



FREE RADICAL SCAVENGING ACTIVITY OF AQUEOUS SOLUTION OF BLACK SALT

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ABSTRACT

In vitro free radical scavenging activity of aqueous solution of black salt was evaluated by using DPPH radical scavenging activity and hydrogen peroxide radical scavenging activity. Ascorbic acid was used as standard. For the DPPH method the IC₅₀ value of black salt was 137µg/ml and for ascorbic acid it was 41.70µg/ml. The IC₅₀ value of black salt was 168µg/ml and the standard showed IC₅₀ value of 58.30µg/ml for hydrogen peroxide radical scavenging activity. Results show that the black salt has got antioxidant activity.

Key words: Black salt, Free radical scavenging, Antioxidant

INTRODUCTION

In the human body the free radicals are continuously produced due to the oxygen utilization by the cells of the body. This generates a series of reactive oxygen species (ROS) like super oxide anion (O₂⁻) and hydroxyl (HO·) radicals and non-free radical species such as H₂O₂, singlet oxygen (O₂) and nitric oxide (NO). Natural antioxidants like catalase, superoxide dismutase, and glutathione peroxidase are present in the body [1]. These antioxidants neutralize ROS by scavenging. There is a balance in the amount of ROS and antioxidants produced in the body. Sometimes under unavoidable circumstances the ROS are produced in such high amounts that the antioxidant defense mechanism proves to be inadequate. Large amount of ROS in the body is harmful. ROS are highly reactive and can easily react with almost all the biological molecules including DNA, proteins, lipids and lipoproteins [2].

The destruction of these biomolecules may lead to the formation of various pathophysiological disorders [3].

Various studies showed that the natural antioxidants have very less toxicity and proved to be safer and effective [4]. The natural antioxidants scavenge the free radicals and avoid the excess ROS formation in the body [5].

Black salt or kala namak as it is called is used in ayurvedic medicine as digestive aid, heart burns and to alleviate flatulence. So far the antioxidant properties of black salt have not been tested. Hence, in the present investigation, the aqueous solution of black salt was screened for in vitro antioxidant activity.

METHODS AND MATERIALS

Test drug

The black salt uncrushed lumps were bought from a local market of Thane city, Maharashtra. The salt was powdered in mortar pestle and dissolved in water to make desired concentration of the solution

IN VITRO ANTIOXIDANT METHODS

DPPH (1, 1-diphenyl-2-picryl hydrazyl) radical scavenging activity [6], [7]: 1ml of (20-200µg/ml) test drug/standard (Ascorbic

acid) was added to 2ml of DPPH (Himedia lab) in methanol (0.33%). After keeping for 30 minutes at 37°C the absorbance at 517nm was measured using UV-spectrophotometer. Corresponding blanks were taken. The experiment was performed in triplicate. The absorbance of DPPH as control was measured at 517nm. Lower absorbance of the reaction mixture indicated higher radical scavenging activity. The scavenging effect (%) was measured using the following formula:

$$\text{Scavenging Effect(\%)} = \frac{(\text{Control absorbance} - \text{Test absorbance})}{\text{Control absorbance}} \times 100 \dots\dots\dots(1)$$

DPPH accepts an electron to become a stable diamagnetic molecule. The methanolic solution of DPPH (violet color) has got a strong UV absorbance at 517nm. The presence of a reducing agent in this methanolic solution pairs the odd electrons of DPPH radical and further the solution losses color stoichiometrically and also the absorbance of the solution decreases at 517nm.

Hydrogen peroxide radical scavenging activity [8]: 1ml of (20-200µg/ml) test drug/standard (Ascorbic acid) was added to 0.6ml of hydrogen peroxide solution (Ashwin fine chemicals and pharmaceuticals) in phosphate buffer (PH-7.4). After incubating for 10 minutes at 37°C the absorbance was measured at 230nm. Corresponding blanks were taken. The experiment was performed in triplicate. The absorbance of hydrogen peroxide in phosphate buffer as control was measured at 230nm. The scavenging effect (%) was measured using equation (1).

Hydrogen peroxide produces hydroxyl radicals in cells. Scavenging of these radicals by the test drug is used as a test for antioxidant activity. The reduction of these radicals is seen by the decreased absorbance at 230nm with increasing concentration of the test drug.

RESULTS AND DISCUSSION

DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging activity

IC₅₀ value of the aqueous solution of black salt was found to be 137µg/ml and for ascorbic acid was 41.7µg/ml.

Table 1: DPPH radical scavenging activity of aqueous solution of black salt and standard (Ascorbic acid)

Concentration(µg/ml)	Scavenging effect of aqueous solution of black salt (%)	Scavenging effect of standard-Ascorbic acid (%)
20	11.27 ± 0.0665	34.81 ± 0.2148
40	36.30 ± 0.3700	49.59 ± 0.2148
80	44.41 ± 0.1648	66.60 ± 0.0982
160	51.87 ± 0.0768	78.36 ± 0.1239
200	55.63 ± 0.0768	82.31 ± 0.4073
400	58.68 ± 0.3038	92.46 ± 1.0350

Values are expressed as a mean ± SEM of three observations.

DPPH radical scavenging activity

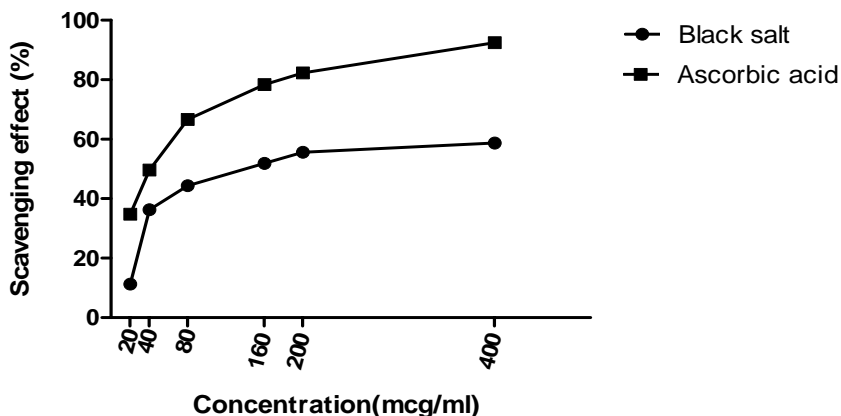


Fig.1: DPPH radical scavenging activity

Table 2: Hydrogen peroxide radical scavenging activity of aqueous solution of black salt and standard (Ascorbic acid)

Concentration($\mu\text{g/ml}$)	Scavenging effect of aqueous solution of black salt (%)	Scavenging effect of standard-Ascorbic acid (%)
20	9.42 \pm 0.0721	33.54 \pm 0.1916
40	15.71 \pm 0.0735	42.67 \pm 0.2201
80	36.67 \pm 0.0145	55.69 \pm 0.5414
160	48.66 \pm 0.1272	64.00 \pm 0.8896
200	54.83 \pm 0.0786	85.61 \pm 0.4994
400	63.22 \pm 0.3897	94.48 \pm 0.4155

Values are expressed as a mean \pm SEM of three observations.

Hydrogen peroxide radical scavenging activity

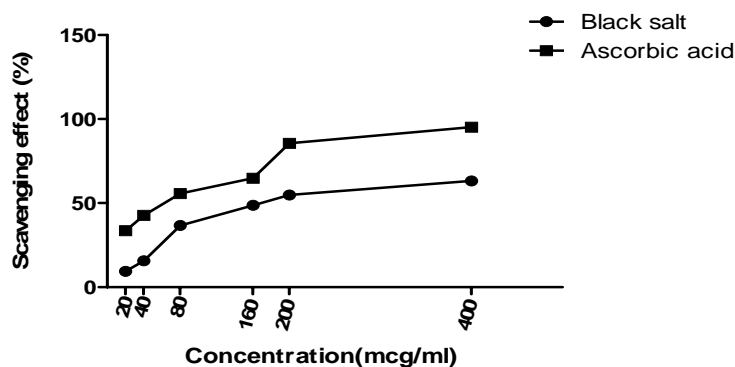


Fig. 2: Hydrogen peroxide radical scavenging activity

Hydrogen peroxide radical scavenging activity

IC₅₀ value of aqueous solution of black salt was found to 168 $\mu\text{g/ml}$ and for ascorbic acid was 58.30 $\mu\text{g/ml}$.

CONCLUSION

Results have revealed that the aqueous solution of black salt has free radical scavenging activity.

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