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Study of Antioxidant Activity of Phyllanthus Emblica L. From Chlorophyllin

KEYWORDS: Chlorophyllin; Phyllanthus emblica L.; Antioxidant; Scavenging activity

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ABSTRACT *The present study was designed to investigate the antioxidant activity of Phyllanthus emblica L. (Leaf), The fresh leaves of this plant were extracted the chlorophyllin content and the potential of this edible plant was evaluated by DPPH assay (1,1 diphenyl – 2 picrylhydrazyl) which is the scavenging assay. The radical scavenging activity of the leaf extract was measured as decolurising activity followed by the trapping of the unpaired electron of DPPH. The percentage decrease of DPPH standard solution was recorded 82.05% of inhibition for Phyllanthus emblica L. when compared with authentic chlorophyllin. In the conclusion of this study Phyllanthus emblica L. possess more potential activity. The result indicated that this plant extract could be an important dietary source with antioxidant.*

Introduction

Chlorophyllin is a semi-synthetic mixture of water-soluble sodium copper salts derived from chlorophyll. Chlorophyllin has been used orally as an internal deodorant and topically in the treatment of slow-healing wounds for more than 50 years without any serious side effects. Chlorophyllin form molecular complexes with some chemicals known or suspected to cause cancer, and in doing so, may block carcinogenic effects. Chlorophyllin can neutralize several physically relevant oxidants in invitro [1,2] and limited data from animal studies suggest that chlorophyllin supplementation may decrease oxidative damage induced by chemical carcinogens and radiation [3,4]. In the biological activity of chlorophyllin are able to tight molecular complexes with certain chemicals known or suspected to cause cancer, including polycyclic aromatic hydrocarbons found in tobacco smoke [6], some heterocyclic amines found in cooked meat [7], and aflatoxin-B1 . Amla is a well-known for its rich vitamin-c (ascorbic acid) and polyphenol compounds. Ascorbic acid shows antioxidant property. Antioxidant may mediate their effect by directly reacting with ROS, quenching them or chelating the catalytic metal ions. [8]. Antioxidants rich diets can reduce oxidative damage to DNA, thus preventing a critical step in the impact of antioxidants. [9] *Phyllanthus emblica*, also known as amla, has been used in Ayurveda, the ancient Indian system of medicine. It has been used for the treatment of several disorders such as common cold, scurvy, cancer and heart disorders. [10-13]. *Phyllanthus emblica* may have the ability to regulate melanin production in the skin, reducing age spots and preventing sun damage. It is used equally as a medicine and as a tonic to build up lost energy and vigor. E. officinalis is extremely nutritious and might be a chief dietary source of vitamin C, amino acids, and minerals. Entire parts of the plant are used for medicinal purposes, particularly the fruit, which has been used in Ayurveda as a powerful rasayana and in customary medicine for the treatment of diarrhea, jaundice, and inflammation. The fruit is used either alone or in combination with other plants to treat many ailments such as common cold and fever; as a diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, alterative, antipyretic, anti-inflammatory, hair tonic; to prevent peptic ulcer and dyspepsia, and as a digestive. Moreover, plant parts show antidiabetic, hypolipidemic, antibacterial, antioxidant, antiulcerogenic, hepatoprotective, gastroprotective, and chemopreventive properties. [14]

Plant anatomy

Kingdom : Plantae
Division : Flowering plant
Class : Magnoliopsida
Order : Malpighiales
Family : Phyllanthacae
Tribe : Phyllantheae
Subtribe : Fluegginae

Materials and Methods

Sample collection

The fresh leaves of *Phyllanthus emblica* were collected in unani hospital, Royapettah, Chennai, Tamilnadu, India.

Extraction of chlorophyllin preparation

10g of fresh leaves were weighed and 1g of sodium carbonate is added to neutralize the activity. The material was ground with 50ml of acetone and filtered using filter paper and the procedure is repeated until the residue is colorless. Finally it is washed with100ml of diethyl ether to wash off acetone. The ether extract is then poured into a separating funnel and acetone is washed off using distilled water and the procedure is repeated until a yellow aqueous layer separated which consists of flavones. In order to remove the remaining flavones, 1% sodium carbonate is added. The solution is poured into a 250ml bottle. To this 10ml of methanol saturated with potassium hydroxide is added and shaken thoroughly and incubated in ice box overnight. The alkaline solution of chlorophyllin salts were poured into a separating funnel. The bottle is washed several times with distilled water and ether to remove traces of pigments. 100ml of diethyl ether is added to the funnel and left for 30min. The chlorophyllin separated as a dark greenish layer below. The greenish layer is removed and the ether layer is washed with distilled water to remove traces of chlorophyllin salts. The filtrate is evaporated to dryness in a rotary evaporator to give an ether extract of leaves. The extracted chlorophyllin is stored in ice box.

Column Chromatography

Place the glass wool at the bottom of the column. Put 10ml 7:3 petroleum ether and acetone solution into the column.

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Weigh 20g silica gel 40 in a 50ml erlenmeyer flask and some amount of petroleum ether acetone solution slowly while mixing the slurry with a glass rod. Place the slurry in the column with a pipette continuously, then place an erlenmeyer flask under the column and the excess solvent to drain.

Thin Layer Chromatography

A solution of 7ml hexane and 3ml acetone was prepared. Enough solution was poured into a TLC jar. TLC plates were taken and 40-50µl of sample was loaded on the plate. When the solvent reached top, it was removed from the tank and distance between the starting point and solvent front, pigment line were noted; using which Rf was calculated.

Rf = Distance between starting point & pigment line/ Distance between starting point & Solvent front

Standard Rf values are: 0.16-Xanthophyll; 0.32-chlorophyll b; 0.44-chlorophyll a; 0.95-β-carotene; 0.49-pheophytin b; 0.60-pheophyina.

Antioxidant Activity of Chlorophyllin (DPPH Free Radical Scavenging Activity) [15]

The ability of the extracts to annihilate the DPPH radical (1,1-diphenil-2-picrylhydrazyl) was investigated by the method described by (Blois, 1958). Stock solution of leaf extracts was prepared to the concentration of 1mg/ml. 100µg of each extracts were added, at an equal volume, to methanolic solution of DPPH (0.1mM). The reaction mixture is incubated for 30min at room temperature; the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid was used as standard controls. An inhibition activity of free radicals was calculated in % inhibition according to the following formula

% of Inhibition = (A of control – A of Test)/A of control * 100

Results and Discussion

Chlorophyllin were extracted from the fresh leaves of *Phyllanthus emblica* and were purified by column chromatography and placed in Thin Layer chromatography were single band were observed shows the result that chlorophyllin is present where as other flavonoids were absent. Then it has been taken for further investigation for the antioxidant activity by comparing with authentic chlorophyllin where as shown in Figure 1 and Table 1. The Table and figure shows the antioxidant activity of *phyllanthus emblica* has more in concentration when compared with authentic chlorophyllin and ascorbic acid used as a positive control.

Table 1: DPPH scavenging activity of chlorophyllin extract and chlorophyllin standards

Concentration (µg)	Control	DPPH Scavenging Assay % of Inhibition		
		Sample (D)	Chlorophyllin STD	Ascorbic Acid
20	0.7344	45.30229	5.310458	27.83224
60	0.7344	67.90577	18.00109	57.78867
100	0.7344	82.05338	27.90033	82.29847

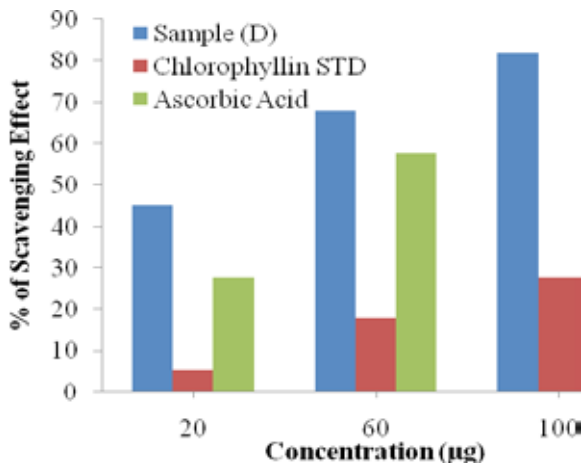

Figure 1: DPPH scavenging activity of Chlorophyllin extract and Chlorophyllin standards and Ascorbic acid was used as Positive control

REFERENCE

1. Kumar SS, Devasagayam TP, Bhushan B, Verma NC. Scavenging of reactive oxygen species by chlorophyllin: an ESR study. Free Radic Res;35(5):563-574 (2001). | 2. Kamat JP, Boloor KK, Devasagayam TP. Chlorophyllin as an effective antioxidant against membrane damage in vitro and ex vivo. Biochim Biophys Acta;1487(2-3):113-127 (2000). | 3. Park KK, Park JH, Jung YJ, Chung WY. Inhibitory effects of chlorophyllin, hemin and tetrakis(4-benzoic acid)porphyrin on oxidative DNA damage and mouse skin inflammation induced by 12-O-tetradecanoylphorbol-13-acetate as a possible anti-tumor promoting mechanism. Mutat Res; 542(1-2):89-97 (2003). | 4. Kumar SS, Shankar B, Sainis KB. Effect of chlorophyllin against oxidative stress in splenic lymphocytes in vitro and in vivo. Biochim Biophys Acta; 1672(2):100-111 (2004). | 5. Tachino N, Guo D, Dashwood WM, Yamane S, Larsen R, Dashwood R. Mechanisms of the in vitro antimutagenic action of chlorophyllin against benzo[a]pyrene: studies of enzyme inhibition, molecular complex formation and degradation of the ultimate carcinogen. Mutat Res;308(2):191-203 (1994). | 6. Dashwood R, Yamane S, Larsen R. Study of the forces of stabilizing complexes between chlorophylls and heterocyclic amine mutagens. Environ Mol Mutagen;27(3):211-218 (1996). | 7. Breinholt V, Schimerlik M, Dashwood R, Bailey G. Mechanisms of chlorophyllin anticarcinogenesis against aflatoxin B1: complex formation with the carcinogen. Chem Res Toxicol;8(4):506-514 (1995). | 8. Sun, J., Y. F. Chu, X. Wu and Liu, R. H. Antioxidant and antiproliferative activities of common fruits. J. Agri. Food.Chem.52:912- 915 (2002). | 9. Zhang, Y., N.P. Seeram, R. Lee, L. Feng and Heber, D. Isolation and identification of strawberry phenolics with antioxidant and human cancer cell antiproliferative properties. J. Agri. Food.Chem. 56:670-675 (2008). | 10. Chopra, R. N., Nayar, S. L. and Chopra, I. C., Glossary of Indian Medicinal Plants, Council of Scientific and Industrial Research, New Delhi, (1956). | 11. Gupta, B. C., The Vanusadhi-darpana, S. C. Auddy & Co, Calcutta, (1908). | 12. Rao, T. S., Kumari, K. K., Netaji, B. and Subhokta, P. K., J. Res. Ayurveda Siddha, 6, 213–214 (1985). | 13. Ghosh, A., Sharma, A. and Talukdar, G., Food Chem. Toxicol., 30, 865–869 (1992). | 14. Krishnaveni M, Mirunalini S. Therapeutic potential of Phyllanthus emblica (amla): The ayurvedic wonder. J Basic Clin Physiol Pharmacol;21:93-105 (2010). | 15. Blois MS. Antioxidant determinations by the use of a stable free radical, Nature, 29: 1199-1200 (1958). |