IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
April 2016 Vol.:6, Issue:1

Anti – Oxidant Activity of Siddha Herbo Mineral Formulation "Pancha Lavana Mezhugu" with the Estimation of Phenol, Flavonoids and Vitamin C

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Dr.A.Lavanya[1], Dr.M.Mohamed Musthafa[2], Dr.N.Anbu[3]

[1]P. G. Scholar, Department of Sirappu Maruthuvam, Government Siddha Medical College, Chennai, Tamilnadu, India.

[2]Guide, Department of Sirappu Maruthuvam, Govt., Siddha Medical College, Chennai, Tamilnadu, India.

[3]Head of the Department, Department of Sirappu Maruthuvam, Govt., Siddha Medical College, Chennai, Tamilnadu, India.

Submission: 5 April 2016
Accepted: 10 April 2016
Published: 25 April 2016

Keywords: PLM, Antioxidant, Radical scavenging, Kayakalpam

ABSTRACT

Pancha Lavana Mezhugu (PLM) is a siddha herbo mineral formulation made up of 5 types of salts and 5 types of herbals which have been therapeutically used for musculoskeletal disorders. Siddha system emphasis mainly on healthy long life (kayakalpam) by preventing aging and degenerative diseases with medicines having antioxidant activity. Oxidative stress is a condition of imbalance between radical generating and radical scavenging systems. Antioxidant has the ability to donate electrons which neutralizes the free radical without forming another free radical. Here, PLM was tested for its antioxidant activity. Vitamin C, phenols, flavonoids were estimated to know the background of antioxidant activity. Due to the presence of Vit C, phenols and flavonoids PLM has potent antioxidant activity.

INTRODUCTION

Siddha medicine is one of the ancient traditional systems that originated in southern part of India in Tamil language. Siddha medicines are mainly made with herbals, metals, minerals and animal products. For that, Siddhars said "food is medicine: medicine is food" by knowing the bioactivity and potential health benefits of phytoconstituents like flavonoid, phenol etc., in the flora which is used as food.

Oxidative stress is a condition of imbalance between radical generating and radical scavenging systems. Oxidative stress disturbs the normal redox cell state which causes harmful effect by the production of free radicals[1]. A free radical is an atom or molecule that has a single unpaired electron in an outer shell, so they are usually unstable having affinity towards losing or picking up an extra electron. These free radicals are responsible for the pathogenesis of several diseases like atherosclerosis and myocardial infarction, degenerative and neurodegenerative disorders, autism and ADHD, even cancer initiation.

So anti- oxidant can protect against oxidative damage by decreasing the number of free radicals which cause chronic diseases and aging process[2].

Siddhars designed many drug formulations with antioxidant activity. One among such formulations is "PANCHA LAVANA MEZHUGU" made out of five salts and five herbals. To reveal its potentiality, antioxidant activity and estimation of phenol, flavonoids and vitamin C were done

PANCHALAVANA MEZHUGU (PLM)[8]:

Ingredients

- INDHUPPU *(Rock salt)*
- KALLUPPU *(Sodium chloride)*
- KARIYUPPU *(Sodium chloride)*
- VALAIYALUPPU *(Sodium silicate)*
- VEDIYUPPU *(Potassium nitrate)*
- PIRANDAI CHARU *(Cissus quadrangularis)*

- KUPPAIMENI CHARU *(Acalypha indica)*
- MURUNGAIPATTAI CHARU *(Moringa olifera)*
- NOCCHI ILAI CHARU *(Vitex negundo)*
- KUMARI CHARU *(Aloe vera)*
- THAEN *(Honey)*
- PERUNGAYAM *(Ferulaasafoetida)*

MATERIALS AND METHODS

EXTRACT PREPARATION FROM PANCHA LAVANA MEZHUGU:

The phytochemical screening of Pancha Lavana Mezhugu extract was assessed by standard method as described by Brinda *et al.,* (1981); Siddiqui and Ali (1997) and Savithramma *et al.,* (2011). Phytochemical screening was carried out on the Pancha Lavana Mezhugu extract using aqueous extract to identify the major natural chemical groups such as flavonoids, phenols. General reactions in these analyses revealed the presence or absence of these compounds in the Pancha Lavana Mezhugu extract tested.

Test for Flavonoids:

For flavonoids identification, 2ml of plant extract, 1ml of 2N sodium hydroxide (NaOH) was added. Formation of yellow colour indicates the presence of flavonoids.

Test for Phenols:

For phenol identification, 1ml of the plant extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue / green colour indicates the presence of phenols.

Estimation of Vitamin C:

Titration with a modification of Tillmans' technique, or with iodine was used to determine the amount of vitamin C [ascorbic acid].

Estimation of total phenol content in of Pancha Lavana Mezhugu:

Total phenolic content in of Pancha Lavana Mezhugu extracts was determined by the Folin–Ciocalteau colorimetric method [Slinkard and Singleton, 1956]. For the analysis, 0.5 ml of aliquot of sample was added to 0.5 ml of Folin–Ciocalteau reagent (0.5 N) and the contents of the flask were mixed thoroughly. Later 2.5 ml of sodium carbonate (2%) was added, and the mixture was allowed to stand for 30 minutes after mixing. The absorbance was measured at 760nm in a UV-Visible Spectrophotometer. Total phenolics contents were expressed as mg gallic acid equivalents (GAE)/g extract.

Estimation of Total Flavonoid Content in Pancha Lavana Mezhugu extracts:

Total flavonoids content in aqueous Pancha Lavana Mezhugu extracts was determined by the aluminium chloride colorimetric method (Mervat *et al*., 2009). 0.5 ml of Pancha Lavana Mezhugu at a concentration of 1mg/ ml was taken and the volume was made up to 3ml with methanol. Then 0.1ml $AlCl_3$ (10%), 0.1ml of potassium acetate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance was recorded at 415nm after 30 minutes of incubation. A standard calibration plot was generated at 415nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

QUALITATIVE ANALYSIS OF ANTIOXIDANT ACTIVITY OF PANCHA LAVANA MEZHUGU EXTRACT:

The antioxidant activity of Pancha Lavana Mezhugu extract was determined by following the method as described by George *et al.,* (1996); Samundeeswari *et al.,* (2013).

4.3.4.2. QUANTITATIVE ANALYSIS OF FREE RADICAL SCAVENGING ACTIVITY OF PANCHA LAVANA MEZHUGU EXTRACT:

The antioxidant activities were determined using DPPH (Sigma-Aldrich) as a free radical. 100µl of Pancha Lavana Mezhugu extract were mixed with 2.7ml of methanol and then 200µl of 0.1% methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH

solution was prepared and measured as a control (Lee *et al.*, 2005). Subsequently, at every 5 min interval, the absorption maxima of the solutions were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicates. Free radical scavenging activity was calculated by the following formula:

% DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance of control)] x 100.

RESULTS

SCREENING PANCHA LAVANA MEZHUGU EXTRACT FOR PHYTOCHEMICALS:

Phytochemicals Tested	Pancha Lavana Mezhugu Extract
	Aqueous
Flavonoids	+
Phenol	++

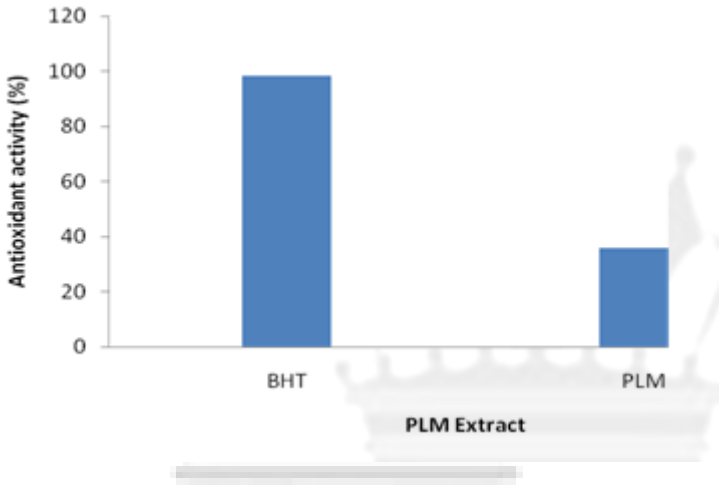
Key : + = positive, ++ = strong positive.

QUANTIFICATION OF VITAMIN, PHENOL, FLAVONOID OF PANCHALAVANA MEZHUGU EXTRACT:

Sample	Vitamin C (mg/g)	Total Phenol content (mg GAE / g)	Total Flavonoid content (mg QE /g)
Pancha Lavana Mezhugu extracts	8.169	2.134	0.625

QUANTITATIVE ANTIOXIDANT ACTIVITY OF PANCHA LAVANA MEZHUGU EXTRACT

Medicines	0	5	10	15	20	25	30
PLM (OD)	0.86	0.86	0.75	0.75	0.73	0.73	0.72
%	23.2	23.2	33.0	33.0	34.8	34.8	35.71
BHT (OD)	0.14	0.11	0.09	0.07	0.06	0.04	0.02
%	87.5	90.1	91.6	93.75	94.6	96.4	98.2



DISCUSSION

Flavonoids are phytoconstituents which consist of large group of polyphenolic compounds having a benzo – y – prone structure and are abundantly present in plants. Flavonoids are proved for its high antioxidant activity *in vitro* & *in vivo*[3]. Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals or by chelating metal ions[5]. Lipid peroxidation is a common consequence of oxidative stress. Flavonoid protects lipids against oxidative damage[4].

Vitamin C is a six carbon lactone[7]. Vitamin C is a potential water soluble antioxidant because it is an electron donor for eight different enzymes. It is a powerful inhibitor of lipid peroxidation. It also regenerates the major antioxidant (Vit E) in lipoproteins and cell membranes.

Recent studies have been shown that polyphenolic constituents derived from plants are more effective antioxidants *in vitro* than Vit E or C[6]. The amount of vitamin C, flavonoids, phenolic compounds of PLM has been showed in Table – Radical scavenging activity of PLM has been shown in Fig.

This shows that the antioxidant activity of PLM is due to its presence of flavonoids, phenolic compounds and Vit C in it.

CONCLUSION

All these above estimations suggest that PLM has antioxidant activity along with its other pharmacological activities like antiinflammatory, antispasmodic and analgesic activities.

Even though many herbals have flavonoids, phenols, vitamin-c and possess antioxidant activity. PLM which is a siddha herbo mineral formulation also has micro elements with long shelf life period works in low dosage itself. Being, it is an herbal mineral formulation can able to supply for growing high population during the shortage of herbals.

A single medicine which has efficacy to cure disease, prevent disease, provide needed nutrition opens the door for future.

REFERENCES

1. Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options Bayani Uttara,[1,*#] Ajay V. Singh,[2,3,#] Paolo Zamboni,[3] and R.T Mahajan[1,*] Author information ► Article notes ► Copyright and License information ►
2. Free radicals, antioxidants and functional foods: Impact on human health V. Lobo, A. Patil, A. Phatak, and N. Chandra Author information ► Article notes ► Copyright and License information ►
3. The Scientific World Journal Volume 2013 (2013), Article ID 162750, 16 pages http://dx.doi.org/10.1155/2013/162750 Review Article Chemistry and Biological Activities of Flavonoids: An Overview Shashank Kumar and Abhay K. Pandey Department of Biochemistry, University of Allahabad, Allahabad 211002, India Received 24 August 2013; Accepted 7 October 2013 Academic Editors: K. P. Lu and J. Sastre.
4. Free radicals, antioxidants and functional foods: Impact on human health V. Lobo, A. Patil, A. Phatak, and N. Chandra Author information ► Article notes ► Copyright and License information ►
5. Free radicals, antioxidants and functional foods: Impact on human health V. Lobo, A. Patil, A. Phatak, and N. Chandra Author information ► Article notes ► Copyright and License information ►
6. Phenolic antioxidants Fereidoon Shahidi[a], P. K. Janitha[a] & P. D. Wanasundara[a] pages 67-103 Publishing models and article dates explained Published online: 29 Sep 2009.
7. Journal of the American College of Nutrition Volume 22, Issue 1, 2003 Review Series: Antioxidants and Their Clinical Applications Vitamin C as an Antioxidant: Evaluation of Its Role in Disease Prevention Sebastian J.

Padayatty MRCP, PhD[a], Arie Katz MD[a], Yaohui Wang MD[a], Peter Eck PhD[a], Oran Kwon PhD[a], Je-Hyuk Lee PhD[a], Shenglin Chen PhD[a], Christopher Corpe Ph D[a], Anand Dutta BS[b], Sudhir K Dutta MD, FACN[b] & Mark Levine MD, FACN[a*] pages 18-35 Publishing models and article dates explained Received: 12 Apr 2002 Accepted: 13 May 2002 Published online: 19 Jun 2013.