

IN VITRO ANTIOXIDANT AND α -AMYLASE INHIBITION ACTIVITIES OF PANCHSAKAR CHURNAAshok Kumar B.S.¹, Gopi Setty Saran², Firdose Banu¹, Harshada R.¹, Archana P.G.¹

Panchsakar Churna is the composition of *Cassia angustifolia*, *Terminalia chebula*, *Zingiber officinale*, *Foeniculum vulgare* and *Saindhava lavana*. Aqueous extract of churna was used to investigate antioxidant activity by ferrous ion chelating assay and ferric reducing power and alpha amylase inhibition activity by dinitrosalicylic acid method (DNSA). Aqueous extract of churna showed maximum ferrous chelating activity - 42.01 and ferric reducing power - 1.5 and 83.33% of inhibition protein denaturation at 1000 $\mu\text{g/ml}$. Panchsakar churna showed significant antioxidant and alpha amylase inhibition activities. *Acta Medica Medianae* 2013;52(4):12-14.

Key words: Panchsakar churna, alpha amylase inhibition activity, ferrous chelating activity

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Introduction

Panchsakar Churna, an Ayurvedic poly-herbomineral formulation, consists of *Cassia angustifolia*, *Foeniculum vulgare*, *Terminalia chebula*, *Zingiber officinale* and *Saindhava Lavana*, traditionally used for managing constipation, rheumatoid arthritis, haemorrhoids, abdominal pain, flatulence, assimilatory disorder and rheumatic conditions, all diseases of Kapha Dosha origin, improves digestion and ensures timely evacuation of faeces, improves liver functions, hyperacidity, heart burn and acidic belching (1).

The main aim of the present study was to investigate antioxidant and alpha-amylase inhibition activity of aqueous extract of Panchsakar Churna.

Antioxidant activity studies**Ferric reducing power**

The ferric reducing power of the aqueous extract of Panchsakar Churna (APC) was determined by using potassium ferricyanide-ferric

chloride method (2). Different concentrations (100–1000 $\mu\text{g/ml}$) of extracts were added to 2.5 ml 0.2 M phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixtures were incubated at 50°C for 20 min, after which 2.5 ml trichloroacetic acid (10%) were added. Two and one half milliliters of the mixture were taken and mixed with 2.5 ml water and 0.5 ml 1% FeCl₃. The absorbance at 700 nm was measured after allowing the solution to stand for 30 min. A graph of absorbance vs. extract concentration was plotted to observe the reducing power.

Ferrous ion chelating activity

The chelating ability of the aqueous extract of Panchsakar Churna (APC) was determined according to the modified method of Minotti and Aust, 1987 (3). In this assay, the plant extract binds Fe²⁺ ion generated in vitro using 500 μM iron (ii) sulphate as ion donor. 0.2ml of sample of different concentration (100–1000 $\mu\text{g/ml}$) of the churna extract was mixed with 0.336ml of Tris HCl (0.1M, pH7.4), followed by the addition of 0.436ml (saline, 0.9% NaCl w/v). The mixture was left to stand at room temperature for 5 min. 0.26ml of 0.25% aqueous 1,10-phenanthroline were added. The absorbance of the solution was read on uv/visible spectrophotometer at 510 nm against control which consists of Tris HCl, saline and phenanthroline without the plant extract.

$$\text{Chelating ability (\%)} = \frac{(A_{\text{control}} - A_{\text{sample}}) \times 100}{A_{\text{control}}}$$

Alpha amylase Inhibition assay (3, 5-dinitro-salicylic acid method)

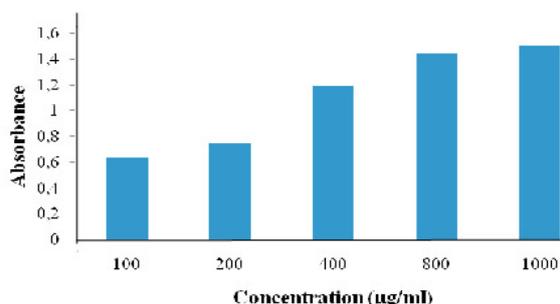
The inhibition assay was performed according to Miller (4) using the DNS method. Aqueous extract of Panchsakar Churna (APC) of varied concentrations in 500 μ l were added to 500 μ L of 0.02 M sodium phosphate buffer (pH6.9 containing 6 mM sodium chloride) containing 0.04 units of alpha amylase solution and were incubated at 37°C for 10 min, followed by addition of 500 μ L of a 1% starch solution in 0.02 M sodium phosphate buffer (pH6.9) in all the test tubes. The reaction was stopped with 1.0 ml of 3,5 DNSA reagent. The test tubes were then incubated in a boiling bath water for 5 min, cooled at room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. The control samples were also prepared accordingly without any plant extracts and were compared with the test samples containing various concentrations of the plant extracts prepared with different solvents. The results were expressed as % inhibition calculated using the formula:

$$\% \text{ Inhibition activity} = \frac{\text{Abs(Control)} - \text{Abs(Extract)}}{\text{Abs(Control)}} \times 100$$

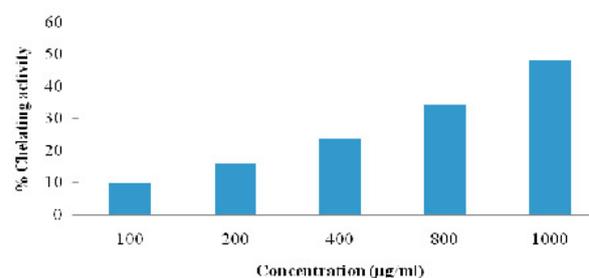
Result and discussion

Iron is an important trace element of the body, being found in a functional form in hemoglobin, myoglobin, the cytochromes, enzymes with iron sulphur complexes and other iron-dependent enzymes (5). Iron has the unique ability to alter its oxidation and redox states in response to liganding, which makes it essential for various cellular processes (6). The cells maintain the free iron concentration to a minimum required level to avoid toxic effects of excess iron. However, in some situations the iron balance is disrupted and results in iron overload which is associated with the oxidative stress inducing several health problems including anemias, heart failure, liver cirrhosis, diabetes, arthritis, depression, infertility, and cancer (7). The body lacks any effective means to excrete excessive iron and therefore the interest has been raised to develop the potent chelating agent capable of complexing with iron and promoting its excretion (8,9). Graph 1 shows ferrous chelating activity of aqueous extract of panchsakra churna.

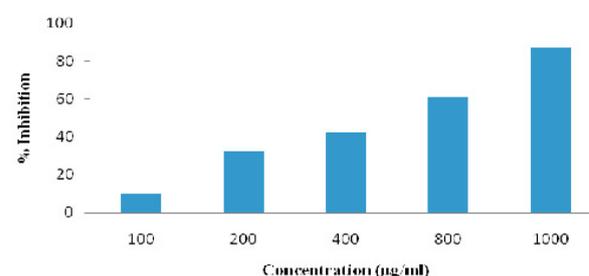
Ferric reduction is frequently used as a marker of electron-donating activity and considered as a significant mechanism of phenolic antioxidant action (10). In the ferric reducing antioxidant power (FRAP) assay, this Fe²⁺ complex can be determined at 700 nm by evaluating the



Graph 1. Ferric reducing activity of Panchsakar Churna



Graph 2. Ferrous chelating activity of Panchsakar Churna



Graph 3. α -amylase inhibition activity of Panchsakar Churna

formation of Perl's Prussian blue in the extracts, if any, which would result in reducing Fe³⁺ to Fe²⁺ by donating an electron. Amount of the reductive ability is directly proportional to the absorbance of the APC at 700 nm (11). This is especially important in neurologic disorders accompanied by increased oxidative damage of neurons (12). Graph 2 shows the ferric reducing power of APC.

Although the acute effects of α -amylase inhibitors may appear to have therapeutic benefit in patients suffering from diabetes mellitus, obesity and other diseases of insulin resistance, chronic administration in animal models has been shown to induce adverse effects including deleterious histological changes to the pancreas (13). Graph 3 shows Panchsakra Churna inhibition of α -amylase enzyme.

Panchsakra Churna has shown potent antioxidant and α -amylase inhibitory activities. Natural health products were clearly indicated as a promising avenue for the prevention of chronic diseases (14).

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IN VITRO ANTIOKSIDACIONE I AKTIVNOSTI PANCHSAKAR CHURNA U INHIBICIJI α -AMILAZE

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U sastavu panchsakar churna nalaze se *Cassia angustifolia*, *Terminalia chebula*, *Zingiber officinale*, *Foeniculum vulgare* i *Saindhava* lavana. Vodeni ekstrakt churna korišćen je da bi se ispitalo antioksidaciono dejstvo pomoću helat analize jonom gvožđa i smanjene moći gvožđa, kao i dejstvo inhibicije alfa amilaze pomoću metoda dinitrosalicilnom kiselinom. Vodeni ekstrakt churna je pokazao maksimalno dejstvo helata gvožđa od 42.01 i smanjeno dejstvo gvožđa od 1.5 i 83.33% inhibicije denaturacije proteina na 1000 μ g/ml. Panchsakar churna je pokazao antioksidaciono i inhibitorno dejstvo alfa amilaze. *Acta Medica Medianae* 2013;52(4):12-14.

Ključne reči: Panchsakar churna, inhibitorno dejstvo alfa amilaze, helaciono dejstvo gvožđa