The Journal of Phytopharmacology 2014; 3(6): 418-422
Online at: www.phytopharmajournal.com

Research Article

ISSN 2230-480X
JPHYTO 2014; 3(6): 418-422
November- December
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Phytochemical and free radical scavenging activity of Poorna chandrodayam chendooram (metallic herbal based drug)

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Abstract

Under most pathological conditions there is generation of reactive oxygen species and other free radicals. An increase in the antioxidant reserves of the organism can reduce oxidative stress and some of the plant-derived Drug may help to reduce it. Poorna chandrodayam chendooram are extensively used in Indian traditional medicine. In the present study we have examined the in vitro antioxidant activity of Drug. The antioxidant activities of different concentrations of Drugs were determined by Total polyphenolic content, Ascorbic acid, Total flavonoids, DPPH Radical Scavenging activity, Hydroxyl Radical Scavenging Activity and Nitric Oxide Scavenging activity. The effective antioxidant activity of Poorna chandrodayam chendooram has found increased with increasing concentration.

Keywords: Poorna chandrodayam chendooram, ROS, Ascorbic acid, DPPH, Hydroxyl Radical Oxide free radical scavenging activity, Nitric Oxide Scavenging activity.

Introduction

Free radicals, often called reactive oxygen species (ROS), are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism.[1] They are generated as by-products of biological reactions or from exogenous factors. When ROS production is greater than the detoxification capacity of the cell, excessively generated ROS causes extensive damage to DNA, proteins, lipids etc. and acts as a mediator of pro-inflammatory and carcinogenic events.[2] Such conditions are considered to be important causative factors in the development of diseases such as diabetes, stroke, arteriosclerosis, cancer and cardiovascular diseases.[3] In the treatment of such diseases, antioxidant therapy has gained an immense importance. These antioxidants interfere with the oxidative processes by reacting with free radicals, chelating catalytic metal ions and also by acting as oxygen scavengers.[4]

Indian alchemy is one of the disciplines in which Parpam, Chedooram and Chunnam were first described as intriguing formulations of metals and minerals such as gold, silver, copper, iron, zinc, mercury, and so forth, apparently associated with organic macromolecules derived from the herbal juices by alchemic processes making these biologically assimilable.[5] Minerals are combined with herbs that assist the assimilation and delivery of the ingredients to the human body.[6] These herbo mineral medicine are prepared by repeated incineration of metals or their salts (preferably oxides) with medicinal herbs or their extracts so as to eliminate their harmful effects and are taken along with honey, milk, butter, or ghee (a preparation from milk).[7] Most of the medicines are mixture of compounds and because of its synergistic action; toxicity is being diminished, thereby increasing bioavailability through the cells of the body. Treating the minerals with herbal juices may lead to reduction in particulate size even up to nano levels (less than 100 nm) enable increased potency.

Poorna chandrodayam chendooram is a well known, mercurial preparation with gold and sulphur[8] widely used for many ailments like tuberculosis, jaundice, fever, rat bite, cancerous ulcer, sprue and male sterility.[9] Hibiscus and Aloe juice is added for titration.[10] These drugs are mostly a mixture of compounds and because of its synergistic action and purification process[11] toxicity is being diminished[12], thereby increasing bioavailability through the cells

of our body.[13] These drugs are known to be effective even in low concentration.[14]

The aim of the present study was investigate the invitro antioxidant activity of the aqueous extract of PCC included Total polyphenolic content, Ascorbic acid, Total flavonoids, DPPH Radical Scavenging activity, Hydroxyl Radical Scavenging Activity and Nitric Oxide Scavenging activity.

Materials and Methods

Chemicals

Na_2Co_3, Ethanol, Folin-ciocalteu reagent, EDTA, TCA, Starch, $CuSo_4$, Aluminium chloride, Potassium acetate, DPPH, H_2O_2, deoxy ribose, Riboflavin, NBT, Sodium nitroprusside, Griess reagent All the chemicals used in this study were of analytical grade.

Drug Preparation

The (Poorna Chandrodayam Chendooram drug obtained from the SKM Siddha and Ayurvedic Medicine's India Private Limited, Saminathapuram, mudakurichi, Erode-638104. Tamilnadu, India. The drug (Poorna Chandrodayam Chendooram) is not soluble in water therefore a suspension of gum acacia is made for oral administration. The 10 g of gum acacia dissolved in 100 ml of distilled water by gradual trituration in a mortar. Then well prepared solution was taken and added Poorna Chandrodayam Chensdooram at the dose of 3 mg/ml/100 g.

Determination of total polyphenolic compounds in PCC

Total polyphenolic compounds were determined according to a protocol.[15] PCC (1 ml) was mixed with 1 ml of 95% ethanol, 5 ml of distilled water and 0.5 ml of 50%. The mixture was allowed to react for 5 min and 1ml of 5% Na_2CO_3 was added. Thereafter, it was thoroughly mixed and placed in the dark for 1 hour and absorbance was measured at 725 nm with UV-Visible spectrophotometer. A gallic acid standard curve was obtained for the calculation of polyphenolic content. The concentration of poly phenols was expressed in terms of mg/ml of sample.

Determination of Ascorbic acid (Vit C)

5 g of the sample was weighed in to an extraction tubes and 100 ml of EDTA/TCA (2:1) extracting solution were mixed and the mixture shaken for 30 min. This was transferred in to a centrifuge tubes and centrifuged at 3000 rpm for about 20 min. It was transferred in to a 100 ml volumetric flask and made up to 100 ml mark with the extracting solution. 20 ml of the extract was pipette in to a volumetric flask and 1% starch indicator was added. These were added and titrated with 20% $CuSO_4$ solution to get a dark end point.[16] The amount of Vitamin C was calculated as mg/100 ml of sample.

Determination of Total flavonoid in PCC

Aluminium chloride colorimetric method was used for flavonoids determination.[17] 1 ml of sample were mixed with 3 ml of methanol, 0.2 ml of 10% Aluminium chloride, 0.2 ml of 1 mole potassium acetate and 5.6 ml of distilled water. It remained at room temperature for 30 mins. The absorbance of the reaction mixture was measured at 415 nm with UV-Visible spectrophotometer. The caliberation curve was prepared by preparing quercetin solution at various concentrations in methanol. The concentration of flavonoids was expressed in terms of mg/100 ml of samples.

DPPH Radical Scavenging assay

Accurately weighed 4.3 mg of DPPH was dissolved in 3.3 ml of methanol in a test tube.[18] Solution was protected from light by covering with aluminum foil. 150 µl of above solution was taken and diluted up to 3 ml with methanol, the absorbance of this solution was taken immediately at 516 nm on UV spectrophotometer using methanol as blank. This reading was served as control reading. For the test and standard, the aliquots of different concentration ranging were prepared. For the assay 150 µl of the test or std solution was added to 150 µl of DPPH solution and diluted up to 3 ml with methanol, the absorbance of this solution was taken after 15 min at 516 nm on UV spectrophotometer using methanol as blank. The absorbance was taken in triplicate manner.

The % Scavenging activity was found by using following formula:

$$\% \text{ scavenging activity} = \frac{\text{O.D of control} - \text{O.D of test}}{\text{O.D of control}} \times 100$$

Hydroxyl Radical Scavenging Activity

The Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+}/ascorbate/ EDTA/ H_2O_2 system.[19] The reaction mixture contained deoxy ribose (2-8mM), Fecl (0.1mM), EDTA (0.1mM), H_2O_2 (lmM), ascorbate (0.1mM), KH PO - KOH buffer (20mM, 2 4 pH 7.4) and various concentrations (25- 400 µm of extracts and std 10 to 80 µm /ml) of standard drug in a final volume of 1 ml. The reaction mixture was incubated for 1hr at 37°C; deoxyribose degradation was measured with spectrophotometer at 532 nm.

The percentage of hydroxyl radical scavenging activity was calculated by the formula:

$$\% \text{ scavenging activity} = \frac{\text{O.D of control} - \text{O.D of test}}{\text{O.D of control}} \times 100$$

Nitric Oxide scavenging assay

Sodium nitroprusside (5 µM) in standard phosphate buffer solution was incubated with different concentration of the test extracts dissolved in standard phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25°C for 5 hr. After 5 hr, 0.5 ml of incubation solution was removed and diluted with 0.5 ml Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in water).[21] The absorbance of chromophore formed was read at 546 nm. The control experiment was also carried out in similar manner, using distilled water in the place of extracts. The experiment was performed (in triplicate) and the activity was compared with ascorbic acid, which was used as a standard antioxidant.

Nitric oxide radical scavenging that is calculated by the Formula:

$$\% \text{ Nitric oxide radical radical scavenging} = \frac{(\text{Control OD} - \text{Sample OD})}{\text{Control OD}} \times 100$$

Statistical Analysis

The statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Dunnet,s t-test, P- values <0.001 were considered as significant

Results and Discussion

In the present study, the phytochemical analysis of PCC was found to be contain rich source of Flavonoids (38.7±4.1 mg/100 ml) Poly phenols (86.2±8.3 mg/100 ml) and Vitamin C (42.2±4.1 mg/100 ml) (Figure 1).

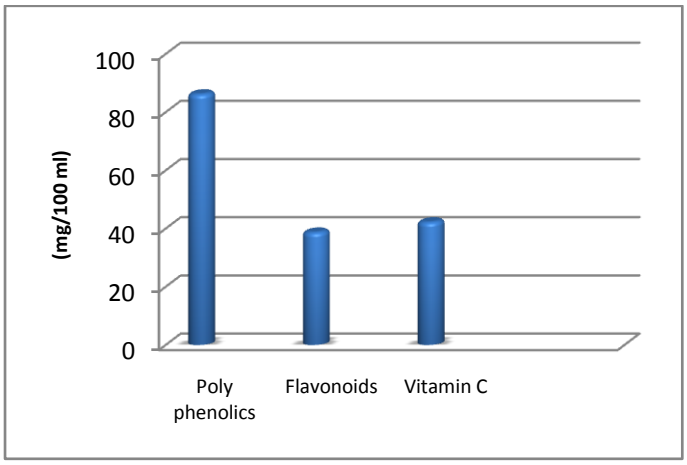

Figure 1: Phytochemical analysis of PCC

Poly phenolics compounds are well known as antioxidant and scavenging agents against free radicals associated with oxidative damage.[22] The presence of these compounds in PCC may give credence to usage for the management of oxidative stress induced ailments.

Flavonoids are important secondary metabolite of plant modulating lipid peroxidation involved in atherosclerosis, thrombosis and carcinogenesis. It has been confirmed that pharmacological effect of Flavonoids is correlating with their antioxidant activities.[23] Further more the medicinal usage of PCC might be attributed to the high concentration of Flavonoids and therefore it could support its usage for the management of oxidative stress induced diseases.

Vitamin C act as antioxidant in biological systems and scavenge the free radicals and thereby increase the antioxidant defense in the body. The effects of Vitamin C and other putative antioxidant on biomarker of oxidation have been studied in many pathological states that mentioned to result from or result in oxidative stress.[24]

The identification of antioxidant is beneficial to biological system against ROS ravage. Recently importance has been given for *in vitro* antioxidant study to understand the pharmacological role of herb mineral drug. *In vitro* techniques have been used for detection of antioxidant which is based on the ability of compounds to scavenge peroxide radicals.

DPPH radical Scavenging assay

In the present study several free radical scavenging activities of Aqueous extract of PCC were evaluated by DPPH scavenging assay. Aqueous extracts of PCC have got profound antioxidant activity. DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, which gets decolorized in the presence of antioxidants.[25] The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, it gets decolorized which can be quantitatively measured from the changes in absorbance at 517nm. The Aqueous extracts of PCC exhibited a significant dose dependent inhibition of DPPH activity. In this study, at 125 µg /ml, the PCC extract showed highest inhibition of DPPH activity shown in (Figure 2). The results of DPPH-free radical scavenging assay suggest that the PCC extract is more capable of scavenging free radicals.

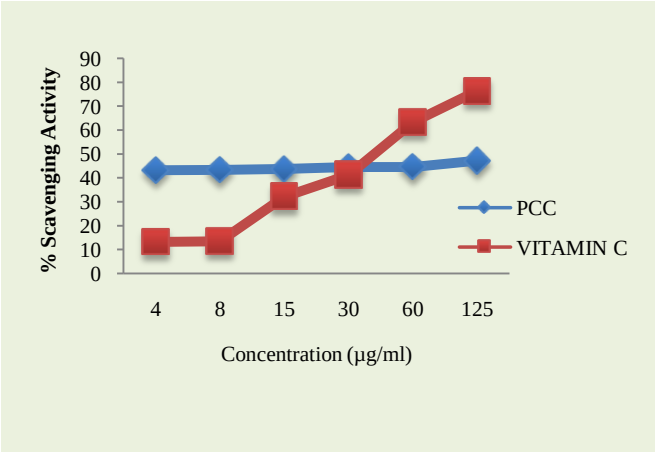

Figure 2: *In vitro* free radical scavenging effect of PCC by DPPH method

Hydroxyl Radical Scavenging Activity

The hydroxyl radical is an extremely reactive free radical formed in biological system. It has been implicated as a major active oxygen centered radical formed from the reaction of various hydro peroxides with transition metal ions, which is capable of damaging almost every molecule found in living system causing lipid peroxidation and biological damage.[27, 28]

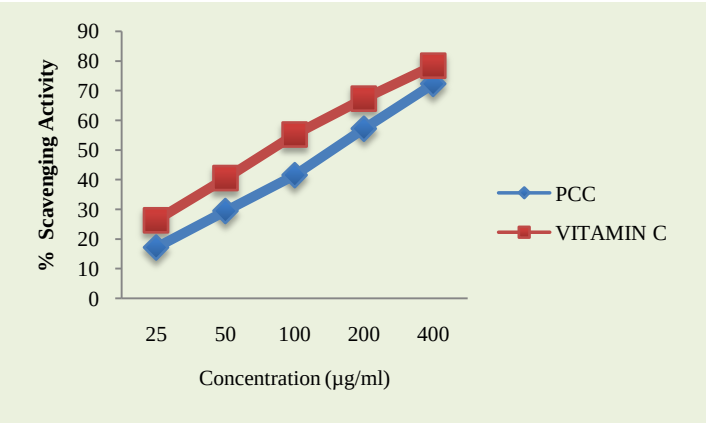

Figure 3: *In vitro* free radical scavenging effect of PCC extract by hydroxyl radical scavenging method

The maximum Hydroxyl radical scavenging effect was found at 400µg/ml concentration. The PCC showed higher scavenging activity shown in Figure 3. This ability of the extracts shows the quenching ability of hydroxyl radicals, which seems to be a good scavenger, of active oxygen species thus reducing the rate of chain reaction.

Nitric Oxide scavenging activity

Nitric oxide (NO) is a potent pleiotropic mediator of physiological process such as smooth muscle relaxant, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical, which plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities.[29] Although nitric oxide and superoxide radicals are involved in host defense, over production of these two radicals contributes to the pathogenesis of some inflammatory diseases.[30] Moreover in the pathological conditions, nitric oxide reacts with superoxide anion and form potentially cytotoxic molecules, peroxynitrite. Nitric oxide inhibitors have been shown to have beneficial effects on some aspects of inflammation and tissue damage seen in inflammatory diseases.[31]

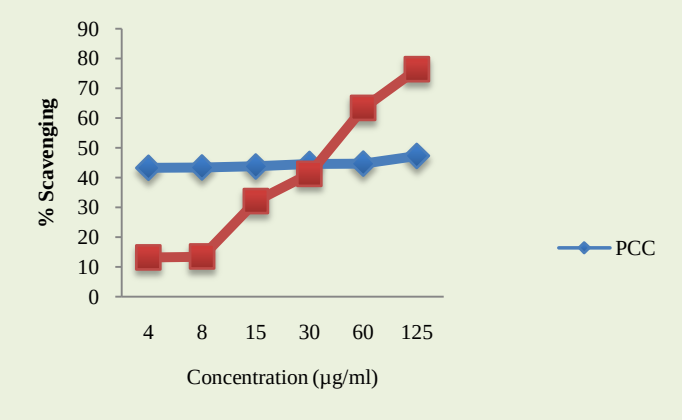

Figure 4: *In vitro* free radical scavenging effect of PCC by nitric oxide scavenging method

PCC significantly inhibited nitric oxide in a dose dependent manner (Figure 4) at a concentration of 125µg/ml. The results indicates that, the extract might contain compounds capable of inhibiting nitric oxide and offers scientific evidence for the use of the drug in the indigenous system in treatment of various diseases.

Conclusion

In conclusion, the results obtained in the present study indicated PCC extract exhibited free radical scavenging activity against hydroxyl, peroxide and DPPH radicals. The overall antioxidant activity of PCC might be attributed to its Polyphenolic content, Flavonoids and Vitamin C constituents. The findings of the present study suggested that PCC could be a potential source of natural antioxidant that would have great importance as therapeutic agents in preventing or slowing the progress of reactive oxygen species and associated oxidative stress related degenerative diseases.

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