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In-vitro antioxidant and reducing potential activity of extracts of C*itrus Limon* and S*olanum Lycopersicum*

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ABSTRACT

Newly, importance has substantially augmented in verdict naturally arising antioxidant to supplant manmade antioxidants, which were circumscribed due to their side effects for instance carcinogenesis. These indicate that the plants are a significant foundation of natural antioxidant, which might be obliging in averting the advancement of innumerable oxidative stresses and Reactive oxygen species (ROS) which have been found to play an imperative role in the commencement and/or advancement of several diseases such as atherosclerosis, inflammatory injury, cancer and cardiovascular disease. Thus, latest studies have scrutinized the potential of plant products as antioxidants against various diseases induced by free radicals. Antioxidant capacity is widely used as a parameter to characterize nutritional health food or plants and their bioactive components. This study is done to evaluate the in vitro antioxidant and free Radical Scavenging potential of the extract of fruits of Citrus limon and Solanum lycopersicum. The plant extract is obtained by cold expression method and the resultant solution is serially diluted to 10, 20, 30, 40, 50 µg/ml. The antioxidant potential was analyzed by hydrogen peroxide scavenging activity seen at 230nm using UV Visible Spectrophotometer. Based on the results obtained in the present study, it is concluded that fruit extracts of Citrus limon and Solanum lycopersicum exhibits high antioxidant activity. The reducing power assay exhibited that reducing capability of the extract were considerably increased with cumulative concentration and were higher compared to the standard ascorbic acid and the antioxidant action was evaluated by scavenging hydrogen peroxide method where the EC_{50} *values of Ascorbic acid, Citrus limon and Solanum lycopersicum were found to be 0.766, 1.418 and 1.3046. Based on the results obtained in the present study, it is concluded that fruit extracts of Citrus limon and Solanum lycopersicum exhibits high antioxidant and free radical scavenging activities.*

Keywords: *Citrus limon*; *Solanumlycopersicum*; hydrogen peroxide; reducing power; ascorbic acid.

INTRODUCTION

Citrus fruits, which belong to the family of *Rutaceae* are one of the foremost fruit tree crop grown throughout the world. Although, *Citrus limon* is the key fruit in this cluster accounting for about 70% of citrus output. India ranks 6th position in the production of citrus fruit cultivation in the world. The precise starting point of citrus fruits is not evidently recognized, although most researchers place its source to be South East Asia[1]. The most recent research indicates an origin in Australia, Southeast Asia, New Caledonia, Northeast Indiaand New Guinea [2] and it is in this area that some profitable species such as oranges, mandarins, and lemons originated.

Citrus trees are perennial trees that yield fruits of diverse customs and sizes (from round to oblong), which are full of cologne, essence and juice. It has an irregular, strong and perky color from green to yellow skin or rind known as epicarp or flavedo, which shields the fruits and protects from damages. The glands comprise the essential oils that provide the fruit its typical citrus scent. It involves a white, thick and spongy mesocarp or albedo which together with the epicarp formulate the pericarp or peel of the fruit. The inner part constitutes the pulp which is distributed into distinct sections or juice sacs (with or without seeds, conferring to diversities) by a thick radical film or endocarp. This portion is rich in solvable sugars, ascorbic acid, pectin, fibers, diverse organic acids and potassium salt that give the fruit its characteristic citrine flavor. [1,3]

Citrus fruits are consumed as fresh or applied for treated citrus products and citrus-by-products. Citrus essential oils are additional by-product of citrus fruits. Essential oils are volatile oils achieved from the citrus fruits peel's sacks. They are recycled by the food industry to give flavor to drinks and foods. They are also an element for the pharmaceutical industry for the preparation of drugs, detergents, colognes and supplementary cosmetics as well as for homemade cleaning products.

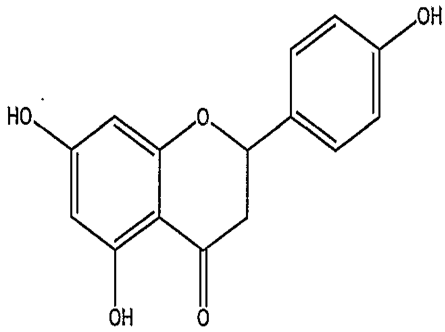
Figure I Showing the slices of lemon and tomato

Solanum lycopersicum belonging to family *Solanaceae* is second most important crop in world next to potato. It is cultivated all over the world. Over20 billion metric heaps of tomatoes are shaped every year on a sphere basis. The main producer countries of tomatoes are Spain, United States, Turkey, India, U.A.R. Italy, and Mexico. As, a processing crop, it grosses first rank amongst the vegetables. It is also referred to as poor man's orange because of its high vitamin, malic acid, citric acid contents, and the fact that it serves as a fine appetizer.

Tomato fruit quality is resolute largely by color, consistency, and aroma. Among these color and flavor are probably the most useful criteria for estimating maturity of tomato fruit. The flavor of a fruit becomes pronounced when the sugar content is at its maximum, at which time the skin acquires its richest color.

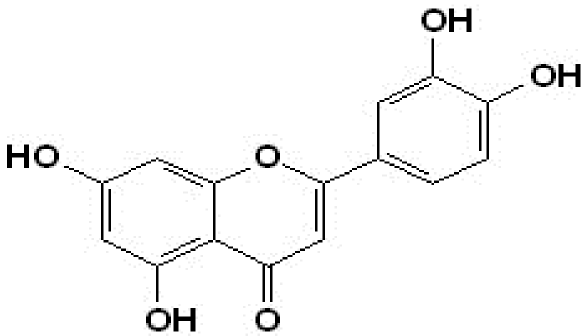
PHYTOCHEMICALS AND THEIR PHARMACOLOGICAL ACTION

The biochemical complexes existing in plants are called phytochemicals. Plants yield chemicals known as secondary metabolites that are not directly involved in the process of growth but acts as disincentives to insects and microbial attack. Alkaloids, cyanogenic glycosides, flavonoids, terpenoids and phenolic compounds all fit in this category. Flavonoids are another phytochemicals found in citrus fruits. The flavonoids have robust intrinsic capability to amend the body's response to allergens, viruses and carcinogens. They display anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity [4]. Flavonoids and some other phenolic compounds constitute the total phenolics of tomato.



NARINGENIN $C_{27}H_{32}O_{14}$

Quercetin, myricitin, rutin, tangeritin, naringin and hesperidin are found amongst the communal flavonoids in citrus fruits [5] although naringenin and quercetin are isolated from the fruit skin of tomatoes. These flavonoids are liable for the bitter taste of certain grape fruits, lemons and oranges.

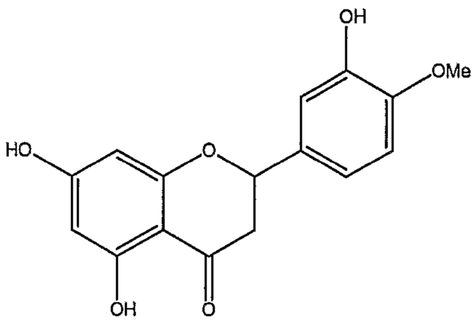


QUERCETIN $C_{15}H_{10}O_7$

Quercetin is a flavonoid and more precisely a flavonol that creates the aglycone of the glycoside rutin. Quercetin is found to be the most active due of the flavonoids and many medicinal plants owe much of their activity due to their high quercetin content [4,6].

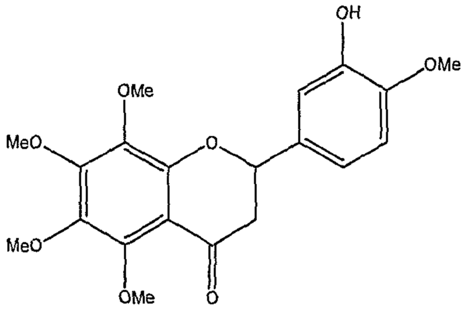
Quercetin has proved important anti-inflammatory activity because of direct inhibition of numerous primary processes of inflammation. For example, it inhibits; the creation of histamine and additional allergic/inflammatory mediators [6]. In addition, it also exerts potent antioxidant activity and ascorbic acid sparing action. Quercetin also shows remarkable anti-tumor properties. Quercetin may have positive effects in combating or helping to prevent cancer, prostatitis, heart diseases, cataracts, allergies/inflammations and respiratory diseases such as bronchitis and asthma[8].

Hesperidin is a flavonoid glycoside originates amply in citrus fruits. It’s a glycone arrangement is called hesperetin. Hesperidin is supposed to play a part in plant defence. It acts as antioxidant rendering to in vitro studies[7,8]. It reduces cholesterol [7,9] and has anti-inflammatory effects[7]. It also presented an ability to perforate the blood brain barrier in an in vitro model[7,8]



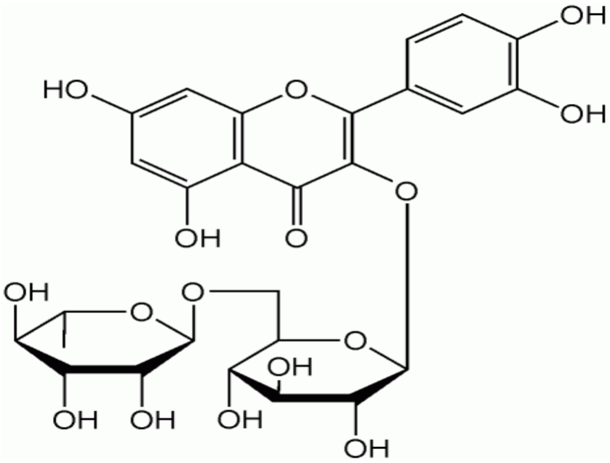
HESPERIDIN $C_{28}H_{34}O_{15}$

Tangeritin is a polymethoxyleted flavone that is originated in tangerine and other citrus peels. Tangeritin shows potential as an anti-cancer agent. It strengthens the cell wall and protects it from invasion[10]



TANGERITIN $C_{20}H_{20}O_7$

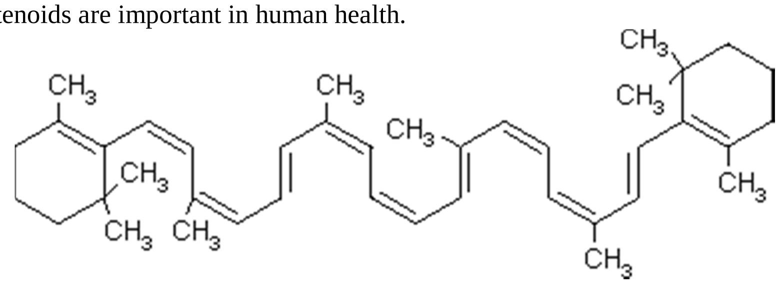
Another useful citrus flavonoid glycoside is rutin and it is also known as rutoside or quercetin-3 rutinoside, In humans, rutin attaches to the Fe2+ ion and it is, averting it from binding to hydrogen peroxide and generating a highly reactive free radical that may harm cells. It is also an antioxidant and therefore, it plays an imperative part in inhibiting various cancers. Moreover, rutin reinforces the capillaries and therefore, it can condense the symptoms of hemophilia. It also supports to inhibit edema of the legs[1]. Rutin, as ferulic acid can diminish the cytotoxicity of oxidized low density lipoprotein (LDL) cholesterol and lessen the risk of heart diseases[7].



RUTIN $C_{27}H_{30}O_6$

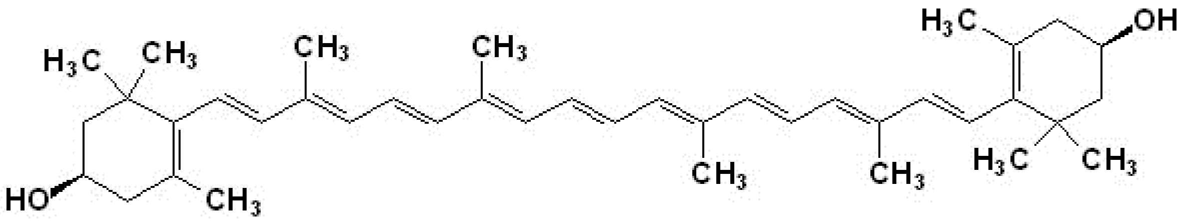
The citrus bioflavonoids that comprise hesperidine, quercetin, rutin (a glycoside of quercetin) and tangeritin, in addition to possess antioxidant activity and an ability to surge intracellular levels of ascorbic acid, rutin and hesperidin exert beneficial effects on capillary permeability and blood flow[7,11]. They also exhibit some of the antiallergic and anti-inflammatory benefits of quercetin[6,9]. Hydroxyethyl rutosides (HER)have been used in the cure of capillary penetrability, easy yellowing, hemorrhoids and varicose veins[7,9,11].

Another group of phytochemicals found in citrus are carotenoids. Carotenoids are the pigments liable for the colors of many plants. Carotenoids are important in human health.



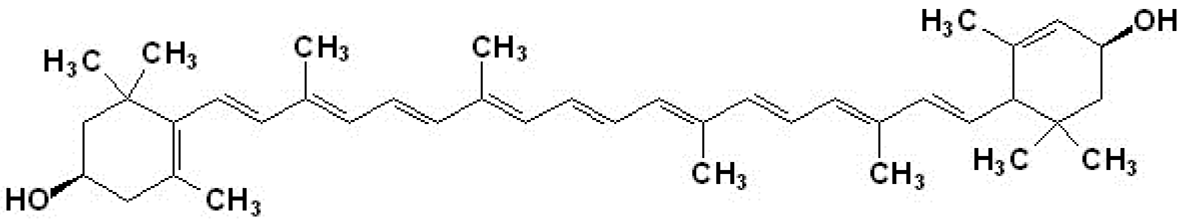
β-CAROTENE $C_{40}H_{56}$

Carotene plays an essential role as sources of vitamin A. The most active role is fortification against severe ailments such as cancer, heart diseases and deteriorating eye diseases. It is an antioxidant and acts as controllers of the immune system[12].

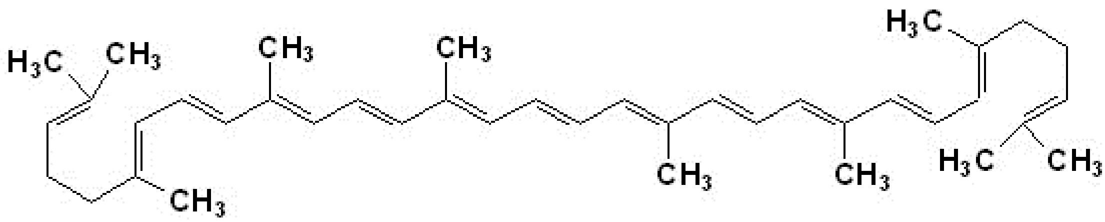


ZEAXANTHIN $C_{40}H_{56}O_2$

Age-related muscular degeneration (ARMD) associated with ageing can lead to a total blindness in healthy people. However, there is a substantial contrary correlation between the frequency of ARMD and the consumption of citrus fruits rich in provitamin A. Citrus carotenoid was demonstrated to have significant reductions in the risk of developing ARMD.

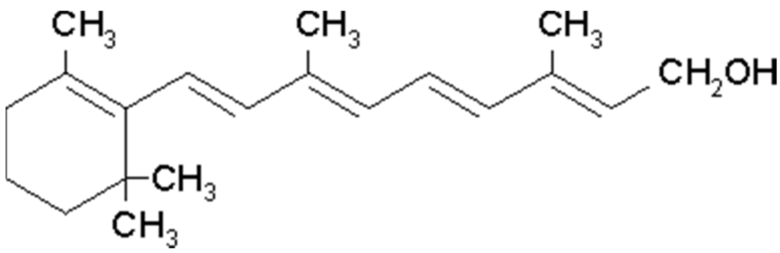


LUTEIN $C_{40}H_{56}O_2$



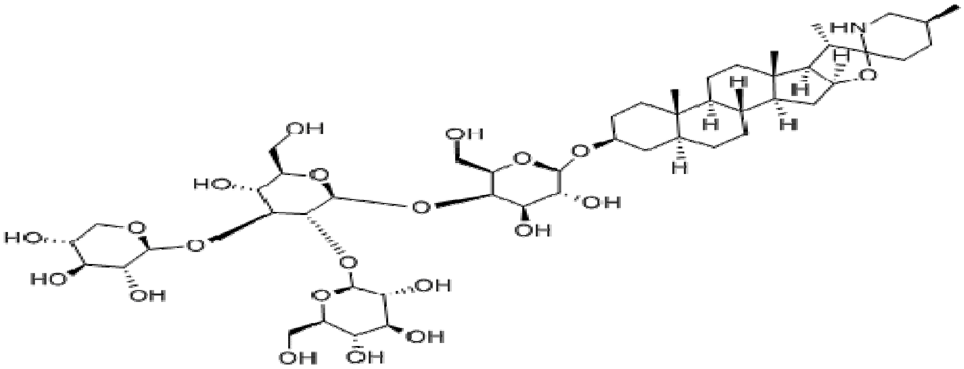
LYCOPENE $C_{40}H_{56}$

The carotenoids present are namely α- carotene, β-carotene, cryptoxanthin, Lutein and zeaxanthin[13,14]



RETINAL $C_{20}H_{30}O$

Citrus is the main source from which primates' device vitamin C. tomato is a rich source of ascorbic acid, as the fruit matures the concentration of amino acid increases. It acts also as antioxidants in the skin by scavenging and quenching free radical produced by ultra violet radiation stabilization. The production of collagens is also dependent on vitamin C. It helps in the elevation and restoration of skin and enhancement of fine wrinkles[3].



TOMATINE $C_{50}H_{83}NO_{21}$

Tomato contains Tomatine, a glycosidal steroidal alkaloid. Traces of solanine are also found. Red ripe tomatoes lose almost all their tomatine when left on the plants for 2-3 days. Besides alkaloids stigmasterol, β-sitosterol are the two main sterols of the tomato fruit.

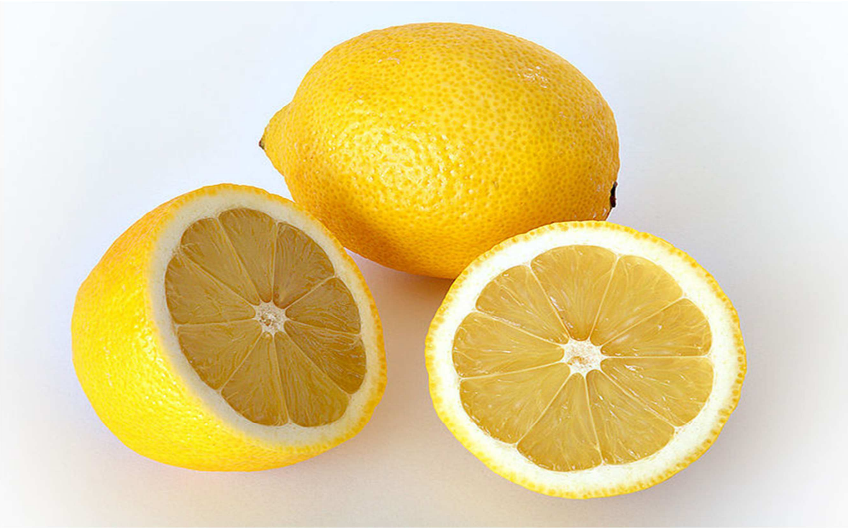
ETIOLOGY OF MAJOR DISEASES

Oxidative stress has been implicated in the etiology of diseases such as cardiovascular ailments and lung cancer, cancer, atherosclerosis, muscular degeneration, Alzheimer's disease, inflammation, and Emphysema, diabetic complications, asthma, and colon cancer[18]. An antioxidant may disturb biological structure by (i) suppressing the formation of ROS and reactive nitrogen species (RNS);(ii) affecting enzyme activities[15]; (iii) inducing *de novo* biosynthesis of defense enzymes and thereby affecting other endogenous antioxidants[16];(iv) preserving NO activity[17], or (v) sequestering transition metal ions.

MECHANISM OF ACTION OF ANTIOXIDANTS:

Antioxidants are secondary metabolites found naturally in plants such as citrus fruits. An antioxidant can be defined as anything that inhibits or prevents oxidation of a substrate[19]. Our body is repetitively exposed to a diversity of oxidizing agents and the body is equally inherent with antioxidants to furnish for the free radicals produced from the oxidants thus upholding a balance between the production of free radicals and deactivation by antioxidants. When there is disproportion between formation and neutralization of free radicals by antioxidants, it outcomes to oxidative stress [20,22].

Oxidative stress plays a major part in the expansion of chronic and degenerative ailments such as cancer, autoimmune disorders, rheumatoid arthritis, cataract, aging, cardiovascular and neurodegenerative diseases[23,24].
The human body has several mechanisms to counteract oxidative stress by generating antioxidants, which are either naturally formed in situ, or externally provided through foods and/or supplements. These antioxidants act as free radical scavengers by avoiding and revamping damages affected by ROS, and therefore can improve the immune defense and lessen the risk of cancer and degenerative diseases[24].



Aerobic cells undergo metabolism producing free radicals. The oxygen ingestion integral in cell growth leads to the generation of a series of free radicals causing oxidative stress. The interface of these class with molecules of a lipid nature produces new radicals: hydro peroxides and different peroxides[7]. This group of radicals comprising hydroxyl, superoxide and lipids peroxides may interact with biological systems in a clearly cytotoxic manner. These species interact with such life essential molecules as nucleic acids and proteins, producing oxidative reactions involving alterations and protein exchange[7,25] Free radicals are created when cells use oxygen to produce energy. These by-products are generally reactive oxygen species (ROS) such as super oxide anion, hydroxyl radical and hydrogen peroxide that result from the cellular redox process. The different radicals responsible for the cell oxidation process are the following: singlet oxygen (1O2); superoxide anion (O2), hydroxyl radical (OH*) and peroxyl radical R-O-O*[7,25,26].The hydroxyl radical is the utmost cytotoxic of all these radicals. Also, polyunsaturated fatty acids existing in cell membranes are easily oxidized by both; enzymatic and oxidative peroxidation through free radical chain reaction[7,26]. Initiation of lipid per oxidation can be induced by free radicals (superoxide, hydroxyl and singlet oxygen) produced in biological systems [7,26]. These electrically inert species have the ability to interact and alter genetic constitution. They exhibit catatonic, mutagenic and carcinogenic actions. At low or moderate concentrations, ROS exert beneficial effects on cellular responses and immune function but at high levels, free radicals and oxidants generate oxidative stress, a deleterious process that can damage cell structures, including lipids, proteins, and DNA[24].

Epidemiological studies have shown that regular consumption of fruits and vegetables reduces the risk incidence of chronic diseases[27,28]. The protection that fruits and vegetables provide against diseases has been attributed to the various antioxidants contained in them. They are good sources of natural antioxidants which include carotenoids, vitamins, phenolic compounds, flavonoids, dietary glutathione, and endogenous metabolites and have been shown to scavenge singlet and * triplet oxygen, free radicals, enzyme inhibitors and decompose peroxides[29,30].

Phenolic compounds are secondary metabolites in fruits and vegetables. They have been reported to exhibit antioxidant activity which allows them to scavenge both active oxygen species and electrophiles, to inhibit nitration and to chelate metal ions, to have the potential for autoxidation and the capability to modulate certain cellular enzyme activities[31-33]

Flavonoids are known for their ability to enhance the effects of ascorbic acid. Plant flavonoids comprise anthocyanins, proanthocyanins, flavones, flavonols, flavonones, biflavinonoids, flavin-3-4-diol, isoflavones and catechins. Flavonoids have been reported to act as quenchers of singlet oxygen[3,26,42]. Flavonoids, a class of phenolic compounds has been shown to possess anti-inflammatory, antiviral, anticarcinogenic, antithrombotic, antiallergic and hepatoprotective[34].

Citrus phenolic compounds particularly flavonoids have been reported to possess an important antioxidant activity toward radicals. The citrus flavonoids have the ability to capture electrons, block and/or scavenge the radicals. The citrus flavonoids forma tautomeric dislocation, which prevents the propagating chain reactions of these oxygen free radicals[3,26]. It has been reported that lipid peroxidation can be inhibited by flavonoids acting as strong radical scavengers and singlet oxygen quenchers [7,26]. It has also been proposed that citrus flavonoids react with peroxyl radicals; thus, bringing about the termination of the radicals reaction [7].

Carotenes have been proved to possess antioxidant activity due to their ability to quench singlet oxygen and inhibit lipid peroxidation [35]. The ability of carotenoids, to act as antioxidant has been reported [36]. Antioxidants are secondary phytoconstituents or plant metabolites that inhibit or prevent oxidation of susceptible substrate. In vitro studies have demonstrated that lycopene has the highest antioxidant activity of all the carotenoids [37,38]. It has the ability to quench singlet oxygen (more than that of β-carotene) to trap peroxyl radicals, to inhibit the oxidation of

DNA and inhibition of lipids per oxidation [39] and in some studies to inhibit the oxidation of low density lipoprotein (LDL)[40,41]. Carotenoids are known to suppress these growths of tumors

In in-vitro (test tube) and in vivo (animal) studies [41]. The various carotenoids such as lycopene, β-carotene, α-carotene, lutein and canthaxanthin can decrease malignant transformation of cells [41].

The antioxidant assays can be classified into two categories: tests that measure the ability to scavenge free radicals and trials that assess the ability to inhibit lipid oxidation, with the ability to eliminate free radicals being one of the mechanisms that contribute to the overall activity providing a synergistic effect[30].

MATERIALS AND METHODS

Chemicals
Hydrogen peroxide, potassium dihydrogenortho phosphate, sodium hydroxide, Ascorbic acid, Potassium ferricyanide, Trichloroacetic acid and ferric chloride. All chemicals are of analytical grade or purer.

Plant extracts
The citrus rind of *Citrus limon* is mechanically pressed to release their content. It is then centrifuged for 10 minutes at 1000rpm which separates the essential oil from juice. It is then filtered using Whattman filter paper.

In *Solanum lycopersicum* the pulp and the serum is separated. It is also micro centrifuged for 10 minutes at 2000 rpm giving an upper layer of crude extract. It is further filtered using the Whattman filter paper.

Scavenging of hydrogen peroxide
A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4).Both the extracts (0.1 - 1 mg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extract and standard compounds was calculated as follows:

$$\% \text{ Inhibition of } H_2O_2 \text{ Scavenging activity} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the control and
A_1 was the absorbance in the presence of the sample of extract and standard

Reducing power assay method
Different concentrations (10-50µg/ml) of *S. lycopersicum* and *C. limon*extract were prepared and 1 ml of each solution was mixed with phosphate buffer (2.5ml, 0.2M, Ph 6.8) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20 minutes. To this mixture, 2.5ml of 10% trichloroacetic acid (TCA) was added and then centrifuged at 3000rpm for 10 minutes. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and ferric chloride (0.5 ml, 0.1%) was added and the absorbance was measured at 700nm.

The percentage of reducing power was calculated by using the formula:

$$\text{Reducing power } (\%) = Abs_{control} - Abs_{sample} / Abs_{control}$$

Where, $Abs_{control}$ was the absorbance of solution without extract and
Abs_{sample} was the absorbance with different dilutions of plant extract.

RESULTS AND DISCUSSION

Hydrogen peroxide assay calculation
The effect of plant extract of *C. limon* and *S. lycopersicum* on hydrogen peroxide radical scavenging activity is shown in fig. I. The extracts showed significant antioxidant activity against H_2O_2 radical with correlation coefficient values (r) of *C. limon*as 0.977 and that of *S. lycopersicum* was found to be 0.945. The IC_{50}values of *C. limon* was 1.418mcg/ml and the IC_{50} value of *S. lycopersicum* was 1.3046 comparable to IC_{50}value of ascorbic acid of 0.766µg/ml.

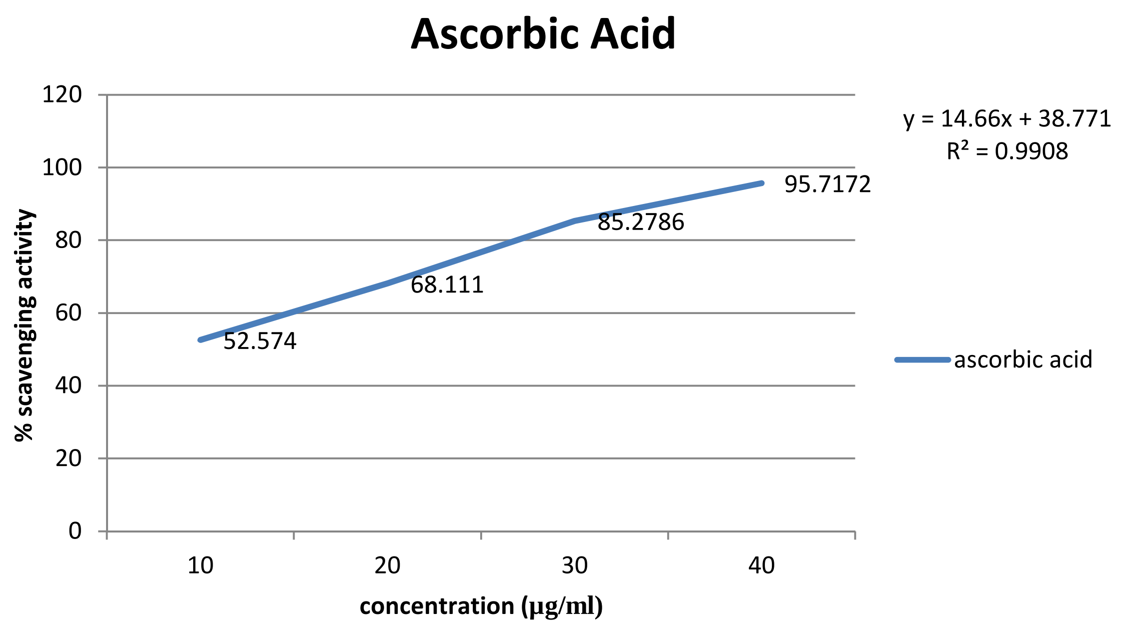


Figure II. Hydrogen peroxide scavenging activity of Ascorbic Acid as standard

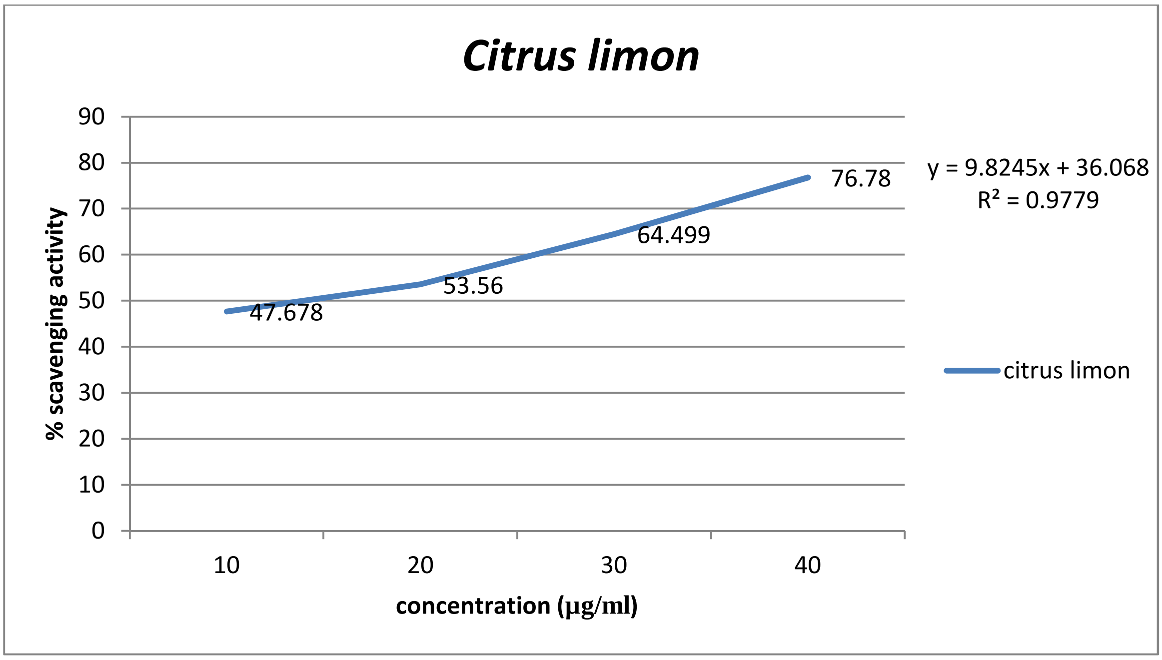


Figure III. Hydrogen peroxide scavenging activity of *Citrus limon*

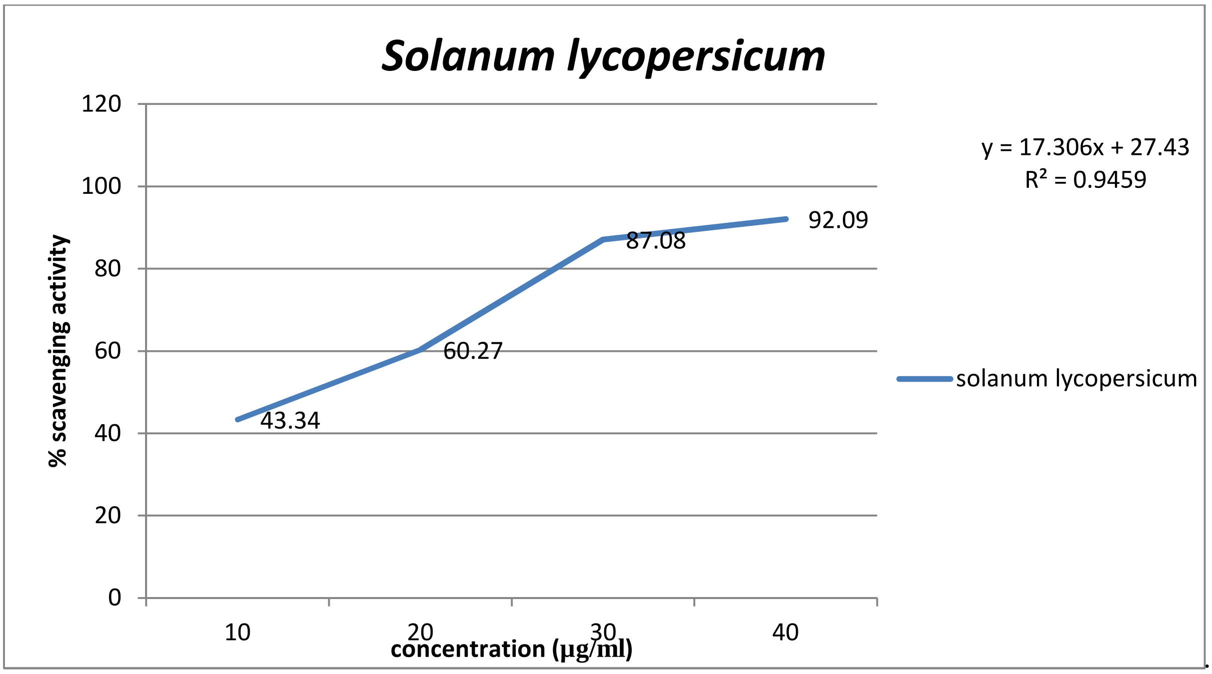


Figure IV. Hydrogen peroxide scavenging activity of *Solanum lycopersicum*

Reducing power assay method

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action. In the reducing power assay, presence of antioxidants in the samples would results in the reducing of Fe^{3-} to Fe^{2-} by donating an electron. Fig. II. shows dose-response curve for the reducing power of the extract. It was found that the reducing power of all the extract also increased with the increase of their concentrations. All extracts had shown good reducing power that was comparable with ascorbic acid. The *S. lycopersicum* and *C.limon* extracts showed significant antioxidant activity with correlation coefficient values (r) of 0.974, 0.994 and EC_{50} value of *lycopersicum* and *limon* was found to be 1.84 µg/ml and 3.48 µg /ml. The EC_{50} value of standard ascorbic acid showed EC_{50} value of 1.87µg/ml.

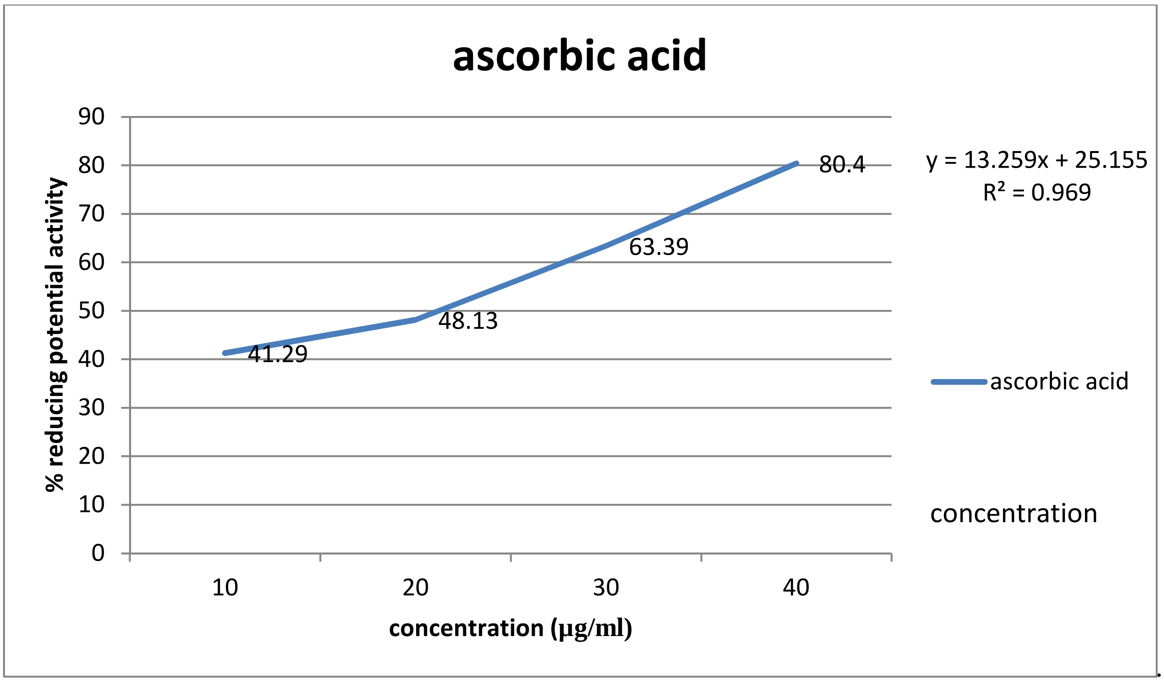


Figure V. Reducing potential activity of ascorbic acid as standard

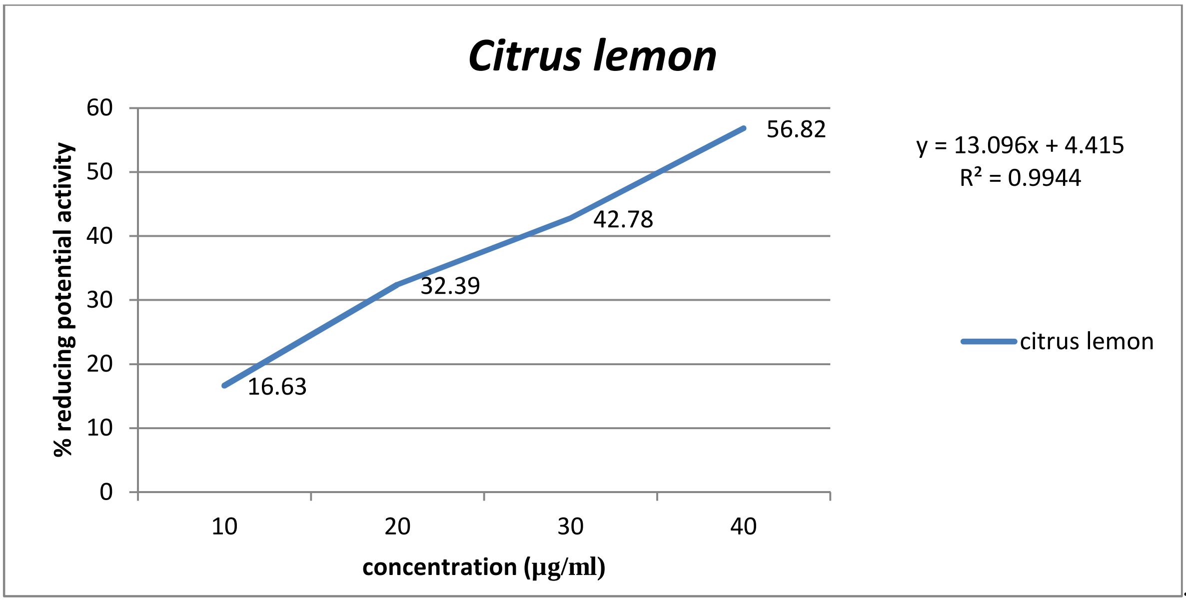


Figure VI Reducing potential activity of *Citrus limon*

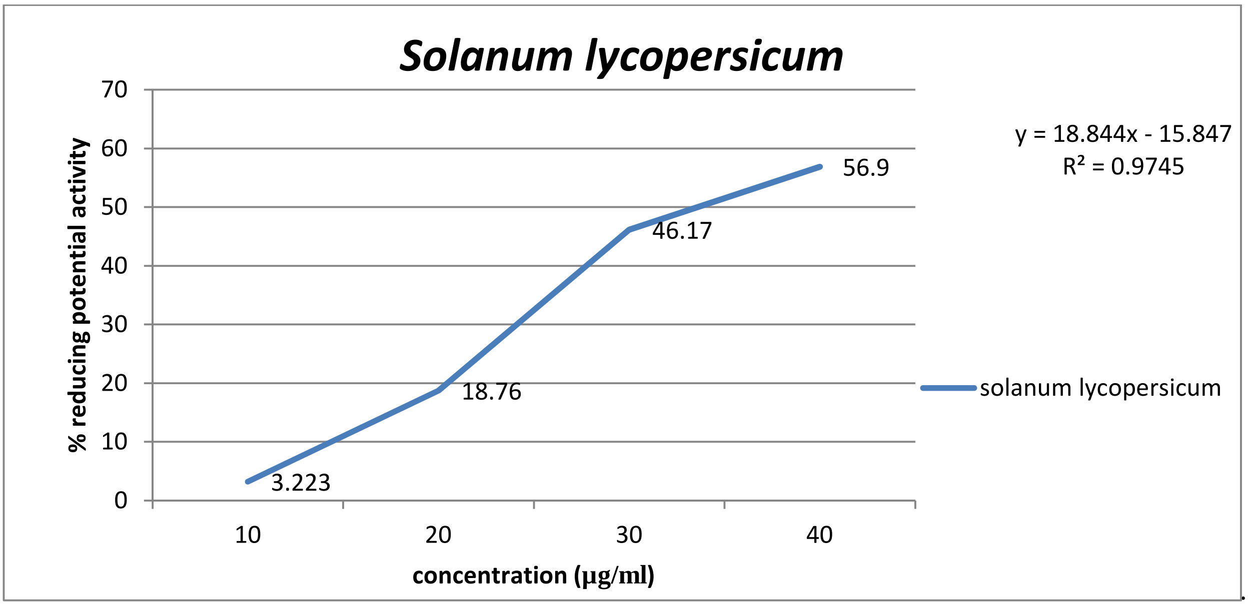


Figure VII. Reducing potential activity of *solanum lycopersicum*

CONCLUSION

This study suggests that the antioxidant activity and chelating activity of *Citrus limon* and *Solanum lycopersicum* is found to be significant when compared with the standard ascorbic acid and thus concluding that the synthetic antioxidants must be replaced by the natural antioxidants which don't have serious side effects but protect from really severe diseases which can occur and the effects of the *Citrus limon* and *Solanum lycopersicum* extract are exerted by scavenging both active oxygen species and electrophiles.

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