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IN VITRO EVALUATION OF THE ANTIOXIDANT POTENTIAL OF *STRYCHNOS POTATORUM* L.

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ABSTRACT

The present study was carried out to investigate the *in vitro* antioxidant activities of the leaves, stem and bark of *Strychnos potatorum*. *Strychnos potatorum* L. an important ethnomedicinal plant in the study region. To evaluate an antioxidant potential of different parts of *S. potatorum* plant, three favorable *in vitro* test methods was used, including nitric oxide, catalase and superoxide dismutase. The results indicated that *S. potatorum* exhibits an excellent antioxidant activity.

Keywords: *Strychnos potatorum*, antioxidant activity, nitric oxide, catalase and superoxide dismutase.

INTRODUCTION

Strychnos potatorum have been used extensively as a folklore medicine and in Ayurvedic practice, notably the fruit as an antidiabetic, antidystentric, emetic, while the pulp is useful as an expectorant. The seeds have been used as a demulcent, emetic, stomachic and tonic, and been found to be effective in the treatment of diabetes, diarrhea and gonorrhea; it has also been found to be locally effective for treatment of eye infections [1]. Phytochemical studies revealed the presence of major alkaloid diaboline and its acetate, triterpenes and sterols and mannogalactans [2]. The seeds have a wide spectrum of established pharmacological activity that includes anti-inflammatory [3], antihypercholesterolemic [4], antiulcerogenic [5] activities. In our traditional medicinal systems like Ayurveda, Siddha and Unani, the S. potatorum is used for treating urinary tract and eye infections; gonorrhoea and kidney troubles, leucorrhoea, tuberculosis, diabetes, venereal diseases and acute diarrhea [6-7]. The ripen seeds are used for clearing muddy water [8]. Hepatoprotective and antioxidant activities of *S. potatorum* seeds was experimentally proved in CCl_4 induced acute hepatic injured experimental rats [5] by using an aqueous extract of seed powder of *S. potatorum* plant. However, there is no literature available on *In vitro* antioxidant potential of different plant parts of *S.* potatorum with enzymatic assay like Superoxide Dismutase (SOD), Catalase (CAT) and Nitric Oxide (NO).

MATERIALS AND METHODS

Plant Material and Preparation of Extracts

Fresh plant parts from four plants of *Strychnos potatorum* were collected in chilled container from Kanpa (Chandrapur district) forest in the month of December. The various plant parts, i.e. leaves, stem, and bark were thoroughly washed in cold water to remove the earthy material and 2 grams of each plant part were weighed and dissolved in 20 ml of 0.1 N Phosphate buffer pH 6.8 and homogenized separately [9]. The homogenized extract was then centrifuged at 10,000 rpm for 20 minutes at 4˚C. All samples were recentrifuged at same condition to avoid the carryover of plant materials. The clear supernatant was then used for an enzymatic analysis.

Enzymatic Analysis: An activity of catalase was estimated following the methodology given by Aebi *et al*. [10]. Total 3ml reaction cocktail contains 1.980 ml of 50mM phosphate buffer, 30mM H_2O_2 1ml and 0.020 ml enzymatic sources. Blank was prepared without enzymatic source. The rate of H_2O_2 decomposition was measured at 240nm. Activity of an enzyme was expressed as units/mg protein. Superoxide dismutase was estimated according to Marklund *et al*. [11]. The reaction cocktail contains 1.480ml of 50mM Tris HCL buffer, 20µl enzyme source, 6 mM 0.5ml EDTA and 0.2 mM 1ml pyrogallol. Control was prepared without enzyme source. The absorbance was recorded at 420 nm for 3 min in smart spectrophotometer. Activity was expressed as units/mg protein.The concentration of nitric oxide was estimated by using methodology provided by Green *et al*. [12]. An equal volume of Greiss reagent (0.1% Napthyl Ethylene Diamine Dihydrochloride and 1% Sulpholylamide in 5% Ortho-Phosphoric acid) and nitric oxide reacts to form a pink

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colored complex which was estimated at 535nm. Concentration was calculated using standard graph of 0.1mM sodium nitrate solution. Protein was estimated as per Lowery *et al.*[13] methodology. Reaction mixture contains 0.010 µL protein source, 990 µL distilled water, 5 ml Alkaline copper sulfate solution and 0.5 ml Folin Ciocalteu reagent. Blank was prepared without protein source. The concentration of an unknown protein sample was estimated using 200µGm of the standard BSA solution. All samples were run in triplicates.

Statistical Analysis

All statistical analysis was done using Med Calc and Epi Info statistical software. Students "T" test for two tailed probabilities assuming unequal variance was used for significant differentiation. The criterion for significant difference was 0.05.

RESULTS

Superoxide dismutase (SOD) constitutes an important link in the biological defense mechanism through dismutation of endogenous cytotoxic superoxide radicals to H_2O_2 [14]. Catalase is an enzymatic antioxidant widely distributed in all animal tissues, including RBC and liver. Catalase decomposes hydrogen peroxide and helps protect the tissues from highly reactive hydroxyl radicals [15]. Nitric oxide radical inhibition assay proved that aerial part of the extract is a potent scavenger of nitric oxide. This nitric oxide generated from sodium nitro-prusside reacts with oxygen to form nitrite. The extract inhibits nitrite formation by competing with oxygen to react with nitric oxide directly and also to inhibit its synthesis. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide [16]. The endogenous antioxidant enzymes are responsible for the scavenging of deleterious oxygen radicals and leads cellular detoxification process.

The above results shows that there is a significant increase in an activity of Catalase in bark as compared to the stem and leaf sample ($p<0.001$, table 1, fig 1) and ($P<0.001$, tab 1, fig 1). Catalase is significantly more active in the stem when it was compared to leaf samples $P<0.01$. SOD is higher in bark than the stem and leaf samples $P<0.01$ and $p<0.01$ respectively. But there is no significant difference in stem and leaf samples in an activity of SOD $p>0.05$. No is higher in stem samples than bark and leaf samples $p<0.001$ and $p<0.01$. NO is higher in leaf samples as compared to bark samples $p<0.05$. The concentration of protein was higher in bark and leaf samples than stem sample $p<0.01$ and $p<0.05$. There is no significant difference in the concentration of protein in bark and leaf sample $p>0.05$.

Table 1. A statistical analysis of Catalase, Superoxide Dismutase and Nitric Oxide in *S. potatorum*

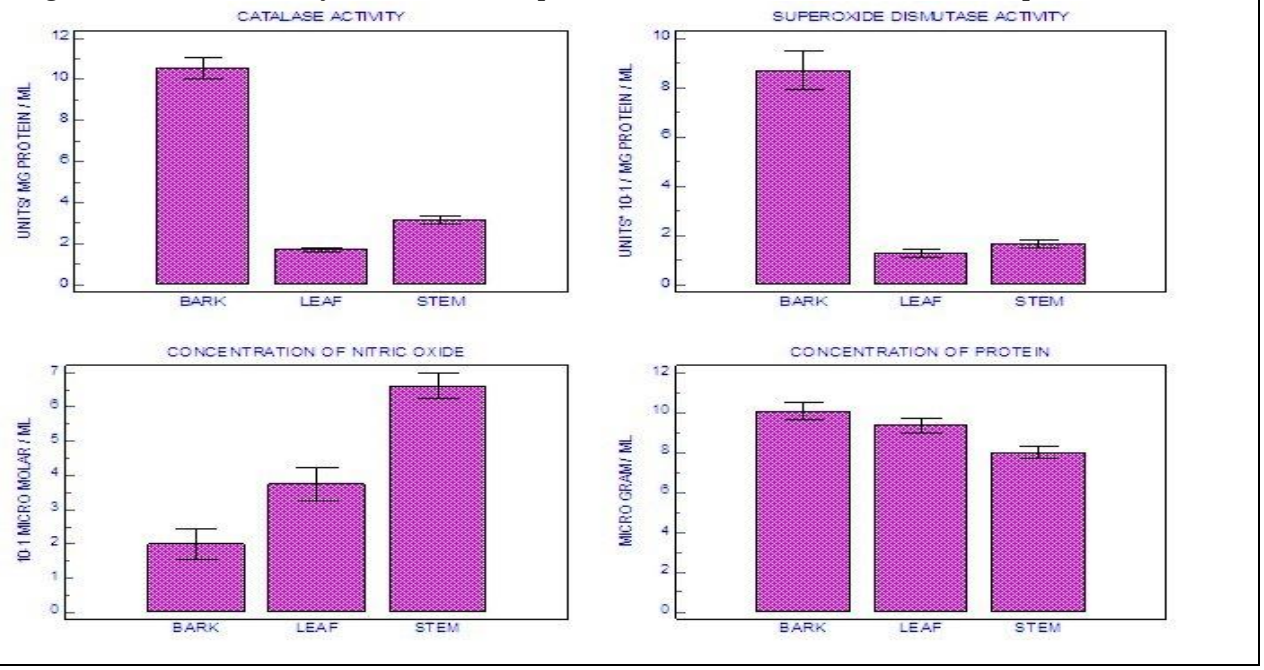
Enzyme	Samples	Samples
Catalase (Units/ mg Protein/ml)	Leaf	1.71±0.06
	Bark	10.54±0.53***
	Stem	3.14±0.20**
Superoxide Dismutase (SOD) (Units/ mg Protein/ml)	Leaf	1.27±0.15
	Bark	8.71±0.77**
	Stem	1.66±0.15*
Nitric Oxide (NO) (µM / ml)	Leaf	3.75±0.47
	Bark	2.00±0.45****
	Stem	6.62±0.37**
Protein (µG/ ml)	Leaf	9.34±0.36
	Bark	10.07±0.41*
	Stem	8.00±0.31****

*p>0.05, **p<0.01, ***p<0.001 and ****p<0.05; p-values of catalase, SOD, NO and protein for the stem and bark samples are p<0.001, p<0.01, p<0.001 and p<0.01 respectively.

Figure 1. The Selected Plant: *Strychnos potatorum* L

Figure 2. Various extracts of *Strychnos potatorum* L.

Figure 3. A statistical analysis of Catalase, Superoxide Dismutase and Nitric Oxide in *S. potatorum*



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