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IN VITRO EVALUATION OF ANTI OXIDANT ACTIVITY OF *LINUM USITATISSIMUM* LEAF EXTRACT

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Abstract:

Antioxidants are compounds that can inhibit or prevent the oxidation of the easily oxidized substrate. One of the plants as a potential source of bioactive compounds and antioxidant activity (Linum Usitatissimum). This plant was commonly found in the Madhya Pradesh, India, Russia, Canada and China has been used by the society. This study aimed to determine the proximate compositions, bioactive compounds and antioxidant activity from large-leafed mangrove fruit which extracted by methanol. The phytochemical screening was carried on the both extracts of leaves of Linum Usitatissimum, revealed the presence of some active ingredients such as Alkaloid, Glycoside, Steroids Gums, Flavonoids, Saponins, Reducing sugar, Tannins. The aqueous and alcoholic leaves extract were also evaluated for their antioxidant activity using FRAP assay, Metal chelating assay, DPPH radical scavenging assay, superoxide-radical scavenging assay and Hydrogen peroxide scavenging assay. The result of the present study showed that the ethanolic leaves extract of Linum Usitatissimum has shown the greatest anti-oxidant activity than aqueous extracts. The high scavenging property of may be due to hydroxyl groups existing in the phenolic compounds. Further work is needful to isolate the exact compound which is responsible for antioxidant activity and biophysical characterization can be done in the future.

Key Words: *Linum Usitatissimum and Antioxidants activity.*

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INTRODUCTION:

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. They may protect cells from damage caused by unstable molecules known as free radicals. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. Free radicals are fundamentals to any biochemical process and represent an essential part of aerobic life and metabolism. Majority of the diseases are mainly linked to oxidative stress due to free radicals [1,2]. Our body is rich in endogenous antioxidants, the substances that have the ability to stop free radicals formation or to limit the damage they cause. The effectiveness of current used exogenous antioxidants arises most probably from the increase of the endogenous free radical scavengers as enzymes (superoxide dismutase and selenium-dependent glutathione peroxidase), vitamins (alpha tocopherol and ascorbic acid). Many plants have been also found to posses free radical scavenging activity (Polyphenols, alkaloids and terpenoids). Low levels of one or more of the essential antioxidants have been shown to be associated with many disorders including cancer, inflammation, atherosclerosis, coronary heart disease and diabetes. Thus, in such cases, the administration of exogenous antioxidants seems to be salutary. Nowadays, a great deal of effort being expended to find effective antioxidants for the treatment or prevention of free radical-mediated deleterious effects.

Oxidative stress is characterized as an imbalance between the production of reactive species and antioxidant defense activity, and its enhanced state has been associated with many of the chronic diseases such as cancer, diabetes, neurodegenerative and cardiovascular diseases[5]. Based on that, many research groups have driven efforts to assess the antioxidant properties of natural products. These properties have been investigated through either chemical (in vitro) or biological (in vivo) methods, or both [6]. The results of these researches have led some to suggest that the long-term consumption of food rich in antioxidants can retard or avoid the ocurrence of such diseases. According to Brewer, the effectiveness of a large number of antioxidant agents is generally proportional to the number of hydroxyl (OH) groups present in their aromatic ring(s). Based on that, the natural compounds would seem to have better antioxidant activity than the currently used synthetic antioxidants, making them a particularly attractive ingredient for commercial foods. Despite the large number of natural products that are currently consumed as antioxidant agents, the search for new chemical entities with antioxidant activity still remains a burgeoning field. In this context, the lichens have played an important role as a source for new antioxidant agents. Lichens are symbiotic organisms consisting of a fungus and one or more photosynthetic partners, the latter usually being either a green alga or a cyanobacterium. They are found in a wide variety of natural habitats or in places with low temperatures, prolonged darkness, drought and continuous light. Lichens produce characteristic and unique secondary metabolites, and most of them occur exclusively in these symbiotic organisms. The most common lichen compounds are aromatic polyketides, particularly depsides, depsidones, depsones, dibenzofurans, and chromones. Lichens have been used in the folk medicine for numerous purposes, among them as astringents, laxatives, anticonvulsives, antiemetics, antiasthmatics, anti-inflammatories, antibiotics, and also for the treatment of cardiovascular, respiratory, and gastric disorders. Furthermore, pharmacological and biotechnological studies have been carried out in order to test and to develop biomaterials containing lichen-isolated natural compounds for humans use.

Medicinal plants with antioxidant potential

1. Rhizophora mangle is a plant from Rhizophoraceae family. The bark extract of the plant showed scavenging activity of hydroxyl radicals and the extract contained polyphenols, carbohydrates and sterols.
2. Diospyros malabarica is a plant from Ebenaceae family. The bark is used for the treatment of fever and fruit juices for healing of wound ulcer5 . The stem extract of the plant competes with oxygen to react with nitric oxide and thus, inhibits the generation of anions. The main phytoconstituents in the extract are phenolic compounds.
3. Asparagus racemosus is a tree from Liliaceae family. It shows antioxidant activity through the free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, nitric oxide scavenging, metal chelation, reduction power and inhibition of lipid peroxidation in rats. The main phytoconstituents are saponins, alkaloids and flavonoids.
4. Auricularia auricular is a tree and known as _tree ear or wood ear‘ from Auriculaceae family: It has shown a potent hydroxyl radical scavenging and lipid per-oxidation inhibitory activities. The main phytoconstituents are flavonoids .
5. Eucalyptus globules is a tree and known as ―Karpura maram‖ from Myrtaceae family. The antioxidant activity of Eucalyptus oil was estimated by two in vitro assays namely diphenyl picryl hydrazyl

radical scavenging activity and inhibition of ascorbate induced lipid peroxidation method.

6. Acacia arabica is a plant from Mimosae family. The antioxidant assays were carried out in vivo and in vitro experimental models. In vitro, lipid peroxidation was carried out by tertiary butyl hydroperoxide (TBH) induced lipid peroxidation. In vivo, experiments were carried out in CCl4-induced hepatotoxicity in rats. The bark of the plant contained quercetin, (+) catechin, (-) epicatechin and gallic acid. The polyphenol rich active fraction of Acacia arabica is a potent free radical scavenger and protects TBH induced lipid peroxidation and CCl4-induced hepatic damage. The bark is used in the treatment of asthma, bronchitis, diabetes, dysentery and skin diseases.

7. Ligustrum vulgare is a plant from Oleaceae family. The leaves antioxidant activity was evaluated using DPPH test. The main phytoconstituents are flavonoids, iridoids, coumarins and essential oil, where flavonoid aglycones are responsible for the antioxidant activity and it shows a potent free radical scavenging activity.

8. Terminalia chebula is a tree and known as Myrobalanus chebula. Combretaceae family. The main phytoconstituents are tannins, chebulinic and gallic acids. The extract was tested by studying the inhibition of radiation induced lipid peroxidation in rat liver microsomes. It shows free radical scavenging activity due to presence of tannins and also It inhibits the development of duodenal ulcer and so the extract has appeared to show a cytoprotective effect on the gastric mucosa.

9. Lobelia nicotianaefolia is a plant from Campanulaceae family. The chemical constituents are alkaloids as lobeline and also it contains volatile oil, resin, gum and fixed oil. It is mainly used in the treatment of asthma and as respiratory stimulant.

10. Citrus lemon is a tree from Rutaceae family. The antioxidant activity was estimated by two in vitro assays, DPPH radical scavenging activity and inhibition of ascorbate induced lipid peroxidation (LPO) method. The main phytoconstituents are citral and limonene. The antioxidant property is shown due to the presence of citral.

MATERIALS AND METHODS:

Reagents

Sodium hydroxide (Analytical grade, FisherChemicals Inc., Fair Lawn, NJ), citric acid (analytical grade), hexanes (HPLC grade, EMD Chemicals Inc., Gibbstown, NJ), methanol (HPLC grade, EMD Chemicals Inc., Gibbstown, NJ), ethyl acetate (HPLC grade, EMD Chemicals Inc., Gibbstown, NJ), BCL3-methanol (Supelco Inc., Belletonte, PA), 98% 2, 2-Dimethoxypropane (Sigma-Aldrich Inc., St. Louis, MO), Anhydrous sodium sulfate (10-60 mesh, Fisher Chemicals Inc., Fair Lawn, NJ), cholesterol (Aldrich Chem. Co., Milw., WI), 5α- cholestane (Sigma-Aldrich Co., St. Louis, MO), heptadecanoic acid (Sigma chemical Co., St.Louis, MO), DHA (cis-4, 7, 10, 13, 16, 19-Docosahexaenoic acid, Sigma-Aldrich Inc., St. Louis, MO)

The solvents were stored at room temperature (20-25ºC) and other reagents were stored at -20ºC freezer. Sodium Hydroxide and citric acid were dissolved in distilled water. All of organic reagents were dissolved in hexanes, except for being particularly noted. Whatman filter papers (Whatman®, 150mm Dia × 100Circles, Cat No 1001 150, Whatman International Ltd, Maidstone, England).

Plant Material Collection

The leaves of *Linum Usitatissimum* were collected and was identified and authenticated from Department. The plant material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

Preparation of plant extracts

Preparation of Aqueous Extract:

Fresh leaves of *Linum Usitatissimum* were collected and washed under tap water. The leaves extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of water. The contents were mixed well and then the mixture was boiled up to 80-100^{0}C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

Preparation of Alcoholic Extract:

Fresh leaves of *Linum Usitatissimum* were collected and washed under tap water. The leaves extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of alcohol. The contents were mixed well and then the mixture was boiled up to 50-60^{0}C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

PHYTOCHEMICAL EVALUTION

The powdered drug was extracted and subjected to qualitative chemical tests.

- **Detection of Carbohydrates**

Small quantities of powdered drug and different extracts were dissolved in distilled water separately and filtered. The filtrates were taken for Molisch's Test, Fehling's Test, Benedict's Test, Barfoed's Test, Test for starch tests to detect the presence of carbohydrates.

- **Test for Gums and Mucilages**

The powdered drug and extracts were treated with absolute alcohol stirred and filtered. The filtrate was dried and examined for its swelling properties.

- **Test for Proteins and Amino Acids**

Small quantities of powdered drug and different extracts were dissolved in few ml of distilled water and subjected to Ninhydrin, Biuret, Million, Xanthoproteic test, test with tannic acid and heavy metals.

- **Test for Fixed Oils and Fats**

The powdered drug and extracts were subjected for Spot Test, Saponification Test.

- **Test for Alkaloids**

Small amount of powdered drug and solvent free various extracts were separately stirred with a few ml of dilute hydrochloric acid and filtered. The filtrates were tested with various alkaloidal reagents such as Mayer's, Dragendroff's, Wagner's and Hager's reagent and Tannic acid.

- **Test for Glycosides**

A small amount of powdered drug and different extracts were dissolved separately in 5ml of distilled water and filtered. Another portion of the extracts were hydrolyzed with hydrochloric acid for one hour on a water bath and hydrolysate was subjected to Legal's, Baljet's, Borntrager's, KellerKilliani's tests and for the presence of Cyanogenetic glycosides.

- **Test for Phytosterols**

The powdered drug and extracts were refluxed with 0.5N alcoholic potassium hydroxide until the saponification was complete. The saponification mixture was diluted with distilled water and extracted with petroleum ether. The ethereal extract was evaporated and unsaponification matter

IN VITRO METHODS OF ANTI-OXIDANT ACTIVITY

Antioxidant activity should not be concluded based on a single antioxidant test model. And in practice several *in vitro* test procedures are carried out for evaluating antioxidant activities with the samples of interest. Another aspect is that antioxidant test models vary in different respects. Therefore, it is difficult to compare fully one method to other one. Researcher has to critically verify methods of analysis before adopting that one for his/her research purpose. Generally *in vitro* antioxidant tests using free radical traps are relatively straightforward to perform. Among free radical scavenging methods, DPPH method is furthermore rapid, simple (i.e. not involved with many steps and reagents) and inexpensive in comparison to other test models. On the other hand ABTS decolorization assay is applicable for both hydrophilic and lipophilic antioxidants. In this work five *in vitro* methods are described and it is important to note that one may optimize logically the respective method to serve his/her experimental objective as no one method is absolute in nature rather than an example.

FERRIC REDUCING-ANTIOXIDANT POWER (FRAP) ASSAY

The FRAP assay was done according to the method described by Benzie and Strain (1999) with some modification. This method is based on reduction of TPTZ-Fe3+ complex to TPTZ-Fe2+ form in the presence of antioxidants. The stock solutions included acetate buffer (300 mM, pH 3.6), 2, 4, 6-tripyridyl s-triazine (TPTZ) solution (10 mM in 40 mM HCl) and ferric chloride ($FeCl_3.6H_2O$) solution (20 mM). The fresh working FRAP solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml $FeCl_3$ solution. Extracts were made up to 2.0 ml with distilled water and 1.0 ml of FRAP solution was added. An intense blue color developed was measured at 593 nm, after an incubation period of 20 min. The absorbance was related to absorbance changes of a ferrous sulphate solution (0 – 100 NM) tested in parallel. All results were based on three separate experiments and antioxidant capacity was expressed as NM FeSO4/ g of dry extract. Quercetin and Butylated Hydroxy Toluene (BHT) were used as positive control.

Metal chelating activity

The chelating capacity of *Linum Usitatissimum* extracts on Fe2+ ions was determined according to the method of Dinis et al (1994), wherein Fe2+ chelating potential of extracts was monitored by measuring ferrous iron – ferrozine complex at 562 nm. Briefly, extracts (0.05 – 1.0 mg/ml), quercetin, BHT and EDTA (10 – 250 Ng/ml) were made up to 4.7 ml with distilled water and then mixture was allowed to react with 0.1 ml of Ferrous chloride (2.0 mM) and 0.2 ml of ferrozine (5.0 mM) for 20 min. Absorbance of mixture was measured at 562 nm against a blank, which contained distilled water, instead of extracts/EDTA/standard antioxidants. The ability of extracts to chelate ferrous ion was calculated using the following equation:

Chelating effect (%) = [Ab control 562 – Ab sample 562/ Ab control 562] x 100.
Experiments were done in triplicate.

DPPH radical-scavenging activity

DPPH radical-scavenging activity of *Linum Usitatissimum* extracts was determined as previously described (Burits and Bucar, 2000). The capacity of extracts to scavenge lipid soluble 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, which results in bleaching of purple color exhibited by stable DPPH radical, is monitored at an absorbance of 517 nm. Briefly, 1.0 ml of extracts (0.05 – 1.0 mg/ml) and quercetin/BHT (10 – 250 Ng/ml) in ethanol were added to 4 ml of 0.004% methanolic solution of DPPH. After incubation for 30 min at room temperature in the dark, absorbance was read against a blank at 517 nm. Tests were carried out in triplicate. The ability of extracts and quercetin/BHT to scavenge DPPH radical was
calculated using the following equation:

Radical scavenging activity (%) = [A0 – A1/ A0] x 100.

Where A0 was absorbance of negative control (containing all reagents except test compounds) at 517 nm and A1 was absorbance of the extracts or quercetin/BHT at 517 nm. DPPH scavenging activity of extracts and standard was expressed as IC50, which was interpolated from a graph constructed, using percent inhibition (Y-axis) against concentration (X-axis) of extracts and standards.

Superoxide radical-scavenging activity

The ability of *Linum Usitatissimum* extracts, quercetin and BHT to quench generation of superoxide radicals was determined according to the method of Nishikimi et al (1972) with a slight change. Superoxide radicals were generated in PMS-NADH system by oxidation of NADH and analyzed by NBT reduction. 0.1 ml of extracts (0.05 – 1.0 mg/ml) and quercetin/BHT (10 – 250 Ng/ml) were mixed with 2.9 ml of phosphate buffer (40 mM, pH 7.4), 1.0 ml of nitroblue tetrazolium (NBT) solution (150 NM in 40 mM phosphate buffer, pH 7.4) and 1.0 ml of NADH (468 NM in 40 mM phosphate buffer, pH 7.4). The reaction was initiated by addition of 1.0 ml of phenazine methosulphate (60 NM in 40 mM phosphate buffer, pH 7.4) to reaction mixture. After incubation at 25°C for 5 min, absorbance was measured at 560 nm. Negative control was subjected to the same procedures as extracts, except that only solvent was added for negative control. All measurements were made in triplicate.The ability of extracts and quercetin/BHT to scavenge superoxide radical was calculated using the following equation:

Superoxide radical scavenging activity (%) = [A0 – A1/ A0] x 100.

Where A0 was absorbance of negative control at 560 nm and A1 was absorbance of the extracts or quercetin/BHT at 560 nm. IC50 value, which represents concentration of extracts and standards that caused 50% inhibition, was determined by a linear regression analysis.

Hydrogen peroxide scavenging activity

The method of Sinha (1972) originally designed for the estimation of antioxidant enzyme, catalase, was adopted to evaluate hydrogen peroxide scavenging effect of *Linum Usitatissimum* extracts. Extracts (0.05 – 1.0 mg/ml) and quercetin/BHT (10 – 250 Ng/ml) were incubated with 0.6 ml of H2O2 (40 mM in a phosphate buffer, 0.1 M pH 7.4) in the dark for 10 min. A negative control was set up in parallel with entire reagent except extract or standard. After incubation, remaining H2O2 was allowed to react with 1.0 ml of dichromate in acetic acid (5% potassium dichromate and glacial acetic acid in the ratio of 1:3) in a boiling water bath for 10 min. Absorbance of green color developed was determined at 620 nm. All experiments were performed in triplicate. The percentage scavenging of H2O2 by *Linum Usitatissimum* extracts and standards were calculated using the following equation:

H_2O_2 scavenging activity (%) = [A0 – A1/ A0] x 100.

Where A0 was absorbance of negative control and A1 was absorbance of the extracts or standards. H_2O_2 scavenging activity of extracts and standards was expressed as IC50, which was interpolated from a graph constructed using percent inhibition (Y-axis) against concentration (X-axis) of extracts and standards.

RESULTS:

Phytochemical screening of *Linum Usitatissimum.*

The present investigation concluded that the isolated compounds from the plant *Linum Usitatissimum* are pure and the plant *Linum Usitatissimum* shows the various antibacterial effects against different bacteria and found that different phytochemical compounds. Further study is needed for the isolation of the constituents present in the plant and its individual pharmacological activity should need to consider and ultimately it should be implemented for the benefit to human beings.

Table: Phytochemical screening of *Linum Usitatissimum*

S.No.	Phytoconstituents	Aqueous	Alcoholic
1.	Alkaloid	+	-
2.	Glycoside	-	+
3.	Steroids	-	-
4.	Gums	+	-
5.	Flavonoid	+	+
6.	Saponins	-	-
7.	Reducing sugar	+	+
8.	Tannins	+	+

Ferric reducing ability of *Linum Usitatissimum*

Table : Ferric Reducing Ability - FRAP (expressed as mM FeSO4/g dry weight) of leaves of *Linum Usitatissimum*

Group	Drugs	IC_{50} value µg/ml
I	Quercetin	13.59±0.098
II	Butylated Hydroxy Toluene (BHT)	3.10 ±0.025
III	AQLU	1.88±0.037
IV	ALLU	2.34±0.065

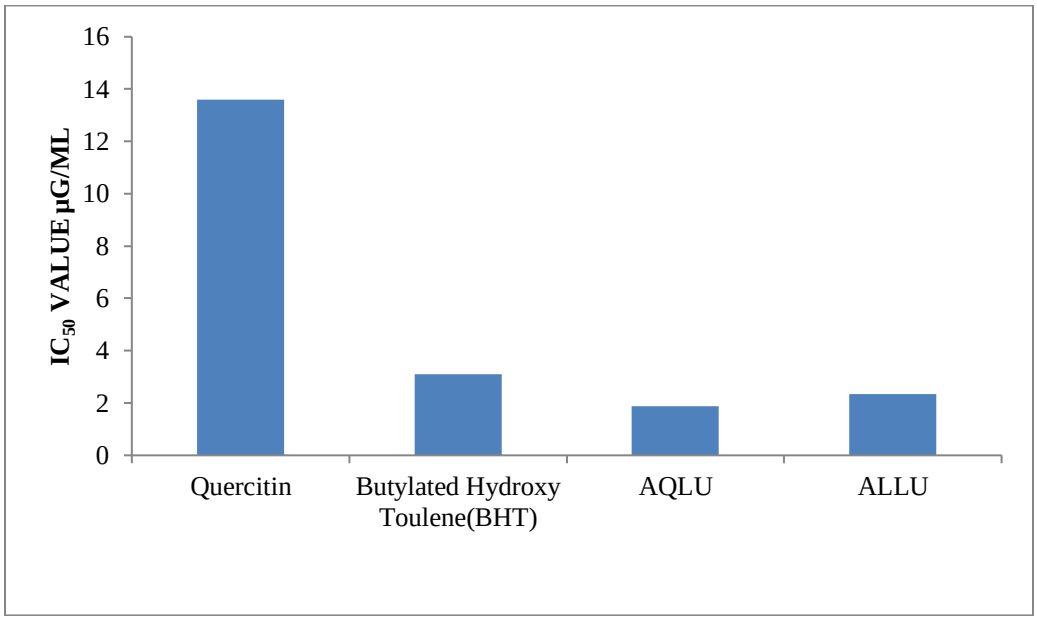

Fig: Reducing power of *Linum Usitatissimum* Quercetin and BHT were used as reference antioxidant Values are means ± SD (n = 3).

Metal chelating activity of *Linum Usitatissimum*

Table 3: Metal chelating activity of leaves of *Linum Usitatissimum*

Group	Drugs	IC_{50} value µg/ml
I	EDTA	6.98
II	Quercetin	143
III	Butylated Hydroxy Toluene(BHT)	89
IV	AQLU	25.18
V	ALLU	31.61

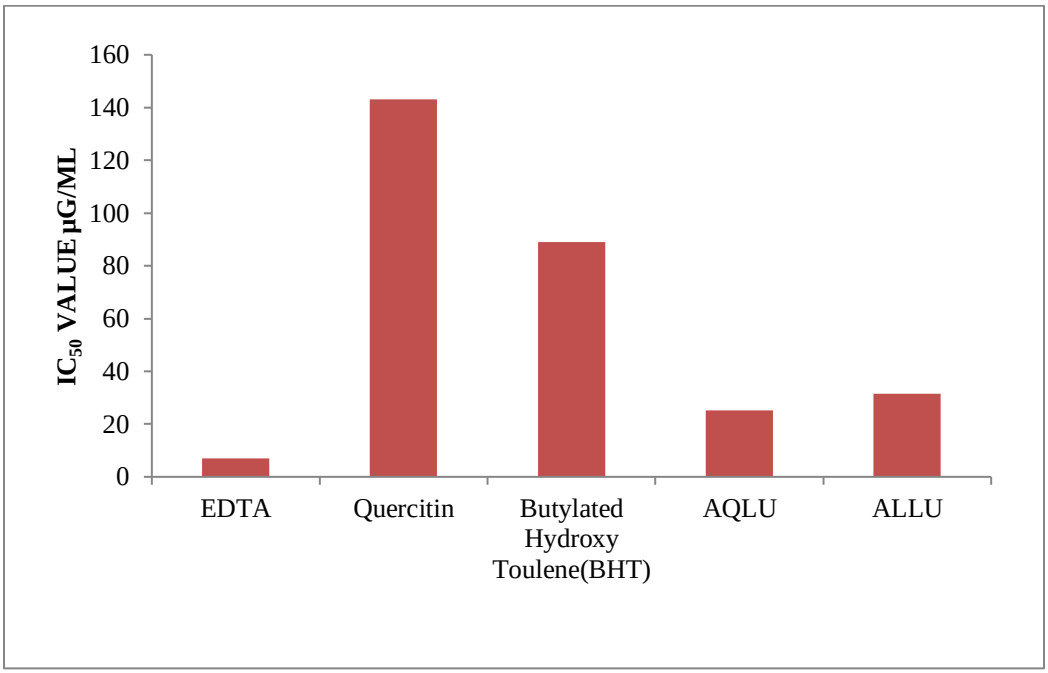

Fig: Metal chelating activity of Linum Usitatissimum EDTA was used as positive control. Quercetin and BHT were used as reference antioxidants. Values are means ± SD (n = 3).

DPPH radical scavenging activity of *Linum Usitatissimum*

Table: Scavenging ability of root, stem and leaves of *Linum Usitatissimum* and standard antioxidants on DPPH as determined by their IC50, expressed as mg/ml.

Group	Drugs	IC_{50} value µg/ml
I	Quercetin	4.2 ±0.024
II	Butylated Hydroxy Toluene (BHT)	2.16 ±0.075
III	AQLU	1.22 ±0.052
IV	ALLU	2.2 ±0.000

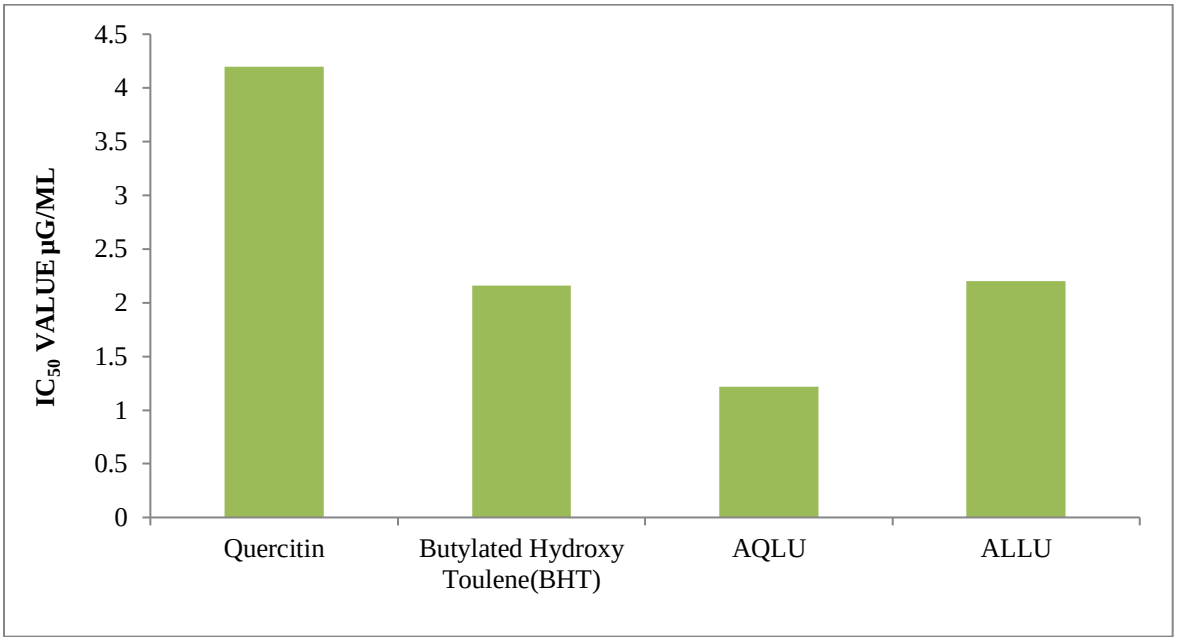

Fig: DPPH radical scavenging activity of *Linum Usitatissimum* Quercetin and BHT were used as reference antioxidant. Values are means ± SD (n = 3).

Superoxide radical scavenging activity of *Linum Usitatissimum*

Superoxide anion is a reduced form of molecular oxygen that is generated during normal metabolic processes. It is known to be destructive to cellular components as a precursor of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical or singlet oxygen (Stief, 2003), contributing to tissue damages and various chronic diseases (Halliwall, 1991). The scavenging activity of *Linum Usitatissimum* extracts on superoxide radicals is shown in Figure 4.9. Extracts from different parts of *Linum Usitatissimum* displayed concentration dependent protective activity against superoxide radicals of which, leaves were the most effective. Alcoholic extracts of leaves (IC50 at 23 Jg/ml) showed potent scavenging activity. Aqueous extracts exhibited moderate activity with IC50 in the range of 131 – 841 Jg/ml. When radical scavenging activity of *Linum Usitatissimum* extracts compared to IC50 values calculated for reference antioxidants BHT (IC50 at 19 Jg/ml), but less effective than quercetin (IC50 at 10 Jg/ml).

Table: Scavenging ability of root, stem and leaves of *Linum Usitatissimum* and standard antioxidants on superoxide radical (O_2•) as determined by their IC50, expressed as mg/ml.

Group	Drugs	IC_{50} value µg/ml
I	Quercetin	0.013 ±0.001
II	Butylated Hydroxy Toluene (BHT)	0.010 ±0.009
III	AQLU	0.34 ±0.008
IV	ALLU	0.312 ±0.001

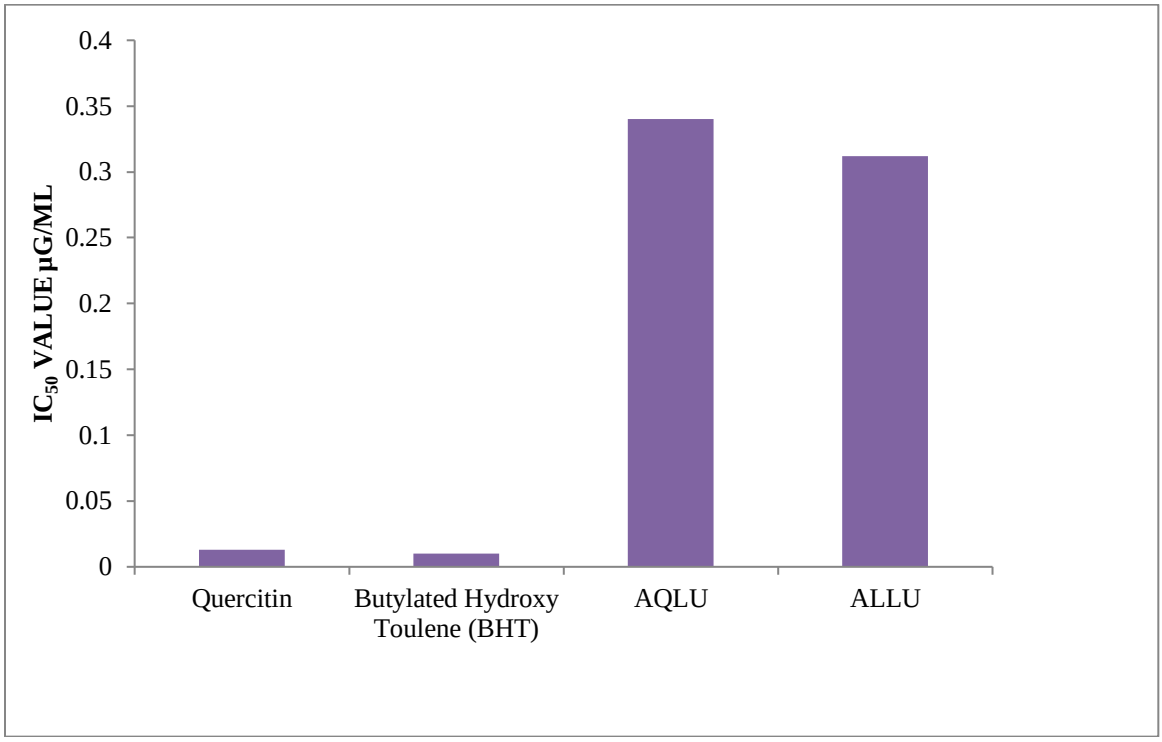

Fig: Superoxide radical scavenging activity of *Linum Usitatissimum* Quercetin and BHT were used as reference antioxidant. Values are means ± SD (n = 3).

Hydrogen peroxide scavenging activity of *Linum Usitatissimum*

Though hydrogen peroxide (H_2O_2) itself is not ver8y reactive, it can occasionally be toxic to cells, since it may give rise to potentially reactive hydroxyl radicals (Halliwell, 1991). The scavenging activity of *Linum Usitatissimum* extracts on H_2O_2 is shown in Figure and compared with Quercetin and BHT as standard antioxidants. *Linum Usitatissimum* extracts were capable of scavenging H_2O_2 in a concentration-dependent manner of different extracts, alcoholic group showed strongest H_2O_2 scavenging activity. The aqueous extract of leaves displayed the most potent activity with IC50 at 67 Jg/ml, which was comparable to Quercetin (IC50 at 34 Jg/ml) and more effective than BHT (IC50 at 89 Jg/ml).

Table: Scavenging ability of leaves of *Linum Usitatissimum* and standard antioxidants on hydrogen peroxide (H2O2) as determined by their IC50, expressed as mg/ml

Group	Drugs	IC_{50} value µg/ml
I	Quercetin	0.040±0.003
II	Butylated Hydroxy Toluene (BHT)	0.063±0.009
III	AQLU	0.072±0.015
IV	ALLU	0.597±0.042

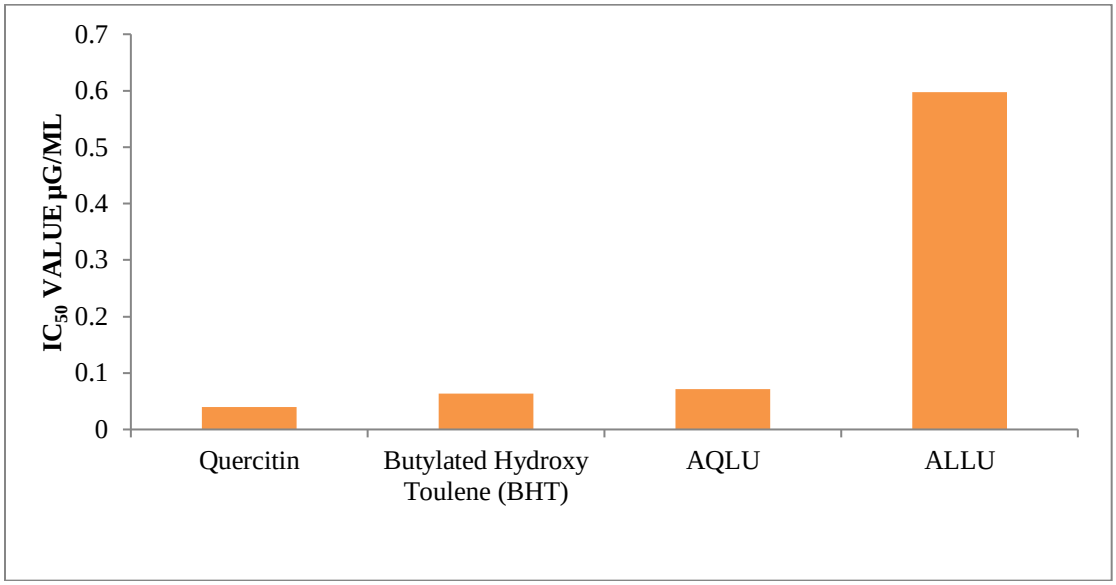

Fig: Hydrogen peroxide scavenging activity of *Linum Usitatissimum* Quercetin and BHT were used as reference antioxidant. Values are means ± SD (n = 3).

SUMMARY:

Phytochemistry has been making a rapid progress and plant products have become increasingly popular in various traditional, complementary and alternative systems as they are pharmacologically potent and have low or no side effects. Food derived products cannot be perceived as "medicine" and are highly interesting for development as preventive and protective agents that may find widespread, long-term use in populations at normal/high risk.

Linum Usitatissimum is a unique plant containing a rich and rare combination of phytochemicals. It is unparalleled in curing multitude of disorders and has aroused great interest for its potential role in helping in maintaining human health. The results obtained in this study led to the conclusion that,

- Leaves of *Linum Usitatissimum* possess substantial biological activities.
- Leaves have high level of polyphenolic and show significant antioxidant activity. *Linum Usitatissimum* could be regarded as a promising source of natural antioxidants and has a potential to be developed as an ingredient in health and functional foods.
- *Linum Usitatissimum* (Alcoholic extracts of leaves) shows negligible cytotoxicity and genotoxicity to normal lymphocytes and exhibits potent protective effect against cell death and DNA damage in cells induced by H_2O_2 under *ex vivo* conditions.
- *Linum Usitatissimum* (Alcoholic extract of leaves) significantly used for astringent.

Our findings suggest the use of *Linum Usitatissimum* extracts in functional foods and food supplements designed for the prevention of various chronic diseases, including The Analgesic and Anti-Inflammatory Activity. However, further studies are needed to prove that the protective effects observed *in vitro* do indeed translate *in vivo*.

CONCLUSION:

The result of the present study showed that the aqueous and alcoholic extract of *Linum Usitatissimum plant*, which contains phenolic and flavonoidal compounds, exhibited the great antioxidant activity. The high scavenging property of methanolic extract of *Linum*

Usitatissimum plant may be due to hydroxyl groups existing in the phenolic compounds' chemical structure that can provide the necessary component as a radical scavenger. Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. A potent scavenger of free radicals may serve as a possible preventative intervention for the diseases. Aqueous and alcoholic extracts of *Linum Usitatissimum plant* in this research exhibited antioxidant. The antioxidant potential may be attributed to the presence of polyphenolic compounds.

In this study, all antioxidant methods (FRAP assay, Metal Chelating assay, DPPH radical-scavenging assay, Superoxide radical scavenging assay and Hydrogen peroxide scavenging assay) showed that the both aqueous and alcoholic extracts of *Linum Usitatissimum* contain more antioxidant activities. More- over, this study demonstrated the important source of phenol compounds, which are a good source of antioxi- dant activity. The phenol component has a high inhibitory effect that prevents lipid peroxidation. However, the solvent type has an important role in detecting phenol compounds and antioxidant factors. Thus, we concluded that *Linum Usitatissimum act* via its free radical scavenging to prevent lipidperoxidation. Therefore, natural antioxidants and phenol compounds in *Linum Usitatissimum have* the capability to be used medically and in food systems to preserve food quality.

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