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Influence of Environmental Factors on Antioxidant Activity of *Linum Usitatissimum*

Sudhanshu[1], Nidhi Rao[1] Sandhya Mittal[1] and Ekta Menghani*[2]
[1]Suresh Gyan Vihar University, Jaipur and [2]Mahatma Gandhi Institute of Applied Sciences, JECRC University, Jaipur-22. India
Email: ektamenghani@yahoo.com

ABSTRACT

Plants are important to the balance of nature. They are the key to life on earth as they directly supply 90% of human calorie intake, and 80% of the protein intake, the remainder been derived from animal products, although these animals have also derived their nutrition from plants. In the search for new natural remedies, similar to those found in the human body, that are useful ininfectious disease, certain plants were investigated for their content in compounds having free radical scavenging properties, as well as for their antioxidant activity: flax seed (Linum usitatissimum).The antioxidant activities of methanol extract of Linum usitatissimum with the different geographical and climatic conditions was measured. The flavonoid contents of extracts were also evaluated. The antioxidant activities were evaluated by 1,1-diphenyl-2- picrylhydrazyl (DPPH) radicals, nitric oxide scavenging and reducing power ability. Antioxidant activity has been compared with BHA, quercetin and vitamin C.

Keywords: *Antioxidant, DPPH, Reducing power, Scavenging activity*

INTRODUCTION

Environment has been a source of medical agents for thousands of years and the use of medicinal plants, especially in conventional medicine, is currently well recognized and established [1]. Environment has been a foundation of remedial agents ever since times immemorial. The consequence of herb in the organization of human ailment cannot be greater than emphasized. It is apparent with the intention of the plant kingdom harbour an infinite resource of vigorous ingredients very useful in the management of countless obstinate diseases. Moreover, the active components of herbal remedies have the advantage of being combined by way of many other substances that become visible to be inactive. Conversely, these complementary components give the plant as an entire safety in addition to effectiveness greatly superior to that of its isolated as well as pure active components [2].

At present days, medicinal plants receive attention to research centers because of their special importance in safety of communities. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites [3-4]. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Various herbs and spices have been reported to exhibit antioxidant activity. In recent years, there has been a growing interest in the so-called functional foods because they can provide physiological benefits additional to nutritional and energetic, as, for instance, antihypertensive, antioxidant or anti-inflammatory [5]. Between the different compounds with functional properties, antioxidants are the most widely studied [6-8]. Oxygen is essential for the survival of all on this earth. Though oxygen is important for life, overload oxygen can have injurious effects. When oxygen is metabolized by the body it creates substances called free radicals and this cause damage to our cells. Free radicals can also be produced by revelation to pollution, fatty foods and cigarette smoke. The development of conditions such as heart and liver disease, some cancers, arthritis, accelerated ageing and eyesight deterioration are thought to be related to the extreme amounts of free radicals. The body has its own natural defenses aligned with free radicals, but these systems from time to time be overwhelmed. Antioxidants are naturally occurring nutrients in food

which helps in destroying these free radicals and minimize damage to our cells [9]. Some examples of antioxidants are beta-carotene, lycopene, vitamins C, E, and A, and other substances

Therefore, in present research work attempts will be made to screen certain Indian Medicinal Plant viz. *Linum usitatissimum* for phytochemical evaluations and their antioxidant potentials. The free radical scavenging activity of these plants probably contribute to the effectiveness of the above plants in various infectious disease. The plants will be screened for the presence of phyto-chemicals viz. alkaloids, flavonoids, tannins, saponins, glycosides etc and their effect on 2,2-Diphenyl-1-picryl-hydraxyl radical (DPPH) will be used to determine their free radical scavenging activity.

MATERIALS AND METHODS

Collection:

Authentic samples: Various market samples of *Linum usitatissimum* were procured from Chunnilal Attar Ayurvedic Store, Ghat Gate, Jaipur in the month of March, 2010.

Identification:

All the samples were authenticated and were given identification number. The identification was as follows:

These samples were authenticated and submitted in Ethnomedicinal Herbarium, Centre of Excellence funded by DST, MGIAS, Jaipur (Rajasthan).

Processing of plant materials:

During the course of the study each sample was screened for its foreign matter and milled, before use.

Experimental details:

Present studies were performed on *Linum usitatissimum* for the following studies-.

1. Phytochemical test of plant extract
2. Antioxidant Potentials of Methanolic extract of plant

1. PHYTOCHEMICAL SCREENING

Phytochemical screening was performed using standard procedure:

TEST FOR REDUCING SUGARS (FEHLINGS TEST)

The aqueous ethanol extract (0.5gm in 5 ml of water) was added to boiling fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

TEST FOR TERPENOIDES (SALKOWSKI TEST)

To 0.5 gm each of the extract was added to 2ml of chloroform. Concentrated sulphuric acid (3ml) was carefully added to form a layer. Reddish brown coloration of the interface indicates the presence of terpenoides.

TEST FOR FLAVONOIDES

4ml of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated Hydrochloride acid was added and red colour was observed for flavonoids and orange color for flavons.

TEST FOR TANNINS

About 0.5 g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

TEST FOR SAPONINS

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously. And observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

TEST FOR ALKALOIDS

Alkaloids solutions produce white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's regent.

The alcoholic extract was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of mayer's reagent. The sample was then observed for the turbidity or yellow precipitation.

2. ANTIOXIDANT ACTIVITY

Preparation of test extracts

All the test plant sample and their adulterants were milled and refluxed in ethanol for 36 h, filtered, concentrated to dryness *in vacuo*. A portion of ethanolic extract was further successively extracted in pet. ether, benzene, chloroform, alcohol and water, concentrated and stored at minimum temperature, until used.

Preparation of DPPH

DPPH (2, 2'-diphenyl-1-picrylhydrazl, $C_{18}H_{12}N_5O_6$; Hi media) 0.8 mg was dissolved in 10 ml methanol to obtain a concentration of 0.08 mg/ml for antioxidative (qualitative and quantitative) assay.

Qualitative assay

Each successive extract (10 mg) was dissolved in 10 ml of its suitable solvent to get a concentration of 1 mg/ml and from this, 0.25µl was taken with the help of micropipette, applied on silica gel G coated plates. These circular spots were sprayed with DPPH solution, allowed to stand for 30 min. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced, and the changes in colour (from deep- violet to light- yellow on white) were recorded at 517 nm on a UV spectrophotometer (Varian Cary PCB 150, Water Peltier System).

Quantitative assay

A concentration of 1 mg/ml of ethanolic extract of each test sample was prepared to obtain different concentrations (10^2µg to 10^{-3} µg/ ml). Each diluted solution (2.5 ml each) was mixed with DPPH (2.5ml). The samples were kept in the dark for 15 min at room temperature and then the decrease in absorption was measured. Absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured. The UV absorbance was recorded at 517 nm. The experiment was done in triplicate and the average absorption was noted for each concentration. Data were processed using EXCEL and concentration that cause 50% reduction in absorbance (RC_{50}) was calculated. The same procedure was also followed for the standards- quercetin and ascorbic acid.

RESULTS AND DISCUSSION

Table 1: Showing Optical density of *Linum usitatissimum* on different concentrations.

CONCENTRATION (µg/ml)	O.D (nm)
0.001	0.989
0.01	0.967
0.1	0.825
1	0.934
10	0.832
100	0.588
1000	0.408

In current presentation, attempts have been made to come across for the methanolic extract which has the potentials as equivalent to antioxidant agents as the methanolic extracts of *Linum usitatissimum* shows the antioxidant activity which is as analogous to ascorbic acid. All the way through the present investigation it was showed that the maximum optical density comes out to be 0.989 nm which is at the concentration 10^{-3} µg/ml and the smallest optical density is 0.408 nm which is at the concentration 10^3 µg/ml where as the other shows comparable O.D at different concentrations i.e. 0.967 nm at 10^{-2}µg/ml, 0.825 nm at 10^{-1} µg/ml, 0.934 nm at 1µg/ml, 0.832 nm at 10^1 µg/ml, 0.588 nm at 10^2 µg/ml.

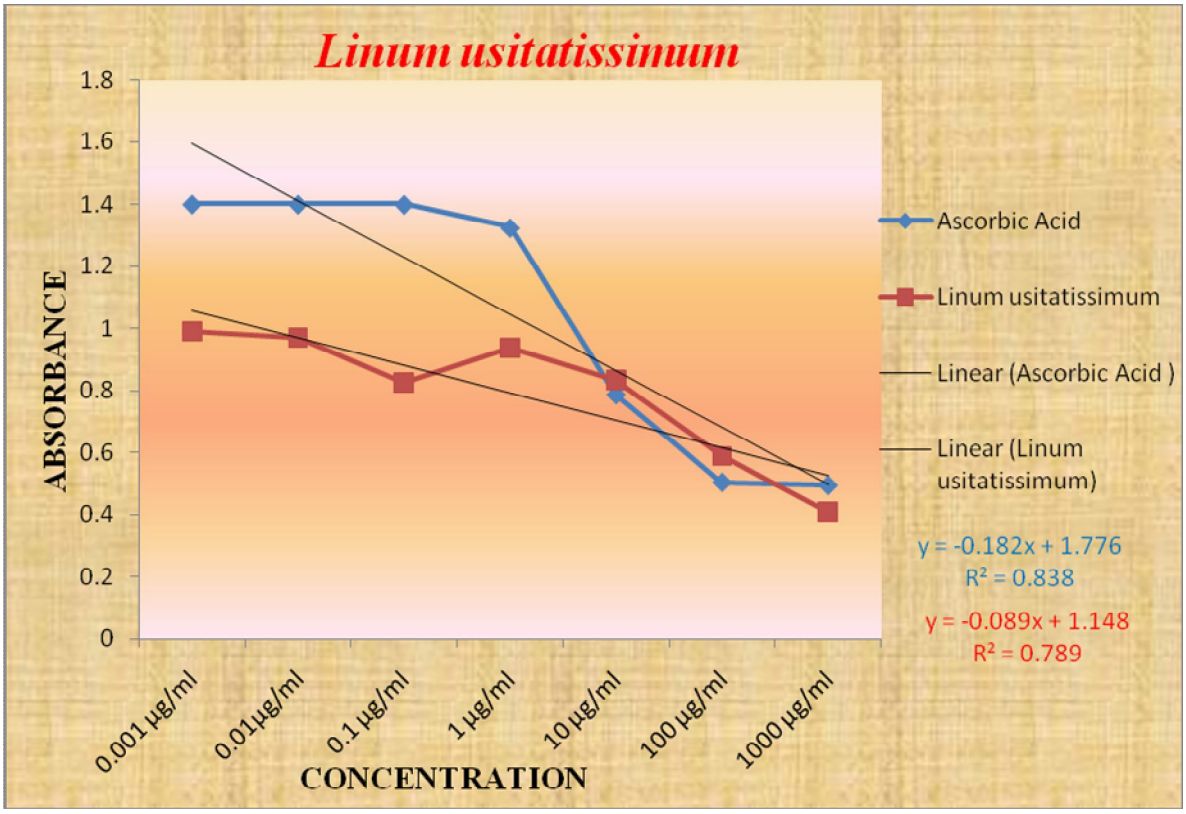

Fig 1: Graph showing Antioxidant Activity of *Linum usitatissimum* at different concentration.

In the current investigations antioxidant activity of *Linum usitatissimum* shows considerable activity associated with the DPPH assay method wherever the regression line perceptible shows the efficacy of it as it has the potentials which are equivalent to ascorbic acid. The antioxidant activity of *Linum usitatissimum* methanolic extract using DPPH assay method shows generous activity which is as similar to standard ascorbic acid. The straight line showed Y= -0.182x+1.776 & regression = 0.838 whereas, in above drug the straight line is Y= -0.089x+1.148 & regression = 0.789.

Table 2: Showing phytochemical screening results of *Linum usitatissimum*.

Linum usitatissimum						
TEST	**Reducing Sugar**	**Saponin**	**Tannin**	**Terpenoides**	**Flavonoides**	**Alkaloides**
	-ve	**-ve**	**-ve**	**+ve**	**-ve**	**-ve**

The phytochemical screening of *Linum usitatissimum* shows the occurrence of Terpenoids whereas it shows the absence of flavonoids, saponin, tannin, alkaloids and reducing sugar respectively. The screening of the *Linum usitatissimum* make only a a small amount of differences in the constituent of the hard-edged plants. The drug shows the substantiation of strong antioxidant activity complementary or in a less important amount. The existence of alkaloids in this plant is credible to be meticulous for the free radical scavenging effects pragmatic.

CONCLUSION

For their credible antioxidant activity, the extract of *Linum usitatissimum* was observed to screening. The consequent test systems, specifically free radical scavenging along with reducing power, was used for the chemical analysis. It was used for observing the radical scavenging effects of extracts. There are significant differences between the constituent of the tested plants. Where *Linum usitatissimum* possess a large amount of terpenoids. The occurrence of these compounds in huge quantity is rationally proportional to the antioxidant activity so it is evidently show that occurrence

of terpenoids will prove the antioxidant activity and promote a drug for treatment ofinfectious diseases caused by environment. The DPPH test provides in sequence on the reactivity of the test compounds with stable free radical and it gives a strong absorption band at 517nm in visible region. Consequently, this type of studies suggests that these plants acquire antioxidant activities which can counteract the damage induced by infectious diseases.

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