K. Thamizhvanan. / Vol 2 / Issue 1 / 2012 / 1-3.

International Journal of

Innovative Drug Discovery

e ISSN 2249 - 7609
Print ISSN 2249 - 7617

www.ijidd.com

EVALUATION OF ANTIOXIDANT ACTIVITY OF WHOLE PLANT OF *IPOMOEA ERIOCARPA* EXTRACT

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ABSTRACT

Antioxidants are vital substances, which possess the ability to protect the body from damages caused by free radical-induced oxidative stress. Therefore the present investigation was carried out to investigate the antioxidant activity of Petroleum Ether Extract of *Ipomoea eriocarpa* whole plant (PEIE). The plant extract was tested for DPPH (2, 2-diphenyl, 2-picryl hydrazyl) radical scavenging, and reducing power assays. The data obtained in the in vitro models clearly establish the antioxidant potency of the Petroleum Ether Extract of *Ipomoea eriocarpa* whole plant.

Keywords: *Ipomoea eriocarpa*, Radical Scavenging, Antioxidant Activity, DPPH, Reducing Power Assay.

INTRODUCTION

One of the documented health promoting activities of many fruits and vegetables is their ability to scavenge naturally produced free radicals and hence acting as antioxidants [1]. Free radicals are normally generated in substantial amounts as a by-product of various internal metabolic processes in aerobic organisms such as phagocytosis, neutrophiles defense, autooxidation of catecholamine and carboxylation or hydroxylation reactions. All of these processes happen in various ways at different times and sites [2]. There is now overwhelming evidence to indicate that free radicals and oxygen species causing oxidative damage to lipid, protein and nucleic acid. Therefore, they have been implicated in the pathogenesis of many human sufferings like cardiovascular and pulmonary diseases, some types of cancer, cataracts, immune/autoimmune diseases, inflammation, arthritis, atherosclerosis and brain dysfuncthion (Parkinson's, Alzheimer's, Huntington's diseases). All are the degenerative condition of old age. The role of ROS as a causative factor in liver cirrhosis, ulcer and reproductive disorders have also been studied extensively.

Humans have evolved highly complex antioxidant systems (enzymic and nonenzymic), which work synergistically, and in combination with each other to protect the cells and organ systems of the body against free radical damage. The antioxidants can be endogenous or obtained exogenously eg, as a part of a diet or as dietary supplements. Some dietary compounds that do not neutralize free radicals, but enhance endogenous activity may also be classified as antioxidants [3]. Many synthetic antioxidant compounds have shown toxic and/or mutagenic effects, which have stimulated the interest of many investigators to search natural antioxidant.

Ipomoea eriocarpa R.Br. (Family: Convolvulaceae) often called annual morningglories, are summer annual or perennial broadleaf plants. Ipomoea eriocarpa R.Br. are often cultivated as ornamentals, however, under favorable conditions they can become troublesome weeds. They are also a major agricultural weed problem in the San Joaquin Valley of California,

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where several species of *Ipomoea* are found. Control is critical from crop emergence to harvest. Destroy seedlings while they are small, because once they have twined up stems they are difficult to control without injuring the crop. Seeds remain viable in soil for long periods. Seeds of Ipomoea species contain many types of alkaloids, including some that are neurotoxins to humans and animals when consumed. Fortunately, there is typically not enough seed in contaminated grain to cause harm to livestock. Most seedlings emerge following irrigation, but they may also appear when surface soil is too dry to allow germination of other annuals. Cotyledons (seed leaves) are butterfly shaped and more deeply notched and much larger than those of field bindweed. First true leaves are heart shaped with deep lobes at the base. Mature plants have long stems that climb and twine. Leaves are large, heart shaped and/or three lobed, and are alternate to one another along the stem. Both leaf types can occur on the same plant. The funnel-shaped flower varies in color depending on the species, from violet or blue to pink and red. Fruit are pods that release seeds through slits. Seeds germinate down to a depth of 4 inches (10 cm) or more, much deeper than most annuals. The whole plant of *Ipomoea eriocarpa* is used for ulcer, fever and rheumatism [4]. From the source of literature documentation and relevant traditional approaches on plant drugs, the present investigation was carried out to investigate the antioxidant activity of the Petroleum Ether Extract of *Ipomoea eriocarpa* whole plant (PEIE) is being reported here.

MATERIALS AND METHODS

Plant material

The whole plant of *Ipomoea eriocarpa* was collected from Tirumala hills, Tirupati, Andhra Pradesh. India. It was identified and authenticated by Prof. *Madhava Chetty*, *K.*, Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India. A voucher specimen has been kept in our laboratory for future reference.

Preparation of plant extract

The collected whole plant was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 100g of powdered materials were extracted with petroleum ether (60°-80°C) using soxhlet apparatus. The extraction was carried out until the extractive becomes colourless. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in tween 80 and used for the experiment. The percentage yield of prepared extract was around 9.5%w/w.

ANTIOXIDANT ACTIVITY

DPPH method

This is the most widely reported method for screening of anti oxidant activity of many plant drugs. DPPH assay method is based on the reduction of methanol solution of coloured free radical DPPH by free radical scavenger. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 516 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. The activity is expressed as effective concentration EC_{50}. The super oxide radical can also be detected by oxidation of hydroxylamine, yielding nitrite which is measured colorimetric reaction.

Reducing power method

This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the anti oxidant activity. In this method anti oxidant compound forms a coloured complex with potassium ferric cyanide, tricloro acetic acid and ferric chloride, which is measured at 700 nm. Increase in the absorbance of the reaction mixture indicates the reducing the power of the samples.

The following methods were used to evaluate antioxidant activity:

DPPH Radical Scavenging Test

The free radical scavenging activity of the petroleum ether extract of *Ipomoea eriocarpa* (PEIE) was determined by using 2, 2 Diphenyl-l-picryl hydrazyl radical (DPPH) using UV-Spectrometry [5] at 517 nm. The DPPH solution was prepared in 95% methanol. The PEIE was mixed with 95% methanol to prepare the stock solution (10mg/100ml or 100µg/ml). From the stock solution 2ml, 4ml, 6ml, 8ml and 10ml of this solution were taken in five test tubes and by serial solution with same solvent were made the final volume of each test tube up to 10 ml whose concentration was then 20 µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml respectively. Freshly prepared DPPH solution (0.004% w/v) was added in each of their test tubes. Containing PEIE (20 µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml) and after 10 min, the absorbance was taken at 517nm, using a spectrophotometer (Shimadzu UV-1700, UV-visible spectrophotometer). Ascorbic acid was used as a reference standard. It is dissolved in distilled water to make stock solution with the same concentration of PEIE control sample was prepared without extract and reference ascorbic acid. 95% methanol was used as blank % scavenging of the DPPH free radical was measured using following equation.

$$\% \text{ DPPH radical Scavenging} = \frac{\text{Absorbance control} - \text{Absorbance of test sample})}{(\text{Asorbance of control})} \times 100$$

Reducing Power Method

The assay of reducing power method [6,7] is one to determine the antioxidant activity. In this 1 ml of plant extract of PEIE solution mixed with 2.5 ml phosphate buffer (0.2M, pH 6.6) and 2.5 ml Potassium Ferricyanide

[K_3Fe (CN6)] (10g/l), the mixture was incubated at 50^0 C for 20 minutes. 2.5ml of Tri chloroacetic acid (100g/l) was added to mixture. This was centrifuged at 3000 rpm for 10 min. Finally 2.5 ml of the supernantant solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (lg/L) and absorbance measured at 700nm in UV- visible spectrophotometer (SHIMADZU UV-1700, UV-visible spectrophotomerer). Ascorbic acid was used as standard and phosphate buffer used as blank.

RESULTS

DPPH radical scavenging activity of petroleum ether extract of *Ipomoea eriocarpa* (PEIE) added to methanol solution of DPPH and radical scavenging activity was measured as 517 nm as compared to standard ascorbic acid. Values are the average of triplicate experiments. Reducing power of petroleum ether extract of *Ipomoea eriocarpa* (PEIE) of as compared to Ascorbic acid. Values are the average of triplicate experiments.

Table 1. Antioxidant activity of petroleum ether extract of *Ipomoea eriocarpa* by DPPH method

S. No	Concentration (µg/ml)	Absorbance of ascorbic acid	Absorbance of PEIE	% scavenging DPPH of Ascorbic acid	% scavenging DPPH of PEIE
1	20µg/ml	0.144	0.130	35.42	41.33
2	40 µg/ml	0.106	0.090	53.57	59.45
3	60 µg/ml	0.088	0.066	60.12	69.65
4	80 µg/ml	0.060	0.040	76.37	82.13
5	100 µg/ml	0.033	0.011	85.84	94.87

Table 2. Antioxidant activity of petroleum ether extract of *Ipomoea eriocarpa* by reducing power method

S. No	Concentration (µg/ml)	Absorbance of ascorbic acid	Absorbance of PEIE
1	0.1	0.17	0.12
2	0.2	0.23	0.22
3	0.3	0.32	0.29
4	0.4	0.40	0.38
5	0.5	0.49	0.42

DISCUSSION AND CONCLUSION

The presence study establishes the antioxidant activity of PEIE by two models i.e by comparing the % of scavenging DPPH (DPPH method) and determining the absorbance (Reducing power method) of PEIE with the standard (Ascorbic acid) at different concentrations of drug. The extract elicited a dose dependent antioxidant activity when compared to standard. Hence invitro tests are conducted to determine the antioxidant activity. The tests conducted are DPPH method and reducing power method. Here the standard substance taken for the comparision is Ascorbic acid which is renowed antioxidant. The results show that the plant extract of *Ipomoea eriocarpa* possess antioxidant activity when compared with standard shows the same. Further development of research may leads to the development of new antioxidant.

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