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Research Article

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Anti-oxidant activity of Siddha Herbo – Mineral formulation of Rasa chendhuram in DPPH assay.

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Abstract

Free radicals and related species have attracted a great deal of attention in recent years. They are mainly derived from reactive oxygen and nitrogen species which are generated during Phagocytosis, can affect the components of the immune system by inducing oxidative damage. Cell damage caused by free radicals appears to be a major contributor in aging and degenerative diseases such as cancer, immune system declination, rheumatoid arthritis, inflammation, brain dysfunction and stress among others. Anti-oxidants are capable of stabilizing or deactivating free radicals before they attack cells. .Rasa chendhuram (RCM) is a herbo mineral formulation in Siddha system of medicine was subjected to antioxidant activity(DPPH Assay). IC50 value of Rasa Chendhuram was 215.82µg/ml (calculated using ED50 plus V1.0 Software) possessed antioxidant activity when compared to the standard ascorbic acid. Hence, proved Rasa Chendhuram is a potent free radical scavenger.

Keywords: Rasa Chendhuram, Antioxidant activity, DPPH assay.

Introduction

Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Free radicals are types of reactive oxygen species (ROS),which include all highly reactive oxygen containing molecules. They are capable of attacking the healthy cells of the body, causing them to lose their structures and function. [1] Cell damage caused by free radicals appears to be a major contributor in aging and degenerative diseases such as cancer, cardiovascular disease, cataract, immune system declination, liver diseases, diabetes mellitus**,** rheumatoid arthritis, inflammation, brain dysfunction and stress among others.[2]

To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex anti-oxidant protection system, that functions interactively and synergistically to neutralize free radicals .Thus, anti-oxidants are capable of stabilizing or deactivating free radicals before they attack cells. Naturally there is a dynamic balance between the amount of free radicals produced in the body and antioxidants that protect the body against deleterious effects. Oftentimes, amount of antioxidant principles present under normal physiological conditions may be insufficient to neutralize free radicals generated.

Therefore, it is obvious to enrich our diet with antioxidants to protect against harmful diseases.

Hence there is an increased interest in the food industry and in preventive medicine in the development of “Natural antioxidants” from plant materials.

Anti-oxidant defense:

It comprises of the agents that catalytically remove free radicals and other reactive species like SOD, CAT, peroxidase and thiols specific antioxidants.[13]Low molecular mass agents that scavenge ROS and RNS, example GSH, ascorbic acid tocopherol.

The antioxidant may be defined as "any substance when present at low concentrations compared with that of an antioxidant substrate that significantly delays or prevent oxidations of that substrate''. Antioxidant defense includes the antioxidant enzymes like SOD,CAT,GSH-px etc, low molecular weight and dietary antioxidants (Halliwell and Gutteridge,1999)[13]

Significance of antioxidants in relation to disease:

Antioxidants may prevent/improve different diseased states (Knight 2000). Zinc is an essential trace element, being a co-factor for about 200 human enzymes, including cytoplasmic antioxidant Cu-Zn SOD, isoenzyme of SOD mainly present in cytosol. It protects the body against free radical damage and also plays an important role in maintaining immune status.

Vitamin E is the considered as the 'standard antioxidant' and tocotrienols. Selenium is also an essential trace elements and co-factor for Glutathione peroxidase.[13]

Materials and Methods

Details regarding sample:

Rasa chendhuram (RCM) is a classic siddha herbo-mineral formulation mentioned in Sikicha Rathna Deepam [9]

Ingredients:

Purified Rasam (Mercury)
Purified Gandhagam (Sulphur)
Purified Paalthutham (Sulphate of zinc)
Mirabilis jalapa-Q.S

Drug collection:

All the ingredients were obtained from country drug shop, Ramasamychetti, Parrys, Chennai, Tamilnadu, India.

Identification and Authentication:

All the raw drugs were identified and authenticated at siddha central research institute (SCRI) Chennai and medicinal botany department, Govt siddha medical college, Arumbakkam, Chennai.

Preparation of the drug:

The flower juice of the yellow variety of Mirabilis jalapa is to be grinded well with the above mentioned raw drugs in the stone mortar for 6 hours(2 saamam) till the juice and the drugs gets spreaded well in the mortar on all sides. Then it is to be collected using the spatula without any wastage. Next the collected medicine is to be placed in a mud jar and is closed with a proper lid and sealed up tightly with 7 layers of mud pasted cloth. After the sealing is dried, the mud jar is placed in the vaalugaendiram. Then it is to be ignited with kamalakini for 6 hours (2 saamam) then for kaadakini for next 6 hours. Then it is to be left aside for the whole night to allow it to cool. Then the settled medicine is to be collected safely and placed in the mortar for grinding to get a fine chendhuram.

Then it is to be collected and placed in a air tight container.

Significant effect on Rasa Chendhuram:

All the ingredients of RCM have antioxidant properties and free radicals scavenging and immunomodulatory effects as follows,

Biochemical functions of sulphur has Antioxidant protection-scavenges or neutralizes free radicals and recycles oxidized antioxidants and enhancing proliferation of lymphocytes, cytotoxic T cell and NK cells.[11] Zinc sulphate is an essential trace element and being a co-factor of several enzymes such as alkaline phosphatase, alchohol dehydrogenase**,** superoxide dismutase (SOD). Itprotects the body against free radical damage.[10] Methonolic extracts of Mirabilis jalapa revealed the immense potential of the methonolic extracts of the aerial parts , flowers and roots for the antioxidant activity and elucidating their tentative mechanisms of action and thereby serve as a free radical inhibitor or scavengers which can be used to treat various oxidative stress related diseases.[12]

Antioxidants Assays:

1. DPPH Assay(2,2-diphenyl -1-picrylhydrazl):

The radical scavenging activity of different extracts was determined by using DPPH assay according to chang et.al(2001).The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm.Ascorbic acid (10mg/ml DMSO) was used as reference.

Principle:

1,1 Diphenyl 2-picryl Hydrazyl is a stable (in powder form)free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity.

The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

$$(DPPH)+(H\text{-}A) \rightarrow DPPH\text{-}H+(A)$$

Antioxidants react with DPPH and reduce it to DPPH-H and as a consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidants compounds or extracts in terms of hydrogen donating ability.

Reagent Preparation:

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

Working Procedure:

Different volumes (1.25-20µl) of extracts were made up to a final volume 20µl with DMSO and 1.48ml DPPH (0.1m M) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20min.After 20 min, the absorbance of the mixture was read at 517nm.3ml of DPPH was taken as control.

Results

The absorbance of the mixture was read at 517nmand the percentage of inhibition was calculated using the formula;

$$\% \text{ inhibition} = \frac{\text{control-test}}{\text{control}} \times 100 \quad \frac{\text{control-test}}{\text{control}} \times 100$$

Ascorbic acid standard:

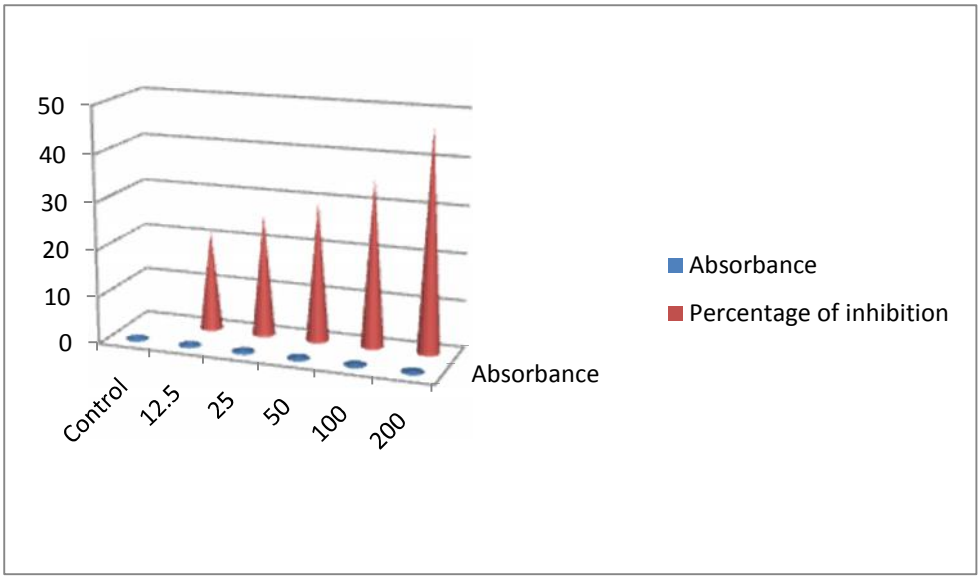
Concentrations(µg)	Absorbance	Percentage of inhibition
Control	1.7983	
12.5	1.4044	21.90
25	1.0782	40.04
50	0.7121	60.40
100	0.2921	83.75
200	0.0692	96.15

Sample Extraction of Rasa Chendhuram:

Concentrations(µg)	Absorbance	Percentage of inhibition
Control	0.9462	
12.5	0.7464	21.11
25	0.7018	25.82
50	0.6671	29.49
100	0.6088	35.65
200	0.4993	47.23

IC50 value of Rasa Chendhuram: 215.82µg/ml (calculated using ED50 plus V1.0 Software)

Chart-1-Antioxidant effect of Rasa Chendhuram in DPPH Assay



Discussion

Siddhars who possessed tremendous powers in themselves and could sustain their bodies for ages. They have explained more about the medicinal characters of all forms of herbomineral formulations and their purification techniques also used in the alchemical rejuvenation medicine, anti-oxidants, corporeal transmutation. Scavenging of 2,2 diphenyl - 1-picrylhydrazyl radical (DPPH assay).This is the simplest and most widely reported method for screening antioxidant activity in foods and many plant drugs. [8]The main limitation of IC50 determination is that the percentage of radical scavenged is dependent of the initial concentration of DPPH. Radical [8]. IC50 value of Rasa Chendhuram was 215.82µg/ml (calculated using ED50 plus V1.0 Software)

Conclusion

The results further support the view that some traditionally used siddha herbo mineral formulations and medicinal plants are important sources of potential antioxidants and may be efficient as preventing agents in some diseases. The results acquired through DPPH assay reveals that the siddha formulations had that the activity is expressed as inhibitory concentration IC50, that is the amount of antioxidant necessary to decrease by 50% the initial DPPH concentration [7,8]. Hence the IC50 value of Rasa Chendhuram was 215.82µg/ml (calculated using ED50 plus V1.0 Software)

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