturonic Acid	-5.6
		D- Glucose	-5
		Gibberellin A1	-6.5
		Methyl Gallate	-5.1
		Octadecanoic Acid, 2-(2-Hydroxyethoxy) Ethyl Ester	-3.8
		Pectin	-5.6
2	VEGFA	D-Glucose	-4.4
		D-Galacturonic Acid	-5
		D-Glucose	-4.4
		Pectin	-5
3	NOS3	2-(3-Methylguanidino) Ethanol	-4.7
		4-(2-Hydroxyethyl)-3-Methyl-2-Pyrazolin-5-One	-5.9
		Glutamic Acid	-5.4
		Nicotinic Acid	-5.9

is proven to act on cardiovascular disease, which is connected to heart contractility, and found to enhance cardiorespiratory fitness and endurance performance. This gene target is considered a biomarker to study the relationship between exercise and cardiac function and rehabilitation. Further clinical studies can be done using *Amalaki* and its combination to substantiate the true potency of the drug as an effective nutraceutical and a natural way of enhancing CRF in sports persons and healthy volunteers.

4. Conclusion

Amalaki is found to have a positive influence on the 12 metabolic pathways that act in enhancing the cardio-respiratory endurance in the human being. PRKCA is analyzed and concluded as the highly modulated gene target with the lowest binding energy. Thus, *Amalaki* is found probably to have an action in enhancing cardiorespiratory endurance.

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6. References

1. Peralta M, Henriques-Neto D, Gouveia ÉR, Sardinha LB, Marques A. Promoting health-related cardiorespiratory fitness in physical education: A systematic review. PLoS One. 2020; 15(8):e0237019. https://doi.org/10.1371/journal.pone.0237019PMid:32745088 PMCid:PMC7398517
2. Aghjayan SL, Lesnovskaya A, Esteban-Cornejo I, Peven JC, Stillman CM, Erickson KI. Aerobic exercise, cardiorespiratory fitness, and the human hippocampus. Hippocampus. 2021; 31(8):817–44. https://doi.org/10.1002/hipo.23337PMid:34101305 PMCid:PMC8295234
3. Tiwari S, Gupta SK, Pathak AK. A double-blind, randomized, placebo-controlled trial on the effect of Ashwagandha (Withania somnifera dunal) root extract in improving cardiorespiratory endurance and recovery in healthy athletic adults. J Ethnopharmacol. 2021; 272(113929):113929. https://doi.org/10.1016/j.jep.2021.113929PMid:33600918
4. Karpovich PV. American Association for Health, Physical Education and Recreation. Research Quarterly American Association for Health, Physical Education and Recreation. 2013; 12(1941):432–50. https://doi.org/10.1080/10671188.1941.10624697
5. Mathews NM. Prohibited contaminants in dietary supplements. Sports Health. 2018; 10(1):19-30. https://doi.org/10.1177/1941738117727736 PMid:28850291 PMCid:PMC5753965
6. Buttar KK, Kacker S, Saboo N. Normative data of maximal oxygen consumption (VO2 max) among healthy young adults: A cross-sectional study. J Clin Diagn Res. 2022; 16(7):cc31-cc34. https://doi.org/10.7860/JCDR/2022/53660.16672
7. Dwivedi V, Lakhotia SC. Ayurvedic Amalaki Rasayana promotes improved stress tolerance and thus has anti-ageing effects in Drosophila melanogaster. J Biosci. 2016; 41(4):697–711. https://doi.org/10.1007/s12038-016-9641-x PMid:27966490
8. Kumar V, Aneesh KA, Kshemada K, Ajith KGS, Binil RSS, Deora N, *et al.* Amalaki rasayana, a traditional Indian drug enhances cardiac mitochondrial and contractile functions and improves cardiac function in rats with hypertrophy. Sci Rep. 2017; 7(1):8588. https://doi.org/10.1038/s41598-017-09225-x PMid:28819266 PMCid:PMC5561106
9. Gupta A, Kumar S, Rajput R, Srivastava R, Rai RK, Sastry JLN. Evaluation of Ratnaprash for its effect on strength, stamina and fatigue using swim endurance test and biochemical estimation in Swiss albino mice. Anc Sci Life. 2015; 35(1):26–31. https://doi.org/10.4103/0257-7941.165626 PMid:26600664 PMCid:PMC4623629
10. Akter R, Khan SS, Kabir MT, Halder S. GC-MS-employed phytochemical characterization, synergistic antioxidant, and cytotoxic potential of Triphala methanol extract at non-equivalent ratios of its constituents. Saudi J Biol Sci. 2022; 29(6):103287. https://doi.org/10.1016/j.sjbs.2022.103287 PMid:35592742 PMCid:PMC9112002
11. U.S. Department of Agriculture, Agricultural Research Service. 1992-2016. Dr. Duke's Phytochemical and Ethnobotanical Databases. Home Page, https://phytochem.nal.usda.gov/
12. Jankowski F, Parthasarathy A, Farah W, Flynn C. Molsoft: Molonglo Telescope Observing Software. 2019.
13. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, *et al.* PubChem in 2021: New data content and improved web interfaces. Nucleic Acids Res. 2021; 49(D1):D1388–95. https://doi.org/10.1093/nar/gkaa971 PMid:33151290 PMCid:PMC7778930
14. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017; 7(1). https://doi.org/10.1038/srep42717PMid:28256516 PMCid:PMC5335600
15. Gfeller D, Michielin O, Zoete V. Shaping the interaction landscape of bioactive molecules. Bioinformatics. 2013; 29(23):3073–9. https://doi.org/10.1093/bioinformatics/btt540PMid:24048355
16. Zhao Y, Huang G, Chen Z, Fan X, Huang T, Liu J, *et al.* Four loci are associated with cardiorespiratory fitness and endurance performance in young Chinese females. Sci Rep. 2020; 10(1):10117. https://doi.org/10.1038/s41598-020-67045-yPMid:32572135 PMCid:PMC7723046
17. Chung HC, Keiller DR, Roberts JD, Gordon DA. Do exercise-associated genes explain phenotypic variance in the three components of fitness? A systematic review and meta-analysis. PLoS One. 2021; 16(10):e0249501. https://doi.org/10.1371/journal.pone.0249501PMid:34648504 PMCid:PMC8516263

18. Peter I, Papandonatos GD, Belalcazar LM, Yang Y, Erar B, Jakicic JM, *et al*. Genetic modifiers of cardiorespiratory fitness response to lifestyle intervention. Med Sci Sports Exerc. 2014; 46(2):302–11. https://doi.org/10.1249/MSS.0b013e3182a66155PMid:23899896 PMCid:PMC405 5466
19. North K. Why is Alpha-actinin-3 deficiency so common in the general population? The evolution of athletic performance. Twin Res Hum Genet. 2008; 11(4):384-94. https://doi.org/10.1375/twin.11.4.384 PMid:18637739
20. Hagberg JM. Do genetic variations alter the effects of exercise training on cardiovascular disease and can we identify the candidate variants now or in the future? J Appl Physiol. 2011; 111(3):916–28. https://doi.org/10.1152/japplphysiol.00153.2011PMid:21565989
21. Ciulei MA. Relationship of DNA Methylation with Cardiovascular Fitness and Body Composition. 2014.
22. Yang J, Tan H, Sun M, Chen R, Zhang J, Liu C, *et al*. Prediction of high-altitude cardiorespiratory fitness impairment using a combination of physiological parameters during exercise at sea level and genetic information in an integrated risk model. Front Cardiovasc Med. 2022;8:719776. https://doi.org/10.3389/fcvm.2021.719776PMid:35071338 PMCid: PMC8782201
23. Kim HJ, Lee SY, Kim CM. Association between gene polymorphisms and obesity and physical fitness in Korean children. Biol Sport. 2018; 35(1):21-27. https://doi.org/10.5114/biolsport.2018.70748
24. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, *et al*. The STRING database in 2021: Customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res. 2021; 49(D1):D605–12. https://doi.org/10.1093/nar/gkaa1074 PMid:33237311 PMCid:PMC7779004
25. Systèmes D. BIOVIA discovery studio modeling environment. San Diego: DassaultSystèmesBiovia; 2016.
26. Ferreira LG, Dos Santos RN, Oliva G, Andricopulo AD. Molecular docking and structure-based drug design strategies. Molecules. 2015; 20(7):13384–421. https://doi.org/10.3390/molecules200713384 PMid:26205061 PMCid: PMC6332083
27. Semenza GL. Hypoxia-inducible factor 1: Oxygen homeostasis and disease pathophysiology. Trends in molecular medicine. 2001; 7(8):345-50. https://doi.org/10.1016/S1471-4914(01)02090-1 PMid:11516994
28. Patergnani S, Suski JM, Agnoletto C, Bononi A, Bonora M, De Marchi E, *et al*. Calcium signaling around Mitochondria Associated Membranes (MAMs). Cell Commun Signal. 2011; 9(1):19. https://doi.org/10.1186/1478-811X-9-19 PMid:21939514 PMCid:PMC3198985
29. Apte RS, Chen DS, Ferrara N. VEGF in signalling and disease: Beyond discovery and development. Cell. 2019; 176(6):1248-64. https://doi.org/10.1016/j.cell.2019.01.021 PMid:30849371 PMCid:PMC6410740
30. Ntovas P, Loumprinis N, Maniatakos P, Margaritidi L, Rahiotis C. The effects of physical exercise on saliva composition: A comprehensive review. Dent J. 2022; 10(1):7. https://doi.org/10.3390/dj10010007 PMid:35049605 PMCid:PMC8775020
31. Kay AM, Simpson CL, Stewart JA Jr. The role of AGE/RAGE signalling in diabetes-mediated vascular calcification. J Diabetes Res. 2016; 2016:6809703. https://doi.org/10.1155/2016/6809703 PMid:27547766 PMCid:PMC4980539
32. Sarwar M, Du X-J, Dschietzig TB, Summers RJ. The actions of relaxin on the human cardiovascular system: Cardiovascular actions of relaxin. Br J Pharmacol. 2017; 174(10):933–49. https://doi.org/10.1111/bph.13523 PMid:27239943 PMCid:PMC5406304
33. Motiejunaite J, Amar L, Vidal-Petiot E. Adrenergic receptors and cardiovascular effects of catecholamines. Ann Endocrinol (Paris). 2021; 82(3–4):193–7. https://doi.org/10.1016/j.ando.2020.03.012 PMid:32473788
34. El Shear F. Novel paradigms in the therapeutic management of heart failure with preserved ejection fraction: Clinical perspectives. Am J Cardiovasc Dis. 2019; 9(5):91-108.
35. Fallo F. Renin-angiotensin-aldosterone system and physical exercise. J Sports Med Phys Fitness. 1993; 33(3):306–12. https://doi.org/10.1016/j.hfc.2018.08.002 PMid:30449384
36. Huang L, Fu L. Mechanisms of resistance to EGFR tyrosine kinase inhibitors. Acta Pharm Sin B. 2015; 5(5):390–401. https://doi.org/10.1016/j.apsb.2015.07.001PMid:26579470 PMCid:PMC4629442
37. Fan W, Waizenegger W, Lin CS, Sorrentino V, He MX, Wall CE, Li H, Liddle C, Ruth TY, Atkins AR, Auwerx J. PPARδ promotes running endurance by preserving glucose. Cell metabolism. 2017; 25(5):1186-93. https://doi.org/10.1016/j.cmet.2017.04.006PMid:28467934 PMCid:PMC5492977
38. Shi X, Wang J, Lei Y, Cong C, Tan D, Zhou X. Research progress on the PI3K/AKT signalling pathway in gynaecological cancer (Review). Mol Med Rep. 2019; 19(6):4529–35. https://doi.org/10.3892/mmr.2019.10121
39. Zardecki C, Dutta S, Goodsell DS, Lowe R, Voigt M, Burley SK. PDB-101: Educational resources supporting molecular explorations through biology and medicine. Protein Sci. 2022; 31(1):129–40. https://doi.org/10.1002/pro.4200PMid:34601771 PMCid:PMC8740840
40. Williams K, Carrasquilla GD, Ingerslev LR, Hochreuter MY, Hansson S, Pillon NJ, *et al*. Epigenetic rewiring of skeletal muscle enhancers after exercise training supports a role in the whole-body function and human health. Mol Metab. 2021; 53(101290):101290. https://doi.org/10.1016/j.molmet.2021.101290 PMid:34252634 PMCid:PMC8355925

41. Panigrahi A, O'Malley BW. Mechanisms of enhancer action: The known and the unknown. Genome Biol. 2021; 22(1):108. https://doi.org/10.1186/s13059-021-02322-1PM id:33858480 PMCid:PMC8051032
42. Østhus IB, Sgura A, Berardinelli F, Alsnes IV, Brønstad E, Rehn T, Støbakk PK, Hatle H, Wisløff U, Nauman J. Telomere length and long-term endurance exercise: Does exercise training affect biological age? A pilot study. PLoS One. 2012; 7(12):e52769. https://doi.org/10.1371/journal. pone.0052769 PMid:23300766 PMCid:PMC3530492
43. Song S, Lee E, Kim H. Does exercise affect telomere length? A systematic review and meta-analysis of randomized controlled trials. Medicina (Kaunas). 2022; 58(2):242. https://doi.org/10.3390/medicina58020242 PMid:35208566 PMCid:PMC8879766
44. Guruprasad KP, Dash S, Shivakumar MB, Shetty PR, Raghu KS, Shamprasad BR, Udupi V, Acharya RV, Vidya PB, Nayak J, Mana AE, Moni R, Sankaran MT, Satyamoorthy K. Influence of Amalaki Rasayana on telomerase activity and telomere length in human blood mononuclear cells. J *Ayurveda* Integr Med. 2017; 8(2):105-112. https://doi.org/10.1016/j. jaim.2017.01.007 PMid:28602428 PMCid:PMC5497001