ISSN 0974-3618 (Print) **www.rjptonline.org**
0974-360X (Online)

RESEARCH ARTICLE

Free Radical Scavenging Activity and Total Antioxidant Capacity of Tin Chlorophyllin from *Morinda citrifolia* L.

Pavithra. S[1]*, N. Banu [2]
[1]Research Scholar, Department of Biotechnology, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Vels University, Chennai, Tamil Nadu, India.
[2]Associate Professor, Department of Biotechnology, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Vels University, Chennai, Tamil Nadu, India.
*Corresponding Author E-mail: **pavithrasgopan@gmail.com**

ABSTRACT:
Medicinal plants are the rich source of harmless medicines, and used for the treatment of various diseases for thousands of years. They can provide biologically active molecules and lead structures for development of modified derivatives with enhanced activity or reduced activity. Green plants synthesize and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for several scientific investigations. Tin chlorophyllin, a water soluble sodium salt of Chlorophyll is one among the family of phytochemical compounds. The central metal atom, magnesium of chlorophyll has been replaced with tin and the phytol chains lost in chlorophyllin. The antioxidant activity of tin chlorophyllin from the leaves of *Morinda citrifolia* L. was investigated by using *in vitro* antioxidant models such as DPPH scavenging assay and Phosphomolybdenum method. The free radical scavenging activity of tin chlorophyllin on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was evaluated and the percentage of inhibition was determined. By phosphomolybdenum method, the total antioxidant capacity of tin chlorophyllin was determined and the absorbance values were expressed as the number of gram equivalent of ascorbic acid. The results demonstrate the potential antioxidant activity of tin chlorophyllin and support its possible role in human health protection and disease prevention.

KEYWORDS: Tin chlorophyllin, *Morinda citrifolia* L., DPPH, Free radicals, phosphomolydenum.

INTRODUCTION:
Free radicals produced in the body cause molecular transformations and gene mutations. The free radical production is continuously balanced by natural antioxidant defense systems in healthy individuals[1]. But when these free radicals are present in higher concentration beyond the antioxidant capacity of a biological system, due to metabolic and other environmental factors; it gives rise to an imbalance known as oxidative stress[2].

In recent years, there has been increasing scientific interest in antioxidants from plants for their side effect free health approach and remedies for prevention and cure of different diseases[3]. Metallochlorophyllin, a water soluble analogue of the ubiquitous green pigment chlorophyll, belongs to a group of compounds, porphyrins that contain a chelated metal ion in the center of the molecule. The sodium and copper salt of chlorophyll in which magnesium has been replaced with tin and the phytol chains lost is the tin chlorophyllin[4]. These metallochlorophyllins also acts as a novel class of catalytic antioxidants that scavenge a broad range of reactive oxygen species (ROS) [5, 6]. Several studies have also demonstrated that metallochlorophyllins have been very effective in mitigating the toxic effects of reactive oxygen or nitrogen species, indicating that they are promising antioxidants for clinical applications[7-10].

Received on 24.11.2016 Modified on 15.12.2016
Accepted on 24.12.2016
Research J. Pharm. and Tech. 2017; 10(2): 453-455.
DOI: 10.5958/0974-360X.2017.00091.9

Moreover metallochlorophyllin not only scavenges hydroxyl radical and singlet oxygen ($^1O_2$), but also prevents lipid peroxidation from oxidative damage[11-14]. It is known to strengthen the antioxidant activity of tetrapyrroles[15].

MATERIALS AND METHODS:

Extraction of Tin Chlorophyllin:

The leaves of *Morinda citrifolia* L. was obtained from the fields of villages in and around Chengalpet District, Tamilnadu. Ten grams of leaf sample was weighed and boiled with water and caustic soda (NaOH) for 30min. 2g of stannous chloride ($SnCl_2$) was added and again boiled for 5min. The solution was filtered and cooled. It was then acidified with HCl and agitated to obtain a green precipitate. The clear liquid was separated and the remaining was filtered. Cold acetone was then added to the liquid and filtered. It was then heated with 50ml of acetone for 10min. The entire acetone was evaporated and 200ml of toluene was added. 3g of sodium bicarbonate and 100ml of acetone was added to obtain a dark precipitate. The extract was then filtered and washed with acetone and dried for storage[16].

COLUMN CHROMATOGRAPHY:

The extracted tin chlorophyllin wad purified by column chromatography with silica gel as the absorbent and toluene: acetone (8:2) as the eluents. The purified tin chlorophyllin was collected and stored at room temperature.

ANTIOXIDANT ACTIVITY:

Free radical scavenging activity- DPPH method:

The free radical scavenging activity (antioxidant capacity) of tin chlorophyllin on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was evaluated by the method reported by Molyneux[17]. 3.7ml of methanol was added to 100µl of extract. To this 200µl of DPPH (0.1 mM) was added. An equal amount of methanol and DPPH without sample was served as a control. After 30 min of reaction at room temperature in the dark, the absorbance was measured at 517 nm. The percentage free radical scavenging activity was calculated according to the following equation:

$$\% \text{ of inhibition} = \frac{(\text{Absorbance of control}) - (\text{Absorbance at test}) \times 100}{(\text{Absorbance of control})}$$

Total antioxidant capacity- Phosphomolybdenum method:

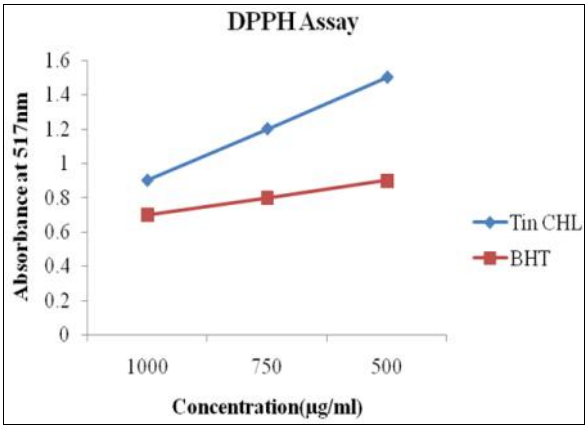
The total antioxidant capacity of tin chlorophyllin was evaluated by phosphomolybdenum method[18]. This assay is based on the reduction of Mo (VI)-Mo (V) by the extract and subsequent formation of a green phosphate or Mo (V) complex at acid pH. A 0.3ml extract was combined with 3ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95ºC for 90min. After cooling to room temperature, the absorbance was measured at 695 nm using a spectrophotometer. The antioxidant activity is expressed as the number of gram equivalent of ascorbic acid.

STATISTICAL ANALYSIS:

All experiments were performed in triplicates and the values were calculated statistically by standard deviation (SD).

RESULT AND DISCUSSION:

The DPPH assay is extensively used in vitro method for the detection of antioxidant activity of plant extracts[19]. The radical scavenging activity of tin chlorophyllin was observed from the decrease in absorbance value with increase in concentration at 517nm (Graph 1). It was observed that the percentage of radical scavenging activity of tin chlorophyllin was higher at 1000µg/ml concentration and compared with Butylated hydroxytoluene (BHT) as positive control (Table 1, Graph 2). The results depicted a concentration dependent scavenging antioxidant activity. This proves tin chlorophyllin as a good antioxidant.



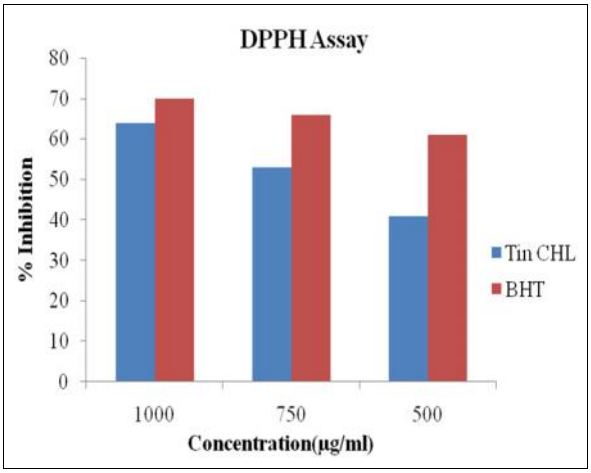
Graph 1 Free radical scavenging activity of tin chlorophyllin

Table 1 Percentage inhibition by tin chlorophyllin

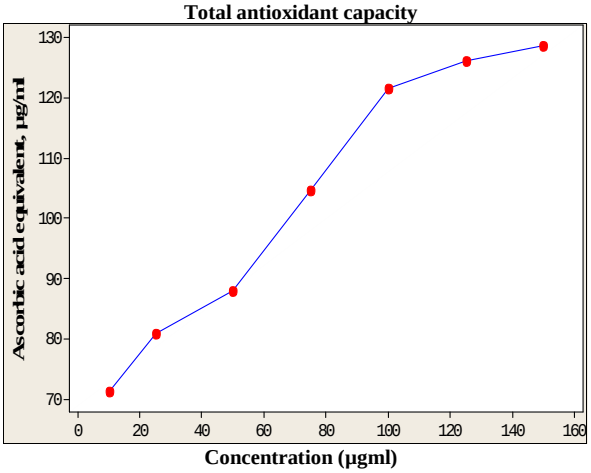
S.NO	CONCENTRATION (µg/ml)	% INHIBITION
1	1000	2.44
2	750	2.19
3	500	2.17

Values are expressed as standard deviation of mean

The total antioxidant capacity (TAC) is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid pH measured at 695nm. The results indicate a concentration dependent total antioxidant capacity (Graph 3). Antioxidant capacity of ascorbic acid has been used as a reference standard from which plant extracts with potential antioxidant activity are compared[20].



Graph 2 Percentage radical scavenging activity of tin chlorophyllin



Graph 3 Total antioxidant capacity of tin chlorophyllin

CONCLUSION:

The results demonstrate the potential of tin chlorophyllin as an important source of natural antioxidants that provide protection against damage caused by free radicals. This further supports its possible role in human health protection and disease prevention.

CONFLICT OF INTEREST:

Conflict of interest declared none.

ACKNOWLEDGEMENT:

The authors thank Dr. Ishari, K. Ganesh, Chancellor, Vels University, Chennai, for facilitating in completion of the research work. We also thank DST, Government of India for their financial support by granting INSPIRE fellowship.

REFERENCES:

1. Gutteridge, JM, Free radicals in disease processes: A compilation of cause and consequence. Free Radical Research, 19: 141–158, (1993).
2. Zima, T, Fialora, L, Mestek, O, Janebora, M, Crkovska, J, Malbohan, I, Journal of Biomedical Sciences, 8: 59, (2001).
3. Gijtenbeek, JMM, Vandenbent, MJ, Vecht, CJ, Cyclosporine neurotoxicity. Journal of Neurology, 246: 339-346, (1999).
4. Dashwood, RH, Pereira C, Bailey GS, Williams D, Jubert C, Mata J, Bench G, Effects of chlorophyll and chlorophyllin on low dose aflatoxin B1 pharmacokinetics in human volunteers: A pilot study. Cancer Prevention Research, 14:1-21, (2009).
5. Rabkin, SW, Klassen, SS, Metalloporphyrins as a therapeutic drug class against peroxynitrite in cardiovascular diseases involving ischemic reperfusion injury. European Journal of Pharmacology, 586: 1–8, (2008).
6. Patel, M, Day, BJ, Metalloporphyrin class of therapeutic catalytic antioxidants. Trends in Pharmacological Sciences, 20: 359–364, (1999).
7. Liang, HL, Hilton, G, Mortensen, J, Regner, K, Johnson, CP, Nilakantan, V, MnTMPyP, a cell-per meant SOD mimetic, reduces oxidative stress and apoptosis following renal ischemia-reperfusion. American Journal of Renal Physiology, 296: 266–276, (2009).
8. Haberle, IB, Cuzzocrea, S, Reboucas, JS, Sueta, GF, Mazzon, E, Paola, RD, Pure MnTBAP selectively scavenges peroxynitrite over superoxide: Comparison of pure and commercial MnTBAP samples to MnTE-2-PyP in two models of oxidative stress injury, an SOD-specifi c Escherichia coli model and carrageenan-induced pleurisy. Free Radical Biology and Medicine, 46: 192–201, (2009).
9. Rabbani, ZN, Spasojevic, I, Zhang, X, Moeller, BJ, Haberle, S, Vasquez, JV, Antiangiogenic action of redoxmodulating Mn(III) mesotetrakis (N-ethylpyridinium-2-yl) porphyrin, MnTE-2-PyP(5), via suppression of oxidative stress in a mouse model of breast tumor. Free Radical Biology and Medicine, 47: 992–1004, (2009).
10. Sheng, H, Yang, W, Fukuda, S, Tse, HM, Paschen, W, Johnson, K, Long-term neuroprotection from a potent redox- modulating metalloporphyrin in the rat. Free Radical Biology and Medicine, 47: 917–923, (2009).
11. Kumar, SS, Devasagayam, TPA, Bhushan, B, Verma, NC, Scavenging of reactive oxygen species by chlorophyllin: An ESR study. Free Radical Research, 35: 563–574, (2001).
12. Kumar, SS, Shankar, B, Sainis, KB, Effect of chlorophyllin against oxidative stress in splenic lymphocytes in vitro and in vivo. Biochimica et Biophysica Acta, 1672: 100–111, (2004).
13. Kapiotis, S, Hermann, M, Exner, M, Laggner, H, Gmeiner, BMK, Copper and magnesium protoporphyrin complexes inhibit oxidative modification of LDL induced by hemin, transition metal ions and tyrosyl radicals. Free Radical Research, 39: 1193–1202, (2005).
14. Kamat, JP, Boloor, KK, Devasagayam, TPA, Chlorophyllin as an effective antioxidant against membrane damage in vitro and ex vivo. Biochimica et Biophysica Acta, 1487: 113–127, (2000).
15. Ma, L, Dolphin, D, The metabolites of dietary chlorophylls. Phytochemistry, 50: 195–202, (1999).
16. Schertz, FM, The extraction and separation of chlorophyll (α+β) carotin and xanthophyll in fresh green leaves, preliminary to their quantitative determination. Plant Physiology, 3: 211-216, (1928).
17. Molyneux, P, The use of free radical DPPH for estimating antioxidant activity. Songklanakarin Journal of Science and Technology, 26: 211-219, (2004).
18. Prieto, P, Pineda, M, Anguilar, M, Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of Vitamin E. Analytical Biochemistry, 269: 337-341, (1999).
19. Ara, N, Nur, H, In vitro antioxidant activity of methanolic leaves and flowers extracts of Lippia Alba. Research Journal of Medical Sciences, 4: 107-110, (2009).
20. Aderogba, MA, Okoh, EK, Idowu, TO, Evaluation of the Antioxidant Activity of the Secondary Metabolites from *Piliostigma reticulatum* (D.C.) Hochst. International Journal of Biological Sciences, 5: 239-242, (2005).