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# Natural Products

*An Indian Journal*

*Full Paper*

NPAIJ, 2(2), 2006 [62-64]

## Antioxidant Activity Of Marketed Herbal Formulation



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*Received: 25<sup>th</sup> March, 2006*

*Accepted: 29<sup>th</sup> April, 2006*

*Web Publication Date : 14<sup>th</sup> June, 2006*



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### ABSTRACT

The antioxidant activity of one herbal formulation (Freezy syrup) was evaluated in two types of free radical scavenging models i.e. (i) DPPH scavenging and (ii) nitric oxide scavenging model. The formulations responded effectively in both of the models tested. The results were also comparable to that of the standard ascorbic acid used as a standard for comparison in this present investigation. Thus, this investigation confirms the use of the Freezy syrup as an antioxidant agent.

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### KEYWORDS

Antioxidant;  
 Freezy syrup;  
 DPPH model;  
 Nitric oxide scavenging  
 model.

### INTRODUCTION

Naturally occurring antioxidants in leafy vegetables and seeds, such as ascorbic acid, vitamin E and phenolic compounds possess the ability to reduce the oxidative damage associated with many diseases, including cancer, cardiovascular diseases, cataracts, atherosclerosis, diabetes, arthritis, immune deficiency diseases and aging<sup>[4-6]</sup>. So, antioxidants are important in the prevention of human diseases. Therefore, the importance of search for natural an-

tioxidants has greatly increased in the recent years. So, many researchers have focused on natural antioxidants and in the plant kingdom numerous crude extracts and pure natural compounds were reported to have antioxidant properties. These crude extracts are then formulated as herbal formulation and available in market. One such formulation was taken to ascertain its antioxidant activity.

## MATERIALS AND METHODS

For the present work a marketed herbal formulation freezy syrup was purchased from local market of Mandsaur (Madhya Pradesh), India for screening of its antioxidant activity. In formulation each 10 ml consist of aqueous extracts of camphor (5 mg), indian zedoary root (50 mg), waterchest nut (50 mg), clove buds (50 mg), dried coriander seeds (100 mg), indian nut grass (100 mg), sandalwood (100 mg), raisins (Black) (100 mg), fennel seeds (100 mg), indian asparagus (100 mg), dried vrukshamla (150 mg), dried cumin seeds (250 mg), indian fumeria (250 mg), vetivera grass (250 mg), indian gooseberry fruit (250 mg), fresh rose petals (600 mg), flavoured syrup base (Q.S.).

Two models DPPH free radical and nitric oxide scavenging were used for the study. For DPPH free radical scavenging activity 0.1 mL sample of various concentrations (5-50 µg/mL) was placed in respective test tubes and 4 mL of ( $6 \times 10^{-5}$ ) mol/L methanolic solution of DPPH· was added. The reaction was allowed to complete in the dark for 20 minutes. The absorbance was taken at 517 nm. Experiment was repeated three times. The decrease in absorbance was measured at 517 nm with the UV-Vis spectrophotometer. Methanol was used to zero the spectrophotometer. The absorbance of the DPPH· radical without antioxidant (i.e. the control) was also measured<sup>[2]</sup>. Nitric oxide scavenging activ-

ity was measured spectrophotometrically<sup>[3]</sup>. Sodium nitroprusside (5 mmol) in phosphate buffered saline was mixed with different concentrations of the formulation (5-50 µg/mL) and incubated at 25°C for 30 min. A control without the test compound was also analyzed. After 30 min, 1.5 mL of the incubation solution was removed and diluted with 1.5 mL of Griess reagent. The absorbance of the chromophore formed during diazotization was measured at 546 nm.

## RESULTS AND DISCUSSION

There was a 94.8% decrease of the DPPH radical at a 50-µg/mL-formulation concentration and it was found to be dose dependent (TABLE 1). DPPH is a stable free radical that can accept an electron or

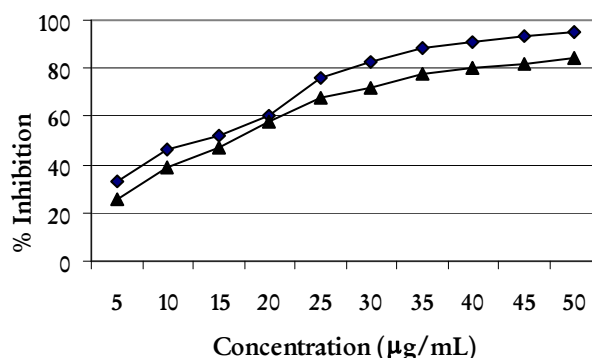


Figure 1: DPPH and nitric oxide scavenging activity of Freezy syrup

TABLE 1: Percentage inhibition of freezy syrup in DPPH and nitric oxide scavenging models

Concentration (µg/mL)	Inhibition (%) <sup>a</sup>		EC <sub>50</sub> (µg/mL)	
	DPPH scavenging	Nitric oxide scavenging	DPPH scavenging	Nitric oxide scavenging
5	33.3 ± 1.1	25.6 ± 1.4		
10	46.2 ± 1.5	39.1 ± 1.6		
15	52.4 ± 1.6	46.8 ± 1.9		
20	60.7 ± 1.1	58.2 ± 2.1		
25	76.2 ± 1.8	67.81 ± 1.8	11.92	16.52
30	82.3 ± 1.7	72.2 ± 1.0		
35	88.2 ± 1.3	77.9 ± 1.2		
40	91.1 ± 1.1	80.2 ± 1.7		
45	93.7 ± 1.2	82.1 ± 1.3		
50	94.8 ± 1.3	84.3 ± 1.2		
Ascorbic acid (100µmol/L)	94.7 ± 3.1	84.6 ± 4.1		

<sup>a</sup> Mean ± SEM of three analysis

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hydrogen radical to become a stable diamagnetic molecule. Due to its odd electron, the methanolic solution of DPPH shows a strong absorption band at 517 nm; reduction of the DPPH radicals can be observed by the decrease in absorbance at 517 nm. DPPH radicals react with suitable reducing agents and then electrons become paired off and the solution loses colour stoichiometrically with the number of electrons taken up<sup>[1]</sup>. Such reactivity has been widely used to test the ability of compounds/plant extracts to act as free radical scavengers.

There was a good inhibition of the nitric oxide formation with the maximum inhibition 84.3% achieved with 50- $\mu\text{g}/\text{mL}$  formulation concentration (TABLE 1). Incubation of solutions of sodium nitroprusside in phosphate buffer saline (PBS) at 25°C for 30 resulted time-dependent nitrite production, which was reduced by the tested formulation. The scavenging of nitric oxide by the Freezy syrup was concentration-dependent. In conclusion, the Freezy syrup is having good antioxidant activity.

## ACKNOWLEDGEMENTS

We are thankful to Director, B.R.Nahata College of Pharmacy, Mandsaur for providing all the required facilities.

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