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IN-VITRO EVALUATIONOF ANTI OXIDANT ACTIVITY OF *EMBLICA OFFICINALIS FRUIT* EXTRACT

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Abstract:
The present study was carried out to evaluate the in vitro antioxidant activity and preliminary phytochemical analysis of alcoholic and aqueous leaf extracts of Emblica Officinalis Fruit. The in vitro antioxidant activity was evaluated by using FRAP assay, Metal chelating assay, DPPH radical scavenging assay, superoxide-radical scavenging assay and Hydrogen peroxide scavenging. Significant antioxidant activity of Ethanolic leaf extract of Emblica Officinalis Fruit Might be due to the presence of Glycosides, Anthraquinone, Phytosterols, Saponins, Tannins & Phenolic compounds, Flavonoids, Steroids found in the preliminary phytochemical analysis.
Our findings suggest the use of Emblica Officinalis Fruit in designed for prevention of various chronic diseases including respiratory disorders, hyperdipsia, epilepsy, fever, sexual debility, paralysis, stomach ulcers, rheumatism, skin diseases, hemorrhagic diseases, and jaundice. However, further studies are needed to prove that the protective effects observed in vitro do indeed translate in vivo.
Key words: *Emblica Officinalis Fruit, Antioxidant activity*

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1. INTRODUCTION:

Antioxidants are compounds capable to either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. They are used for the stabilization of polymeric products, of petrochemicals, foodstuffs, cosmetics and pharmaceuticals.
Antioxidants are involved in the defense mechanism of the organism against the pathologies associated to the attack of free radicals.

Endogenous antioxidants are enzymes, like superoxide dismutase, catalase, glutathione peroxidase or nonenzymatic compounds, such as uric acid, bilirubin, albumin, metallothioneins. When endogenous factors cannot ensure a rigurous control and a complete protection of the organism against the reactive oxygen species, the need for exogenous antioxidants arises, as nutritional supplements or pharmaceutical products, which contain as active principle an antioxidant compound. Amongst the most important exogenous antioxidants, vitamin E, vitamin C, β-carotene, vitamin E, flavonoids, mineral Se are well known, but also vitamin D and vitamin K3.

Exogenous antioxidants can derive from natural sources (vitamins, flavonoids, anthocyanins, some mineral compounds), but can also be synthetic compounds, like butylhydroxyanisole, butylhydroxytoluene, gallates, etc [1].

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, as well as the deterioration of fats and other constituents of foodstuffs [2,3].

Antioxidants or inhibitors of oxidation are compounds which retard or prevent the oxidation and in general prolong the life of the oxidizable matter [4]. The oxidants / free radicals are species with very short half life, high reactivity and damaging activity towards macromolecules like proteins, DNA and lipids. These species may be either Oxygen derived (ROS) or Nitrogen derived (RNS). The most common reactive oxygen species include superoxide anion (O2), hydrogen peroxide (H2O2), peroxyl radicals (ROO) and reactive hydroxyl radicals (OH). The nitrogen derived free radicals are nitric oxide (NO), peroxynitrite anion (ONOO), Nitrogen dioxide (NO2) and Dinitrogen trioxide (N2O3) [5,6] Free radicals are constantly generated resulting in extensive damage to tissues and biomolecules leading to various disease conditions. So the medicinal plants with antioxidant property are employed as an alternative source of medicine to mitigate the diseases associated with oxidative stress.[7,8]

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electron from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants are the substances that inhibit oxidation and are capable of counteracting the damaging effects of oxidation in body tissue. They prevent damage caused by free radicals. Free radicals are very unstable molecules with an unpaired electron and are important intermediates in natural processes involving control of vascular tone, cytotoxicity and neurotransmission. Free radicals cause many human diseases like cancer, Alzheimer's disease, cardiac reperfusion abnormalities, kidney disease and fibrosis etc. Antioxidants play many vital functions in a cell and have many beneficial effects when present in foods [9,10]. Medicinal plant parts are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins.
These compounds have multiple biological effects including antioxidant activity.

Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. Although initial studies suggested that antioxidant supplements might promote health. 8 On the basis of solubility anti-oxidants are of two types.
(a). Hydrophilic antioxidants:- They are soluble in water. Water soluble antioxidants react with oxidants in the cell cytoplasm and blood plasma.
(b). Hydrophobic antioxidants:- They are soluble in lipids. Lipid soluble antioxidants protect cell membranes from lipid peroxidation.
Plants with medicinal and antioxidant property :
Ocimum sanctum Linn. (Tulsi, Sacred Basil)
Family - Lamiaceae
Chemical constituents:-Eugenol (an essential oil) and ursolic acid. volatoil oil, Estragole, thymol. Medicinal and antioxidant property:- Ocimum sanctum has anti-stress, antioxidant, hepatoprotective,immunomodulating,antiinflammatory, antibacterial, antiviral, antifungal, antipyretic, antidiuretic, antidiabetic, antimalarial and hypolipidemic properties with a wide margin of safety. In Ayurvedic medicine, Tulsi is being used either alone or in combination with others in various clinical conditions like anxiety, chronic cough, bronchitis, fever, snake and scorpion bites. The aqueous extract decreased LPO formation (thiobarbituric acid reactive substances TBARS) and increased antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidise (GPX), glutathione transferases (GT). It also increased antioxidant like reduced glutathione (GSH) levels in plasma and liver, lung, kidney and

brain of rat. Tulsi has been found to have therapeutic potential as antidiabetic, hypolipidemic, and antioxidant medicine.[11-13]

Fig :1 Ocimum sanctum Linn

Daucus carrota Linn. (Carrot)

Family :- Apiaceae

Chemical Constituents:- Seed oil of Daucus carrota contain Carotol, daucol, terbenolene, sabinene, carotenoid, carotene, flavonoids, sugars. Alanine, α tocopherol, ascorbic acid, camphene,γ-terpinene, histidine Antitoxin.

Medicinal and antioxidant property:- Used in bronchitis, chest troubles, urinary camplaints, piles, leprosy, tumours, jaundice. Seeds useful in diseases of kidney and in dropsy. Antioxidant and radical scavenging activities are much higher in carrot peel than phloem and xylem tissue .Phenolic acids and flavonoids made greater contribution to the total antioxidant capacity. The quality of the antioxidants in the extracts is determined by the IC50 values. A low IC50 indicates strong antioxidant activity .[14]

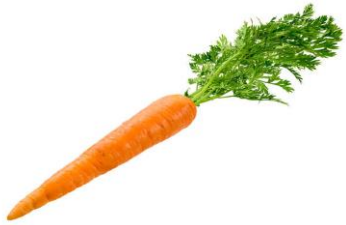

Fig :2 Daucus carrota Linn.

Camellia sinensis (Green tea)

Family: - Theaceae

Chemical Constituents: Polyphenols constitute the most interesting group of green tea leaf components, and in consequence, green tea can be considered an important dietary source of polyphenols, particularly flavonoids. The United States Department of Agriculture (USDA) has recently published a Database for the Flavonoid Content of Selected Foods. The main flavonoids present in green tea include catechins (flavan-3-ols). Green tea shows several medicinal activity like Anti-Aging, Neurodegenerative Diseases such as Anti Alzheimer, Antiparkinson , Antistroke, Cardiovascular Diseases, Anticancer, Antidiabetic, Anticaries, Skin Disorders, Obesity and Weight Loss. In Green tea catechin ,EGCG is a powerful antioxidant that is capable of protecting erythrocyte membrane bound ATPases against oxidative stress, a significant increase in plasma antioxidant capacity in humans after consumption of moderate amounts (1–6 cups/day) and enhanced blood antioxidant potential leads to reduced oxidative damage in macromolecules such as DNA and lipids.[15-18]

Fig :3 Camellia sinensis

Withania somnifera (Ashwagandh)

Family :- Solanaceae

Chemical Constituents: Ascorbic acid,α-tocopherol and reduced glutathione,phenolics,alkaloids, withaferin, superoxide dismutase ,ascorbate peroxidase, catalase, peroxidase & polyphenol oxidase. Medicinal and antioxidant property: Ashwagandh shows Hepatoprotactive, analgesic activity also increase immunity. The free radical scavenging and antioxidant activity of the extract of Withania somnifera was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH(2, 2, diphenyl 1- picryl hydrazyl). Glycowithanolides (WSG) consisting of sitoindosides VII to X and withaferin. Major oxidative free radical scavenging enzymes are superoxide dismutase (SOD), Catalase (CAT) and glutathione peroxidise (GPX).[19]

Fig :4 Withania somnifera

Glycyrrhiza glabra: - (Licorice,Mulathi)

Family: - Leguminosae.

Chemical constituents : Glycyrrhizin, flavones, coumarins,saponin, aroma of licorice is due to mixture of estragole,anethole,eugenole,indole,and cumic alcohol.

Medicinal and antioxidant property: Its medicinal uses are in acute case of conjunctivitis, diuretic, demulcent, antiinflamatory, in peptic ulcer, vomiting, asthama, bronchitis, in curing wounds, tonic10,genitourinary diseases, cough and sore throat. Its extract was tested by studying the inhibition of radiation induced lipid peroxidation in rat liver microsomes. It shows its activity through free radical scavenging property.[14]

Curcuma longa (Turmeric)

Family:- Zingiberaceae

Chemical constituents:- Rhizome contains pigments curcumin, beta-pipene, camphene, Eugenol. Medicinal and antioxidant property: Turmeric is used in India to treat anorexia, liver disorders, cough, diabetic wounds, rheumatism, and sinusitis, antifungal, antibacterial, insecticide. curcumin is a potent antioxidant, it may scavenge the epoxides and prevent binding to macromolecules. In other words, this spice's cell-protective properties are similar to nutrient antioxidants, vitamins C and E, which inhibit free radical reactions.[20,21]

Fig : 5 Curcuma longa

Zingiber officinale (Ginger)

Family:- Zingiberaceae

Chemical constituents:- zingiberene, the main terpenoid and 6-gingerol, the pungent principle, volatile oil, starch, acrid resinous matter, shagoals, zingerone, peradols etc.3 Medicinal and antioxidant property:- prevent nausea, vomiting, coughs and asthma, anti inflammatory, laxative and digestive, appetiser, relief. in diarrhoea, headache, toothache and elephantiasis.12 Ginger extracts possess strong antioxidant radical activities as evidenced by the ABTS assay .Both aqueous and ethanol extracts of ginger have significant natural antioxidant activity. Therefore, consumption of ginger might be helpful in combating the progression of various diseases with oxidative stress components such as atherosclerosis, diabetes mellitus among others.[22]

Fig : 6 Zingiber officinale

Melia azedarach L. (Margosa)

Family - Meliaceae

Chemicalconstituents:- Azaridine, sterols, tannins, paraisine, rutin, seeds are rich in fatty oil consisting of palmitic, oleic, linoleic acid.12

Medicinal and antioxidant Property :- Root bark is used in ascariasis,skin disease, eczema, leucoderma, malarial fever, wounds, diabeties, insecticidal, intestinal worms. Antioxidant activity is evaluated by DPPH radical scavenging assay and free radical scavenging ability of the extracts. The result showed that the extract of Melia azedarach. which contains highest amount of phenolic compounds exhibited the greatest antioxidant activity. The high scavenging property may be due to hydroxyl groups existing in the phenolic compounds chemical structure that can provide the necessary components as a radical scavenger. IC50 value and the total polyphenol content indicating that increasing the polyphenol content strengths the antioxidant activity.[23]

Fig : 7 Melia azedarach L.

Health Benefits of Antioxidants

Recently, antioxidants have attracted considerable attention in relation to radicals and oxidative stress, cancer prophylaxis and therapy, and longevity [24]. Phenols and polyphenols are the target analytes in many such cases; they may be detected by enzymes like tyrosinase or other phenol oxidases, or even by plant tissues containing these enzymes [25].

The recommendations based on epidemiological studies are such, that fruits, vegetables and less processed staple foods ensure the best protection against the development of diseases caused by oxidative stress, such as cancer, coronary heart disease, obesity, type 2 diabetes, hypertension and cataract [26]. The explanation consists in the beneficial health effect, due to antioxidants present in fruit and vegetables [27]. There are numerous antioxidants in dietary plants: carotenoids, phenolic compounds, benzoic acid derivatives, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans, and lignins [28]. Of the 50 analysed food products with high antioxidant content, 13 were spices, 8 were fruits and vegetables, 5 were berries, 5 were chocolatebased, 5 were breakfast cereals, and 4 were nuts or seeds. Considering the typical serving sizes, blackberries, walnuts, strawberries, artichokes, cranberries, brewed coffee, raspberries, pecans, blueberries, ground cloves, grape juice and unsweetened baking chocolate were at the top of the classification .

Fruit juices, beverages and hot drinks contain high amounts of antioxidants, like polyphenols, vitamin C, vitamin E, Maillard reaction products, β-carotene, and lycopene. The consumption of fruit juices, beverages and hot drinks was found to reduce the

morbidity and mortality caused by degenerative diseases. Antioxidants are known to play a key role in the protective influence exerted by plant foods. Epidemiologic studies that analyse the health implications of dietary components rely on the estimation of intakes of sample populations, which are found in databases that provide the compounds found in commonly consumed foods. Thus, the availability of appropriate and complete food composition data is vital. Due to the diversity of chemical compounds with antioxidant activity present in foodstuffs, complete databases of antioxidant contents are not yet available. In addition, levels of single antioxidants in foodstuffs do not necessarily reflect their total antioxidant potential (TAP); the total antioxidant potential depends on the synergic and redox interaction among the different molecules present in food [29,30].

MATERIALS AND METHODS:

7.1 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 (20-25°C) and other reagents were storedat -20°C 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).

7.2 Plant Material Collection

The *Emblica Officinalis Fruit* was collected from the Local Market. 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.

7.3. Preparation of plant extracts

7.3.1 Preparation of Aqueous Extract:

Fresh *Emblica Officinalis Fruit* were collected and washed under tap water. The *Fruit* 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.

7.3.2 Preparation of Alcoholic Extract:

Fresh *Fruit* of *Emblica Officinalis* 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.

8. RESULTS AND DISCUSSION:

8.1 Phytochemical screening of *Emblica Officinalis Fruit*

The present investigation concluded that the isolated compounds from the plant *Emblica Officinalis Fruit* are pure and the plant *Emblica Officinalis Fruit* 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 1: Phytochemical screening of *Emblica Officinalis Fruit*

S.No.	Phytoconstituents	Aqueous	Alcoholic
1.	Alkaloids	+	+
2.	Glycosides	-	+
3.	Anthraquinone	+	+
4.	Phytosterols	+	+
5.	Saponins	+	+
6.	Tannins & Phenolic compounds	+	+

7.	Flavonoids	+	-
8.	Steroids	+	+

8.2 ANTIOXIDANT PROPERTIES OF *EMBLICA OFFICINALIS FRUIT*

Several mechanisms have been proposed to be involved in antioxidant activity such as hydrogen donation, termination of free radical mediated chain reaction, prevention of hydrogen abstraction, chelation of catalytic ions and elimination of peroxides (Gordon, 1990). Antioxidant activity is system- dependent and characteristic of a particular system can influence outcome of analysis. Hence, a single assay would not be representative of antioxidant potential of plant extracts. In this present study, different models of antioxidant assays were employed, which could provide a more consistent approach to assess antioxidant activity of *Emblica Officinalis Fruit*

8.2.1 Ferric reducing ability of *Emblica Officinalis Fruit*

FRAP assay is based on a redox-linked reaction, whereby antioxidants present in plant extracts act as reductants while ferric ions in reagents serve as oxidants. Reduction of ferric-tripyridyltriazine to ferrous complex forms an intense blue color with maximum absorption at 593 nm, which is related to amount of antioxidants in the sample. The ferric reducing ability of *Emblica Officinalis Fruit* is shown in Table. Water and alcohol extract reduced ferric ions efficiently and had reducing activity in the range of 151 – 2.10mM/g, which was greater than or comparable to synthetic antioxidant BHT (2.10 mM/g). Both extracts were less effective, when compared with reducing activity of Quercetin (14.12mM/g).

Reduction of ferric to ferrous ion is frequently used as an indicator of electron donating activity, which is considered to be an important factor dictating antioxidant activity of plant. Figure 4.5 shows dose-response curves for reducing power of different extracts from *Emblica Officinalis Fruit* fruits. Extracts showed significant ability to reduce ferric ions in a dose-dependent manner. Water and alcohol extract showed highest reducing power. Quercetin and BHT revealed potent reducing power, which were distinctly higher than that of any of *Emblica Officinalis Fruit* extracts.

Antioxidant activity has been reported to be concomitant with reducing power of plant extract (Gordon, 1990). Significant ferric reducing ability of *Emblica Officinalis Fruit* extracts observed in this study suggest that polyphenolics present in the extracts have the ability to donate electrons to free radicals by acting as reductones and thus could terminate free radical-mediated oxidative reactions. Catechin, sinapic acid, ferulic acid, Quercetin and myricetin, which were identified in *Emblica Officinalis Fruit* have been shown to possess significant ferric reducing ability in their pure form, suggesting that ferric reducing ability of *Emblica Officinalis Fruit* could have been partly contributed by these phenolics (Pulido et al, 2000). Present findings are in line with those of other investigators, who have also reported that antioxidant properties are concomitant with development of reducing power (Chung et al, 2005).

Table 2: Ferric Reducing Ability - FRAP (expressed as mM FeSO4/g dry weight) of *Emblica Officinalis Fruit*

Group	Drugs	IC_{50} value µg/ml
I	Quercetin	14.12±0.012
II	Butylated Hydroxy Toluene (BHT)	2.10 ±0.021
III	AQEO	1.51±0.054
IV	ALEO	2.10±0.041

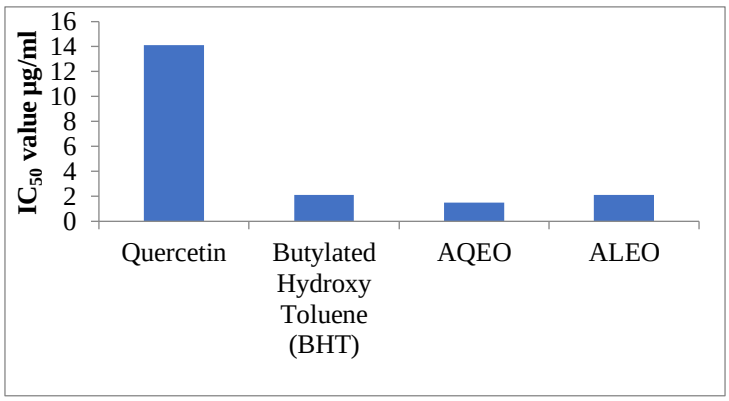

Fig 8: Reducing power of *Emblica Officinalis Fruit* Quercetin and BHT were used as reference antioxidant. Values are means ± SD (n = 3).

8.2.2 Metal chelating activity of *Emblica Officinalis Fruit*

Emblica Officinalis Fruit extracts were evaluated for their ability to chelate ferrous ion by competing with ferrozine in free solution. All extracts displayed an ability to chelate ferrous ion in a dose-dependent manner. However, estimated IC50 was very high (more than 2.0 mg/ml); particularly, in comparison with positive control EDTA (7.14Jg/ml). Quercetin and BHT showed moderate metal chelating activity when compared with EDTA with an IC50 of 142Jg/ml and 76 Jg/ml respectively. Water and alcohol extract showed a chelating ability of 25.12and 38.53% respectively at 1.0 mg/ml. In case of extract, metal chelating activity varied from 2.15% to 30.83%. Alcoholic extracts were the highest, followed by water extract. EDTA, Quercetin and BHT exhibited 99.23%, 60.54% and 71.36% of chelating activity respectively, which were significantly higher than that of *Emblica Officinalis Fruit* extracts.

Transition metal ions gain utmost significance in biological system due to their ability to generate reactive free radicals. They can initiate Fenton type reaction with production of hydroxyl radicals or Haber-Weiss reactions with superoxide radicals (Kehrer, 2000; Wong and Kitts, 2001). They hasten peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate chain reaction of lipid peroxidation (Halliwell and Gutteridge, 1984; Halliwell, 1991). Metal chelating capacity is imperative as it decreases concentration of catalyzing transition metal ions in Fenton type reaction and protects system from oxidative damage through inhibition of metal-dependent processes. Chelating agents that form bonds with metals are effective as secondary antioxidants because they can reduce redox potential by stabilizing oxidized form of metal ion (Gordon, 1990).

Table 3: Metal chelating activity of *Emblica Officinalis Fruit*

Group	Drugs	IC_{50} value µg/ml
I	EDTA	7.14
II	Quercetin	142
III	Butylated Hydroxy Toluene (BHT)	76
IV	AQEO	25.12
V	ALEO	38.53

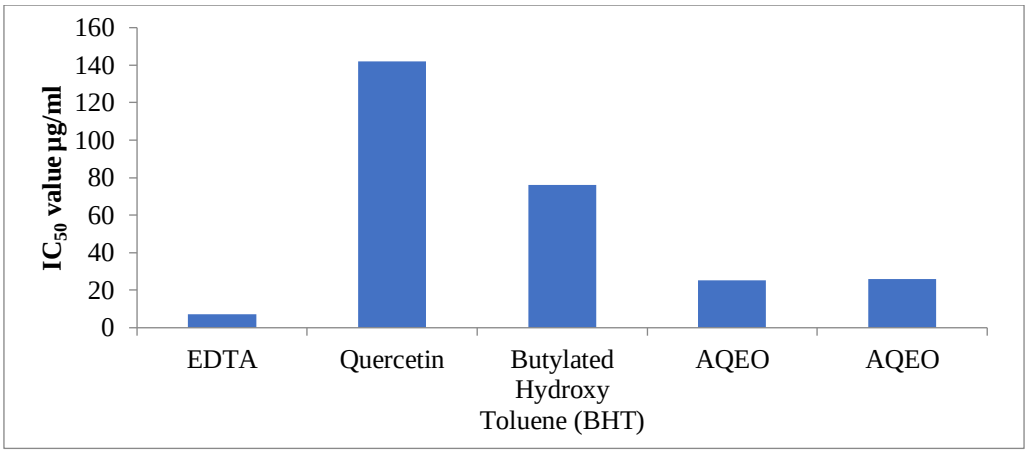

Fig-9 Metal chelating activity of *Emblica Officinalis Fruit* EDTA was used as positive control. Quercetin and BHT were used as reference antioxidants. Values are means ± SD (n = 3).

7.2.3 DPPH radical scavenging activity of *Emblica Officinalis Fruit*

Basic information on efficacy of compounds in *Emblica Officinalis Fruit* extracts to quench free radicals can be deduced from DPPH assay. The DPPH is a stable free radical, which is recognized as a tool for evaluating radical scavenging ability of compounds and antioxidant activity of foods (Sanchez-Moreno, 2002). It accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capacity of DPPH is determined by decrease in its absorbance at 517 nm,

induced by antioxidants. It has also been used to quantify antioxidants in complex biological systems, because of its ease and convenience. Even though, DPPH radicals may not be biologically pertinent, it presents an indication of hydrogen/ electron-donating capacity of plants and provides a useful means to measure in vitro antioxidant activity. *Emblica Officinalis Fruit* extracts revealed a concentration-dependent scavenging of DPPH radicals, with presenting strongest effect followed by Fruit. Of aqueous and alcoholic extracts showed strongest effect (IC50 at 31 Jg/ml for Fruit), followed by water and alcohol extracts. Comparison of DPPH radical scavenging activity with standard antioxidants showed that the most potent Emblica Officinalis Fruit extracts had scavenging ability higher than BHT (IC50 at 493 Jg/ml), but lower than quercetin (IC50 at 11 Jg/ml).

Effective DPPH radical scavenging activity exhibited by *Emblica Officinalis Fruit* extracts could be explained by the presence of polyphenolics in them, whose radical scavenging properties were reported previously in various model systems (Fukumoto and Mazza, 2000). Radical scavenging ability of polyphenolics is attributed to their ability to donate a hydrogen atom from a phenol to give DPPH-H and a phenoxyl radical. Alcoholic extracts contained more amounts of ferulic acid and sinapic acid, which could partially explain higher ability to scavenge DPPH (Kim et al, 2008), in comparison with water and alcohol extracts. Catechin, the major component of water extracts was found to be moderately active as an antioxidant in DPPH assay (Hwang et al, 2001). A comparison between DPPH radical scavenging activities of *Emblica Officinalis Fruit*. *Emblica Officinalis Fruit* extracts were more potent in terms of radical scavenging activity whereby their IC50 values were comparatively much lower than these BHT (Lee et al, 2008; Koksal and Gulcin, 2008; Borowski et al, 2007), thus further demonstrating effectiveness of *Emblica Officinalis Fruit* fruit as natural antioxidants.

Table -4 Scavenging ability of root, stem and *Emblica Officinalis Fruit* and standard antioxidants on DPPH as determined by their IC50, expressed as mg/ml.

Group	Drugs	IC_{50} value µg/ml
I	Quercitin	2.0 ±0.012
II	Butylated Hydroxy Toluene(BHT)	3.12 ±0.024
III	AQEO	1.50 ±0.042
IV	ALEO	4.1 ±0.021

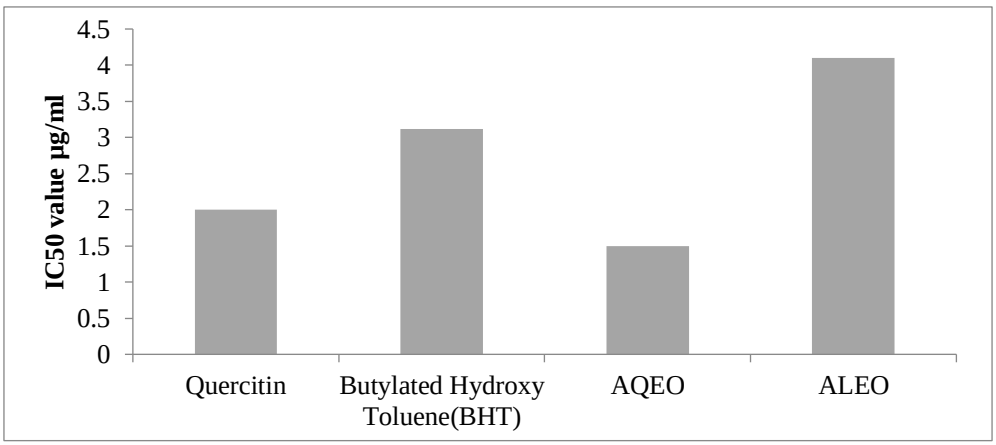

Fig-10 DPPH radical scavenging activity of *Emblica Officinalis Fruit* Quercetin and BHT were used as reference antioxidant. Values are means ± SD (n = 3).

7.2.4 Superoxide radical scavenging activity of *Emblica Officinalis Fruit*

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 *Emblica Officinalis Fruit* extracts on superoxide radicals is shown in Figure. Extracts from different parts of *Emblica Officinalis Fruit* displayed concentration dependent protective activity against superoxide radicals. Of which, Fruit were the most effective. Alcoholic extracts of Fruit (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 *Emblica Officinalis Fruit* 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-5 Scavenging ability of *Emblica Officinalis Fruit* and standard antioxidants on superoxide radical (O2•) as determined by their IC50, expressed as mg/ml.

Group	Drugs	IC_{50} value µg/ml
I	Quercetin	0.05 ±0.001
II	Butylated Hydroxy Toluene(BHT)	0.030 ±0.001
III	AQEO	0.301 ±0.005
IV	ALEO	0.028 ±0.002

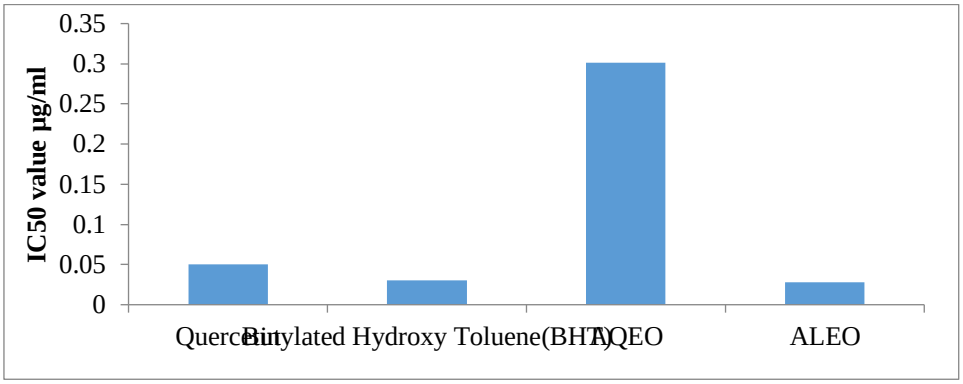

Fig-11 Superoxide radical scavenging activity of *Emblica Officinalis Fruit* Quercetin and BHT were used as reference antioxidant. Values are means ± SD (n = 3).

7.2.5 Hydrogen peroxide scavenging activity of *Emblica Officinalis Fruit*

Though hydrogen peroxide (H2O2) 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 *Emblica Officinalis Fruit* extracts on H_2O_2 is shown in Figure and compared with Quercetin and BHT as standard antioxidants. *Emblica Officinalis Fruit* 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 Fruit 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-6 Scavenging ability of *Emblica Officinalis Fruit* 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	0.038±0.003
II	Butylated Hydroxy Toluene(BHT)	0.080±0.002
III	AQEO	0.072±0.001
IV	ALEO	0.519±0.008

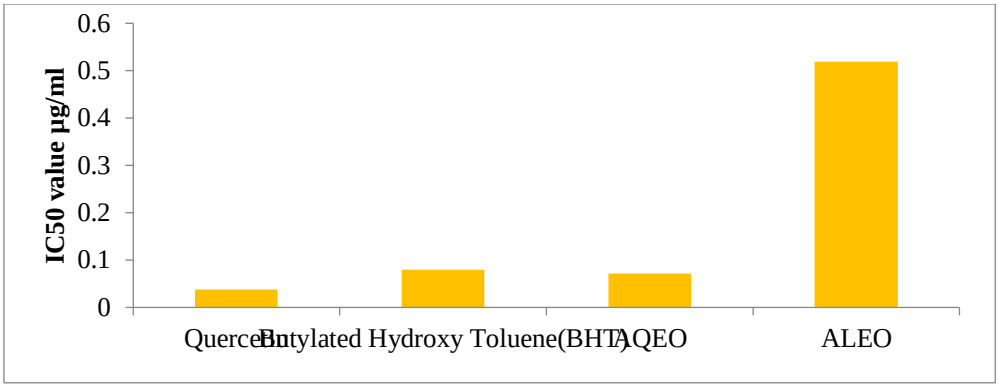

Fig-12 Hydrogen peroxide scavenging activity of *Emblica Officinalis Fruit* Quercetin and BHT were used as reference antioxidant Values are means ± SD (n = 3)

9. 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.

Emblica Officinalis Fruit is a unique plant containing a rich and rare combination of phytochemical. 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 *Emblica Officinalis Fruit* possess substantial biological activities.
- Leaves have high level of polyphenolic and show significant antioxidant activity. *Emblica Officinalis Fruit* 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.
- *Emblica Officinalis Fruit* (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.
- These could be related to the presence of polyphenolic in *Emblica Officinalis Fruit* extracts as they possess significant capacity to remove reactive species by virtue of their ability to induce antioxidant enzyme system in the cells.
- *Emblica Officinalis Fruit* (alcoholic extract of leaves) significantly inhibited the proliferation of several human cancer cells through induction of apoptosis.

Our findings suggest the use of *Emblica Officinalis Fruit* extracts in functional foods and food supplements designed for the prevention of various chronic diseases, including eye diseases, allergies, constipation, cough, dental caries, obesity, stomach ailments, and viral infections. However, further studies are needed to prove that the protective effects observed *in vitro* do indeed translate *in vivo*.

10. CONCLUSION:

The result of the present study showed that the aqueous and alcoholic extract of *Emblica Officinalis Fruit*, which contains phenolic and flavonoidal compounds, exhibited the great antioxidant activity. The high scavenging property of methanolic extract of *Emblica Officinalis Fruit* 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 *Emblica Officinalis Fruit* 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 *Emblica Officinalis Fruit* contain more antioxidant activities. More- over, this study demonstrated the important source of phenol compounds, which are a good source of antioxidant 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 *Emblica Officinalis Fruit act* via its free radical

scavenging to prevent lipidperoxidation. Therefore, natural antioxidants and phenol compounds in *Emblica Officinalis Fruit* have the capability to be used medically and in food systems to preserve food quality.

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