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Research Article

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IN VITRO ANTIOXIDANT POTENTIAL OF VARIOUS EXTRACTS OF *SOLANUM NIGRUM L*

Manik Sharma[1], Malik Romana[2]*, Jeevan Menaria[5], Sangeeta Devi[4], Muzaffar Ahmed Sheikh[3]

[1] P.G. Department of Zoology, Bhoj Mahavidyalaya, Bhopal (M.P)
[2]* P.G. Department of Zoology, S. S. L. Jain College, Vidisha (M.P)
[3]Govt. NMV, Hoshangabad (M.P.)
[4]Unique College, Bhopal (M.P.)
[5]Geetanjali Institute of Pharmacy, Udaipur-313001, India

Abstract In the present investigation we were aimed to investigate the antioxidant potential of various crude extracts of leaves of *Solanum nigrum*. The extraction was carried out by different solvent such as petroleum ether, ethyl acetate and chloroform. The antioxidant property of these extracts was assessed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method.

Keywords Antioxidant, *Solanum nigrum,* 2, 2-diphenyl-1-picrylhydrazyl (DPPH).

Introduction

Solanum nigrum is a species in the *solanum* genus, native to Eurasia and introduced in the America, Australaia and South Africa. Parts of this plant can be highly toxic to livestock and humans, and it's considered a weed. Nonetheless, ripe berries and cooked leaves of edible strains are used as food; and plant parts are used as a traditional medicine [1]. The plant has a long history of medicinal usage, dating back to ancient Greece. In the fourteenth century, the plant under the name of Petty Morel being used with Horehound and wine taken for dropsy. It was a traditional European medicine used as a strong sudorific, analgesic and sedative with powerful narcotic properties, but was considered as dangerous remedy [2].

Solanum nigrum L is an important ingredient in traditional Indian medicines. Infusions are used in dysentery, stomach complaints and fever. The fruits are used as a tonic, laxative, appetite stimulant; and also for treating asthma and "excessive thirst" [3]. Traditionally the plant was used to treat tuberculosis [4]. The boiled extracts of leaves and berries are also used to alleviate liver-related ailments, including jaundice. Solanum *nigrum* is a widely used plant in oriental medicine where it is considered to be antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, antipyretic [3].

Plant derived natural products such as flavonoids, terpenoids, steroids etc have diverse pharmacological properties including antioxidant activity. Antioxidants are compounds that help to inhibit many oxidation reactions caused by free radicals such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals etc, thereby preventing or

delaying the damage to the cells and tissues. As antioxidants play an important role in inhibiting and scavenging radicals, thereby providing protection to humans against infection and degenerative diseases [5]. However, these synthetic antioxidants have side effects such as liver damage and carcinogenesis [6]. Therefore, there is need for isolation and characterization of natural antioxidants having less or no side effects, for medicinal materials to replace synthetic antioxidant. Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains and other metabolites, which are rich in antioxidant activity [7, 8].

Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine [9]. Approximately 20% of the plants found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi-synthetic resources [10].

Material and Methods

Plant material

The plant of *Solanum nigrum* was collected from the local surroundings at Bhopal (M.P) and was acknowledged by a Senior Botanist Dr. Jagrati Tripathi Head of the Department of Biotechnology, Unique College Bhopal (M.P).

Preparation of extracts

The powder of the plant (2 kg) was successively Soxhlet extracted using Petroleum Ether, Ethyl acetate and Chloroform for 70 hr. The plant was collected and washed thoroughly under running tap water and then was rinsed in distilled water; they were allowed to dry for some time. Then these leaves were shade dried without any contamination for about 3 to 4 weeks. The dried powdered (coarse) was subjected to Soxhlet using petroleum ether, ethyl acetate and chloroform respectively. Almost all the chlorophyll and lipid is deposited on the side of the flask and was removed carefully. The extraction was done with each solvent (3500ml) until the supernatant in the Soxhlet became transparent (approximately for 36 hours). Every time before taking the solvents of higher polarity, to remove the traces of previous solvents, exhausted marc was completely dried. All the extracts were filtered, dried and weighed. The extracts obtained were evaporated in rotary evaporator to get a powdery mass. The extracts were dried under reduced pressure using rotator evaporator to get the crude. It was stored below 4 ^{o}C until further used.

Antioxidant Activity

DPPH Radical-Scavenging Activity

The DPPH (2, 2-diphenyl-1-picryl hydrazyl radical) analysis is one of best-known, accurate, and frequently employed methods for evaluating antioxidant activity. It is a stable free radical because of its spare electron delocalization over the whole molecule. A solution of 200 µM in methanol was prepared as a control and 0.5 ml of methanol solution was added to 2.95 ml of all extract solutions prepared in different concentrations (50 to 750 µg/ml). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Percentage inhibition was calculated and the IC_{50} values were estimated by using a non-linear regression algorithm [11- 12].

$$\% \, inhibition = \frac{\text{Absorbance of control} - Absorbance\ of\ Sample}{\text{Absorbance of control}} \text{ X } 100$$

Results

Table 1: Comparison of % Inhibition data of DPPH free radical Scavenging assay by petroleum ether, ethyl acetate and methanol extract

S. No.	Concentration (µg/ml)	% Inhibition		
		Pet ether	**Ethyl acetate**	**Chloroform**
1	50	18.67±0.42	27.85±0.34	18.67±0.45
2	100	25.04±0.21	40.89±0.22	41.48±0.34
3	250	29.63±0.33	55.41±0.12	56.00±0.44
4	500	35.41±0.22	58.22±0.14	60.44±0.21
5	750	49.33±0.45	62.22±0.26	68.74±0.37
IC_{50} Value		**801.58 µg/ml**	**355.5 µg/ml**	**346.15 µg/ml**

The order of DPPH scavenging of standard ascorbic acid and the three extracts was found to be in the order of Chloroform> Ethyl Acetate> Petroleum Ether. These results showed that greater rate of DPPH scavenging activity of chloroform extract may be due to presence of high phenolic or flavonoid compounds. IC_{50} value was determined from the plotted graph of scavenging activity. The Higher percentage inhibition or the lowest IC_{50} indicates the strongest ability of the extracts to act as DPPH radical scavengers. Out of all the extracts, chloroform extract showed the lowest $IC_{50,}$ 346.1µg/ml as compared to ethyl acetate and petroleum ether extracts having IC_{50} values 355.5 µg/ml and 801.5µg/ml respectively (Table 1). These results showed that the lowest IC_{50} value of chloroform extract may be due to high content of phenolic or flavonoid contents and will exhibit more potent antioxidant activity as compared to ethyl acetate and petroleum ether extracts having IC_{50} values.

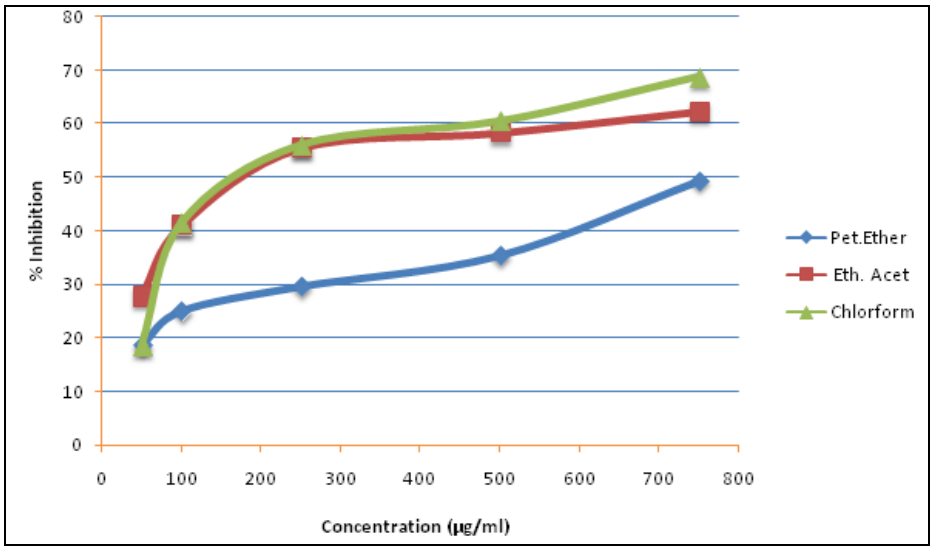

Figure 1: % Inhibition data of DPPH free radical Scavenging assay by extracts

Discussion and Conclusion

The capacity of petroleum ether, ethyl acetate and chloroform extracts to scavenge was measured and the results are shown in Table 1. The antioxidants react with DPPH, a purple colored stable free radical, and convert it into a colorless α-α-diphenyl-α-picryl hydrazine. The amount of reduced DPPH could be quantified by measuring the

decrease in absorbance at 517 nm [13]**.** The results of antioxidant activity indicates that the plant is potently active and the plant leaf extracts contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity.

On the basis of our results it is concluded that *Solanum nigrum* bear potent antioxidant activity. The antioxidants act as defense mechanism that protects against oxidative damage, and include compounds to remove or repair damaged molecules and sufficient intake of antioxidants is supposed to protect against diseases. The phytochemical antioxidants have potent potential to neutralize free radicals or oxidants responsible for the cell damage. From the above antioxidant parameters assayed, *Solanum nigrum* extracts were found to be better antioxidants in DPPH radical scavenging activity. The present study thus scientifically validates and strengthens the candidature of *Solanum nigrum* in the preparation of medicinal aids to combat the wide spectrum of myriad diseases arising due to oxidative stress.

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