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IN-VITRO ANTIOXIDANT ACTIVITY OF - AN AYURVEDIC BHUNIMBADI CHURNA

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Abstract: Medicinal plants represent a rich source of antioxidant agents and used medicinally in different countries as source of many potent and powerful drugs. The present study was undertaken to evaluate DPPH radical scavenging assay and ferric-reducing antioxidant power assay. The antioxidant activity of the methanol extract increased in a concentration-dependent manner. It is concluded that these results suggest that the Methanolic extract of Bhunimbadi Churna possess antioxidant effect in DPPH radical scavenging assay and ferric-reducing antioxidant power assay.

Keywords: Antioxidant, DPPH radical scavenging assay, Churna, Medicinal plants

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INTRODUCTION

Antioxidants are recognized for their potential in promoting health and lowering the risk for cancer, hypertension and heart disease. [1,2,3] The uses of natural antioxidants from plant extracts have experience growing interest due to some human health professionals and consumer's concern about the safety of synthetic antioxidants in foods [4]. Antioxidant activities in plants have been identified by many researchers. [5] The natural occurring antioxidant is focused more on edible plants, especially spices and herbs. Spices and herbs are an excellent source of phenolic compounds (flavonoids, phenolic acid and alcohols, stilbenes, tocopherols, tocotrienols), ascorbic acid and carotenoids which have been reported to show good antioxidant activity.[6, 7]

However, the Himalayan plant, *Swertia chirata* Buch. Ham. Ex Wall resulted in the isolation of seven polyoxygenated xanthones, fraction Sc-1 – Sc-7. Out of seven compounds extracted from this plant, five components (Sc - 3 - Sc-7) exhibited antioxidant activity at different magnitude of potency.[8] The paper deals with these facts. Effect of Solvent extraction on Phenolic content, Antioxidant and Amylase Inhibition activities of Swertia chirata.[9] The methanolic and ethanolic extracts from ginger (*Zingiber officinale* L.) seed (ZOS) were investigated for their antioxidant and radical scavenging activities in eight different assays, namely, total antioxidant activity, reducing power, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, total flavonoid content, total phenolic compound and metal chelating activities.[10]. The black pepper containing oil, the antioxidant aspects will be evaluated by means of the determination of the total phenolic content by the Folin-Ciocalteau method and by the antioxidant activity by the DPPH, ABTS and β-carotene methods.[11] *Cyperus rotundus* was extracted by using different extraction solvents and evaluated for their antioxidant activity using different *in vitro* antioxidant assays.[12] The protective effect of P. kurroa might be ascribable to its membrane-stabilizing property and/or antioxidant nature.[13] The isolation and spectral data for new flavonoid 2-(2, 4-Dihydroxy-phenyl)-3, 6, 8- trihydroxy-chromen-4-one from the roots of *Plumbago zeylanica* were determined and the antioxidant activity were studied by free radical scavenging and superoxide radical scavenging assays.[14] The present study was carried out to investigate the antioxidant effect of the methanolic extract, its alkaloidal and non-alkaloidal fractions along with petroleum ether soluble, ether soluble and ethyl acetate soluble sub-fractions of non-alkaloidal part of the bark of *Holarrhena pubescens.*[15]

MATERIALS AND METHODS

Materials

'Bhunimbadi Churna' containing the plant parts and their powder of fresh plant *Swertia chirata* (Whole plant), *Holarrhena antidysenterica* (Seed), *Zingiber officinale* (Rhizome), *Piper nigrum*

(Fruit), *Piper longum* (Fruit), *Cyperus rotundus* (Rhizome), *Picrorrhiza kurroa* (Rhizome), *Plumbago zeylanica* (Root), and *Holarrhena antidysenterica* (Stem bark) were collected from local market of Vadodara, India. Ascorbic acid, 1,1-diphenyl-2- picrylhydrazyl (DPPH) and Potassium ferricyanide, Ferric Chloride, Potassium hydrogen phosphate were purchased from Sd-fine chemicals limited, Mumbai, India.

Determination of free radical scavenging using DPPH method [16, 17]

Product extract and standard ascorbic acid solution (0.1 ml) of different concentrations viz. 10, 20, 40, 60, 80, 100µg/ml was added to 3 ml of a 0.004% methanol solution of DPPH. An equal amount of methanol and DPPH served as control. After 30 minutes incubation in the dark, absorbance was recorded at 517 nm using UV spectrophotometer (Shimadzu-UV-1601). Samples were measured in three replicates and the percentage inhibition activity was calculated from [(A0-A1)/A0] ×100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract/standard.

Determination of Ferric Reducing Power determination method [18]

Different concentrations of Churna extract and standard ascorbic acid solution viz. 10, 20, 40, 60, 80, 100 µg/ml in 1ml of methanol were mixed with phosphate buffer (2.5ml, 0.2M pH 6.6) and potassium ferricyanide [K3Fe(CN)6] (2.5 ml, 1%). The mixture was incubated at 50oC for 20 min. A portion (2.5 ml) of tricholoroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 g (rpm) for 10 min at room temperature. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (FeCl3) (0.5 ml, 0.1%) and the absorbance of the reaction mixture indicated increased reducing power. The absorbance was measured at 700nm using UV spectrophotometer (Shimadzu-UV-1601). All the tests were performed in triplicate and the graph was plotted with the average of three observations.

Statistical analysis

Experiment data were analyzed using Excel (Microsoft Inc.) and SPSS version 17.0 software. Significant differences between samples were analyzed using analysis of variance (ANOVA) and Dunnett's test using GraphPad Instat 3 software ($P< 0.05$). Data obtained were reported as mean ± standard deviation.

RESULTS AND DISCUSSIONS

Antioxidant capacity

DPPH radical was used as a stable free radical to determine antioxidant activity of natural compounds. [19] The antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals [20]. Thus, the purple colour of 2,2-diphenyl-1- picryl hydrazyl (DPPH) will reduce to α, α-

diphenyl- β-picrylhydrazine (yellow coloured). [21] The scavenging of the stable radical (DPPH) is considered a valid and easy assay to evaluate scavenging activity of antioxidants.[22]

Result indicated the significant decrease in the concentration of DPPH radicals due to the scavenging ability of Methanol extract of Churna and Ascorbic acid, as a reference standard. The IC_{50} values in DPPH radical scavenging model were 14.26 % and 62.80 % for Ascorbic acid and Methanol extract of Churna respectively shows in figure 1.

In this study, the antioxidant activity is also determined on the basis of the ability of antioxidant in this plants extracts to reduce ferric (III) iron to ferrous (II) iron in result shows figure 2 illustrates that Methanolic extract of the Churna had ferric reducing capacity and also comparable to Ascorbic acid.

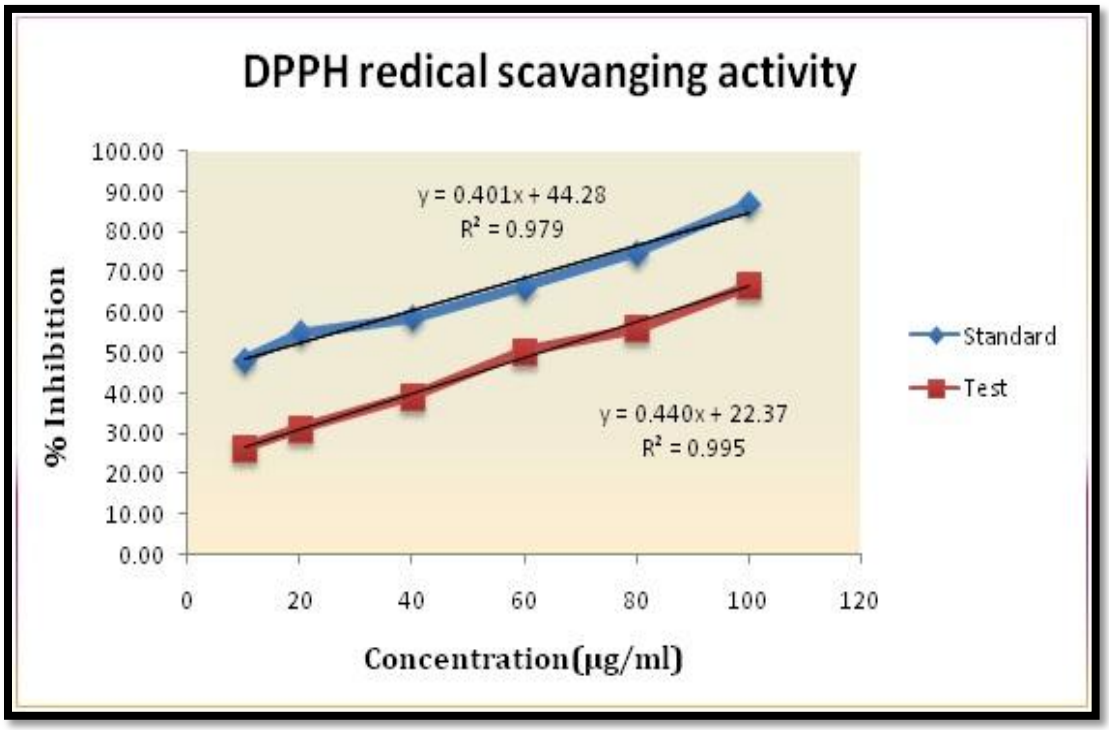

Figure 1: Chart of DPPH radicals scavenging activity

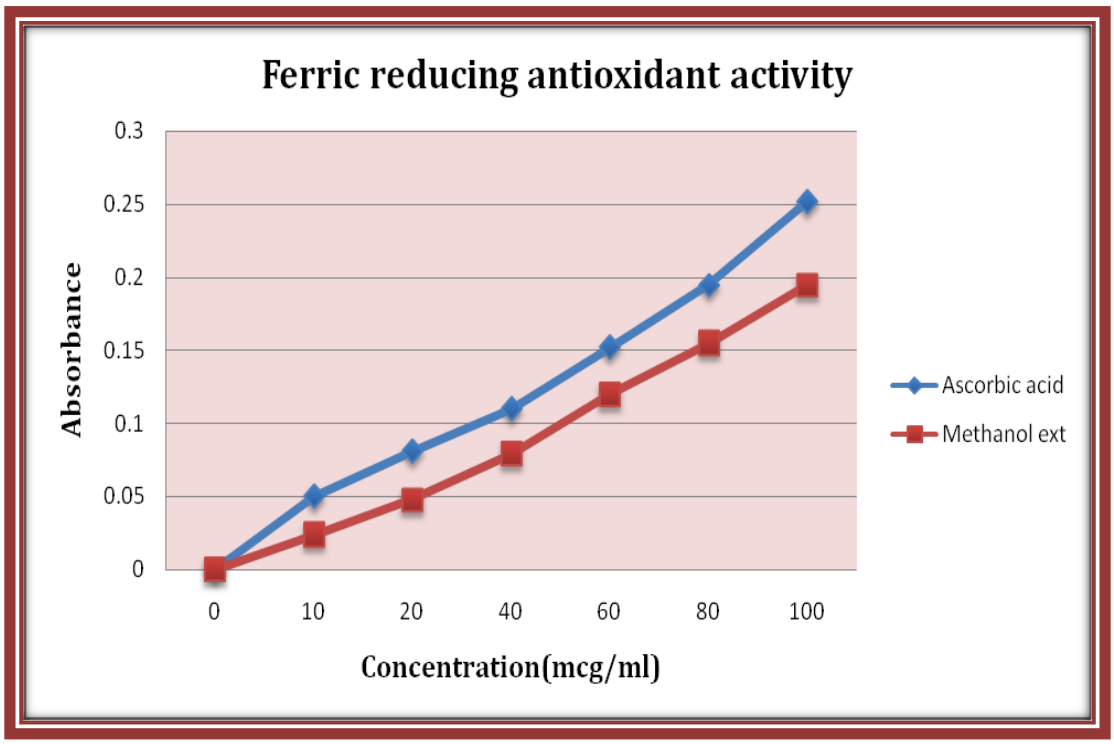

Figure 2: Chart of Ferric reducing antioxidant activity

CONCLUSION:

The results obtained demonstrated that Bhunimbadi Churna had show antioxidant activity in free radical scavenging using DPPH method Ferric Reducing Power determination method. The mixture of plants extracts had showed no synergism effect.

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