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α-Amylase inhibition and antioxidant activity of Singhnad Guggul *in vitro*

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ABSTRACT

Background & Aim: Singhnad Guggulu is a traditional Ayurvedic formula for detoxifying and rejuvenating the joints. The present study was to investigate the α-amylase inhibition and antioxidant activities of aqueous extract of Singhnad Guggul (AESG).
Experimental: α-Amylase inhibition assay by dinitro-salicylic acid method and antioxidant activity by ferric chelating and ferrous reducing assay.
Results: AESG indicated that potent α-amylase inhibition activity (IC_{50}= 84.00 µg/ml). AESG also showed ferric chelating and ferrous reducing activities.
Recommended applications/industries: Our studies showed that Singhnad Guggulu possess alpha- amylase and antioxidant properties.

1. Introduction

Singhnad Guggulu is a traditional Ayurvedic formula for detoxifying and rejuvenating the joints. Singhnad Guggul tablets contain Guggulu resin (*Commiphora mukul* (Arn.) Bhandari), Amalaki fruit (*Emblica officinalis* Gaertn.), Bibhitaki fruit (*Terminalia belerica* Roxb.), Haritaki fruit (*Terminalia chebula* Retz.), Gandhak (sulphur), and castor oil (*Ricinus communis* L.). It combines the potent cleansing ingredients of castor oil and Triphala, which remove natural toxins from the joints, blood and GI tract. The soothing and lubricating qualities of the herbs work to nourish and strengthen the joint tissue and support their proper function. This formula also promotes healthy digestion and elimination helping to maintain a clean and balanced system. In addition, Singhnaad Guggulu is traditionally used in Ayurveda for treatment of difficult skin disorders, psoriasis, eczema, etc. It is also useful for supporting treatment of mild infections of the upper respiratory tract. It primarily reduces Pitta and Vata doshas (Anonymous, 2012). The present study was aimed to investigate α-amylase inhibition and antioxidant activities of aqueous extract of Singhnad Guggul.

2. Materials and Methods

2.1. Preparation of extract

Singhnad Guggul tablets were crushed and extracted with water and dried the extract. Five gram of extract is dissolved in water and used for study.

2.2. α-Amylase inhibition activity

2.2.1. 3, 5-dinitrosalicylic acid assay (DNSA). The inhibition assay was performed using the according to Miller (1959) using DNSA method. The total assay mixture composed of 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 0.04 units of α-amylase solution and extracts at concentrations from 50 to 1000 µg/ml were incubated at 37°C for 10 min. A total of 500 µl of test sample extract (25 to 800 µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5 mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 DNSA reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Controls represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

2.3. Antioxidant activity

2.3.1. Ferric reducing power. The ferric reducing power of the aqueous extract of Singhnad Guggul was determined by using potassium ferricyanide–ferric chloride method (Oyaizu, 1986). Different concentrations (100 to1000 µg/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%) was added. Two and one half milliliters of the mixture was taken and mixed with 2.5 ml water and 0.5 ml 1% $FeCl_3$. 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.

2.3.2. Ferrous ion chelating activity. The chelating ability of the aqueous extract of Singhnad Guggul was determined according to the modified method of Minnoti & Aust (1987). In this assay, the plant extract binds with Fe^{2+} ion generated *in vitro* using 500 uM iron (ii) sulphate as ion donor. 0.2ml of sample of different concentration (100 to 1000 µg/ml) of the plant extract was mixed with 0.336ml of TrisHCl (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 of 5min. 0.26ml of 0.25% aqueous 1,10-phenanthroline was added. The absorbance of the solution was read on UV/visible spectrophotometer at 510 nm against control which consists of TrisHCl, saline and phenanthroline without the plant extract.

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

Whereas Ab means Absorbance.

3. Results and Discussion

Singhnad Guggul is made of natural herbs, which are very helpful in treating the diseases of joints such as rheumatic arthritis, gout, swelling and irritation, backache, cervical spondylitis.

Drugs that reduce post-prandial hyperglycaemia by suppressing hydrolysis of starch such as PPA inhibitors have been found useful in the control of diabetes mellitus (Layer *et al*., 1986; Tundis *et al*., 2010). Many herbal extracts have been reported for their anti-diabetic activity and are currently being used in Ayurveda for the treatment of diabetes. However, such medicinal plants have not gained much importance as medicines due to the lack of sustained scientific evidence. In the present study, Singhnad Guggul was screened for their α-amylase inhibitory potential by chromogenic DNSA method and it was noted that Singhnad Guggul extracts exhibit significant α-amylase inhibition (Fig. 1).

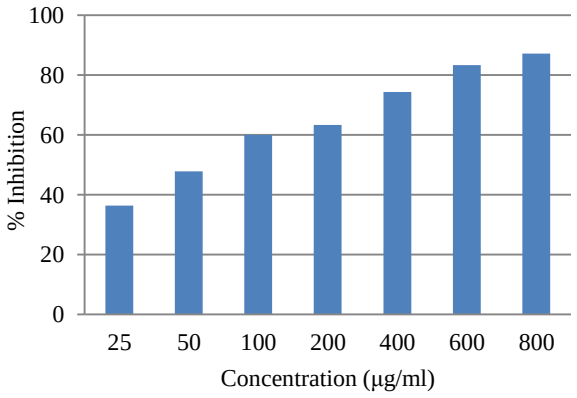

Fig 1.Effect of Singhnad Guggul on α-amylase inhibition activity

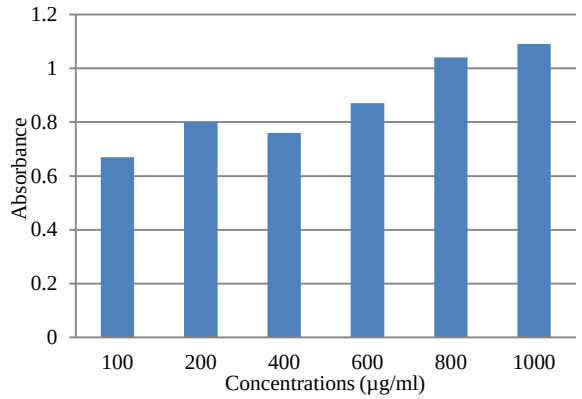

Fig 2. Effect of Singhnad Guggul on ferric reducing assay

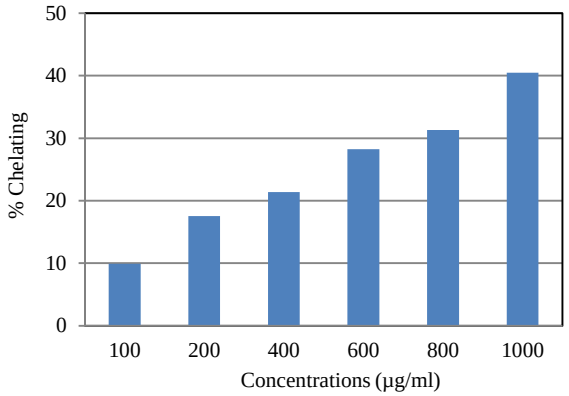

Fig 3. Effect of Singhnad Guggul on ferrous ion chelating activity

The reduction capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir *et al*., 1995). The FRAP assay treats the antioxidants contained in the samples as reductants in a redox-linked colorimetric reaction and the value reflects the reducing power of the antioxidants (Li *et al*., 2006). A higher absorbance corresponds to a higher ferric reducing power. In the present study, the Singhnad Guggul extracts showed increased ferric reducing power with the increased concentration (Fig. 2).

Metal chelating activity is claimed as one of antioxidant mechanisms, since it reduces the concentration of the catalyzing transition metal in lipid peroxidation. Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. Hence, the high ferrous ion chelating ability of the extracts would be somewhat beneficial. As presented in Figure 3, the aqueous extract Singhnad Guggul indicated 40.45%ferrous ion chelating ability. Generally, flavonoids and phenolics are known to act as antioxidants, both as radical scavengers and as metal chelators (Chu *et al*., 2008).

4. Conclusion

Our results suggest Singhnad Guggul possess α-amylase inhibition. However, *in vivo* studies need to be performed to confirm these observations.

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