IN VITRO ANTIOXIDANT ACTIVITY OF AQUEOUS EXTRACTS OF *EMBLICA OFFICINALIS*, *CITRUS LIMON* & *SOLANUM LYCOPERSICON*

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***Abstract*: *Emblica Officinalis, Citrus Limon* & *Solanum Lycopersicon* all these fruits are used in our daily life. All of these plants are used in ayurvedic and herbal medications for many diseases. These plants are content of high rich amount of Vitamin C, Flavanoids, Tannins, Poly Phenolic compounds and Lycopene which are possess antioxidant activity. The aim of the present study was to evaluate the in vitro antioxidant activity of aqueous extracts of *Emblica Officinalis, Citrus Limon* & *Solanum Lycopersicon* by using DPPH radical scavenging activity and Hydrogen Peroxide radical scavenging activity. The antioxidant activity is compared with ascorbic acid as standard. The results are showed that all these extracts are possess antioxidant activity. When used the combination of these *Emblica Officinalis* plus *Citrus Limon* & *Solanum Lycopersicon* plus *Citrus Limon* are showed potent activity when compare to the individual extracts. If daily intake of these plant extracts reduces free radical generation.**

***Keywords*: Antioxidant, DPPH, Vitamin C, Lycopene and Plant extracts**

Introduction

Free radicals are found to be a product of normal metabolism. Although oxygen is essential for aerobic forms of life, oxygen metabolites are highly toxic. As a consequence, reactive oxygen species (ROS) are known to be implicated in many cell disorders and in the development of many diseases including cardiovascular diseases, atherosclerosis, chronic inflammation etc. Although organisms have endogenous antioxidant defenses produced during normal cell aerobic respiration against ROS, other antioxidants are taken both from natural and synthetic origin. Synthetic antioxidants are widely used but their use is being restricted nowadays because of their toxic and carcinogenic effects. Thus, interest in finding natural antioxidants, though any Undesirable effect has increased greatly [1].

There are various normal reactions within our bodies that produced free radicals as by products. Some of these reactions are generation of calories, the degradation of lipids, the catecholamine response under stress, and inflammatory processes. An antioxidant can be defined as any substance which significantly delays or prevents oxidation of oxidizable substrate when present at low concentration compared to that of an oxidizable substrate. There are two groups named as natural enzymatic antioxidants and non-enzymatic ones. Superoxide dismutase and catalase are natural enzymatic antioxidants that are located mostly in peroxisomes. Vitamin E, Vitamin C, BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene), carotenoids, glutathione and derivatives, phenolic compounds, flavonoids and alkaloids are natural and synthetic antioxidants. Through our diet, we can open ourselves to more antioxidants that it is extremely easiest and best way. Consuming fruits and vegetables, we can reduce the risk of oxidative damages to cells. Fruits and vegetables are very good source of natural antioxidants which consist of many different antioxidant components. Hence those are alluded to as "super foods" or "functional foods". These antioxidants are carotenoids, vitamins, phenolic compounds, flavonoids, dietary glutathione and endogenous metabolites. These function as free radical scavengers, singlet and triplet oxygen quenchers, enzyme inhibitors, peroxide decomposers and synergists. Eg: Carotenoids demonstrate photo protection that originates from their ability to quench and inactivate reactive oxygen species [2].

Plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies [3]. This plant-based, traditional medicine system playing an essential role in health care, and about 80% of the world's population relying mainly on traditional medicines for their primary health care [4]. The use of plant extracts and phytochemicals with known antioxidant properties can be of great significance in therapeutic treatments.

Antioxidant refers to a compound that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions and which can thus prevent or repair damage done to the body's cells by oxygen [5]. It act by several mechanisms such as, inhibition of scavenging activity against reactive oxygen species (ROS), reducing power, metal chelation , activity as antioxidative enzymes, inhibition of oxidative enzymes[6]. In recent years, there has been a considerable interest in finding natural antioxidants from plant materials. The antioxidant phytochemicals from plants, particularly flavonoids and other polyphenols, have been reported to inhibit the propagation of free radical reactions, to protect the human body from disease [7]. In addition, the use of synthetic antioxidants has been questioned because of their toxicity [8]. Based upon previous study we selected some home remedies which are act as antioxidant activity. They are *Emblica Officinalis*, *Citrus Limon* & *Solanum Lycopersicon* fruit extracts

Methodology

Plant material

The plant samples were Amla (Fruit), Lemon (Fruit) and Tomato (Fruit) commonly cultivated in South India was undertaken for this study. These procured from the local market and identified in the laboratory Based on local names and pharmacognosy literature.

Apparatus and chemicals

In this study involved the use of a UV-VIS spectrophotometer and centrifuge.

All reagents used were of analytical grade.

DPPH (1,1-Diphenyl-2-picryl hydrazyl) , Ascorbic acid, Dimethyl Sulphoxide, Methanol, Hydrogen Peroxide, Potassium Dihydrogen Phosphate, Potassium Hydroxide, Phosphate Buffer Saline (PBS, Ph 7.4)

Aqueous extractions of Plant materials

- **Aqueous extraction of Amla (Fruit)**

Extracts of Amla were prepared by homogenizing 5 g of each material in 50 ml of distilled water, filtering them through a Whattman filter paper, and centrifuging them at 3,000 rpm at room temperature for 10 minutes. The clear supernatant thus obtained was used for the antioxidant activity [29].

- **Aqueous extraction of Lemon (Fruit)**

The fresh fruits were washed in running tap water in laboratory, surface sterilized with 70% alcohol, rinsed with sterile distilled water and cut open with a sterile knife and the juice pressed out into a sterile universal container separately and then filtered (using Millipore 0.45 filter paper) into another sterile container to remove the seeds and other tissues and used freshly as crude and then centrifuged with 3000rpm at room temperature for 10 minutes. The clear supernatant thus obtained was used for the antioxidant activity [30].

- **Aqueous extraction of Tomato (Fruit)**

In *Solanum lycopersicum* the pulp and the serum is separated. It is further filtered using the Whattman filter paper. It is also micro centrifuged for 10 minutes at 3000 rpm giving an upper layer of crude extract [31].

Phytochemical Screening

The extracts were subjected to various phytochemicals tests to determine the active constituents present in the all Aqueous extracts. It showed the presence of citric acid, Ascorbic acid, minerals, flavonoids, essential oils, carbohydrates, vitamins, tannins are the major components are present in the samples.

In vitro methods [32]

- **DPPH radical scavenging activity**

The antioxidant activity of the aqueous extract was determined on the basis of their scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. 1 ml of various concentrations of the extracts in methanol was added to 4 ml of a 4 mg/100 ml (0.004% w/v) methanol solution of DPPH. After 30 minutes the absorbance of the preparations were taken at 517 nm by a UV spectrophotometer which was compared with the corresponding % inhibition of standard ascorbic acid concentrations (10-100 µg/ml. Ascorbic acid (AA) was used as positive controls. The free radical scavenging activity (FRSA) was calculated by using the following equation:

$$\% \text{ Inhibition DPPH Scavenging activity} = [(A0 - A1)/A0] \times 100$$

Where A0 was the absorbance of the control and

A1 was the absorbance in the presence of the sample of extract and standard

- **Scavenging of Hydrogen Peroxide**

Hydrogen peroxide is the most stable ROS and may be generated directly by divalent reduction of O_2 or indirectly by univalent reduction of O_2 by numerous oxidases, such as xanthine oxidase, uricase, and α-hydroxy acid oxidase localized in the peroxisome. H_2O_2 is decomposed to H_2O and O which can induce cell injury and cause DNA damage in the form of chromosomal aberrations rather than superoxide ions.

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4).The extracts (10- 100 µg/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:

$$\% \text{ Inhibition of } H_2O_2 \text{ Scavenging activity} = [(A0 - A1)/A0] \times 100$$

Where A0 was the absorbance of the control and

A1 was the absorbance in the presence of the sample of extract and standard

Preparation of DPPH solution

4.3mg of DPPH was dissolved in 3.3 ml methanol: it was protected from light by covering the test tubes with aluminum foil.

Standard Ascorbic acid solution – 1 mg/ml in methanol.

Preparation of Hydrogen Peroxide -4.53 g in 1000 mL is 40 mM

Results & Discussion

Antioxidants protect cells against damage caused by molecules known as free radicals the antioxidant effects of plant extracts are mainly due to the presence of phenolic compounds such as flavonoids, phenolic acids, & tannins. Phenolic are the largest group of phytochemicals and have been touted as accounting for most of the antioxidant activity of plants or plant products.

In the present study we observed the reduction capacity of DPPH radical which is induced by antioxidant was determine by the decrease in its absorbance. It is visually noticeable as a change in color from purple to yellow. Hence, DPPH is usually used as a substance to evaluate the antioxidant activity. In the DPPH free radical scavenging assay, all aqueous extract preparations were evaluated for their free radical scavenging activity compared with ascorbic acid as standard compound. The radical scavenging activity of all aqueous extractions increased with increasing concentrations respectively. The scavenging effect increased with the increasing concentrations of test compound. These all results showed that all aqueous extracts are exhibit the Free radical scavenging activity by using DPPH method. In the same manner the extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner.

Lemon

Citrus fruits are rich sources of useful phytochemicals, such as vitamins A, C and E, mineral elements, flavonoids, coumarins, limonoids, carotenoids, pectins, and other compounds (Zhou, 2012). These phytochemicals, consumed through fresh fruits or their derived products, have been suggested to have a wide variety of biological functions including antioxidant, antiinflammation, antimutagenicity, anticarcinogenicity and anti-aging to human health (Ke et al., 2015; Rajendran et al., 2014; Zhang et al., 2015).

Citrus fruits may exert their antioxidant capacity by inhibiting the oxidant enzymes via the bioactive compounds they contain Oxidant enzymes, such as nitric oxide synthase (NOS), lipoxygenase (LOX), xanthine oxidase (XO), cyclooxygenase (COX), NADPH oxidase (NOX), and myeloperoxidase (MPO), have played important roles in redox reactions of a biological system, and are also the main promoters of cellular ROS/RNS (López-Alarcón & Denicola, 2013). The inhibition of XO has been suggested to be one of the key mechanisms of antioxidant action in natural products (López-Alarcón & Denicola, 2013). In Citrus, Nakao et al. (2011) found that hesperetin can directly decrease cellular free radical production by inhibiting XO. Lin et al. (2008) reported that coumarins can directly decrease cellular free radical production by inhibiting XO. Therefore, polyphenols and other phytochemicals potentially contribute to the antioxidant capacity of Citrus fruits.

Amla

Amla is one of the richest sources of vitamin-C and low molecular weight hydrolysable tannins which make Amla a good antioxidant. The tannins of amla like emblicanin-A (37%), emblicanin-B (33%), punigluconin and pedunculagin are reported to provide protection against oxygen radical included haemolysis of rat peripheral blood erythrocytes.38 The mechanism behind antioxidant activity is due to the recycling of sugar moiety and conversion of the polyphenol into medium and high molecular weight tannins. The powerful antioxidant Ellagic acid, present in *Amla*, can inhibit mutations in genes and repairs the chromosomal abnormalities.

The amla fruit contains more than 80% water. It also has protein, carbohydrate, minerals and vitamins. Amla restores the vitality and rejuvenates all body systems. It is a rich source of vitamin C and has been used as a powerful antioxidant agent which also boosts immunity. Vitamin C is important for human beings as it is necessary for the synthesis of intercellular cement "collagen". Collagen is responsible for keeping the cells of the body together. Hence, vitamin C helps to preserve the normal immune function and promotes rejuvenation of cells.

Nature has gifted us with defensive antioxidant mechanisms- superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), GSH peroxidase, reductase, vitamin E (tocopherols and tocotrienols), vitamin C, etc., along with several dietary components. Higher consumption of

Components/nutrients with antioxidant capabilities have been associated with lower frequency of numerous human morbidities or mortalities are per many epidemiological studies. Diverse potential applications of antioxidant/free radical manipulations in prevention or control of disease has been revealed by ongoing research. Natural products from dietary components such as Indian spices and medicinal plants are known to possess antioxidant activity. The study by Poltanov *et al*., investigated the chemistry and antioxidant properties of *E. officinalis* fruit extracts. Extracts produced positive responses in the total phenol, total flavonoid and total tannin assays. Reddy *et al*., suggested that the amelioration of alcohol-induced oxidative stress might be due to the combined effect of phytophenols such as tannins, flavonoid compounds and vitamin C. Shivananjappa *et al*., demonstrated that *E. officinalis* aqueous extracts have potency to modulate basal oxidative markers and enhance endogenous antioxidant defenses using a

hepatocyte cell line (HepG2). Substantial reduction in the levels of lipid hydroperoxide and reactive oxygen species (ROS) was observed in the study that incubated *E. officinalis* for 24 h. Moreover, *E. officinalis* increased the levels of GSH, antioxidant capacity and activities of antioxidant enzymes (SOD; CAT; GSH peroxidase; GSH reductase; and GSH S-transferase).Additionally, when administered once daily for 7 days the active tannoids of *E. officinalis* induced a rise in both frontal cortical as well as striatal SOD, CAT and GSH peroxidase (GPX) activity, with associated reduction in lipid peroxidation in these brain areas. The results also specify that the antioxidant activity of *E. officinalis* may reside in the tannoids of the fruits of the plant, which have vitamin C- like properties, rather than vitamin C itself [33, 34].

Tomato

The tomato juice possessed good quantities of lycopene and polyphenols, which exhibited antioxidant activity and hence can be utilized in food preparations for enhancing their functional properties. The study also favors for the production of a value added novel spread product from the local tomato with lower total soluble solids and higher acidity. The spread was also found to be rich in total polyphenols and lycopene content. Hence, the processing of local tomatoes will help to increase the economy of producers which in turn improve the health of consumers.

Tomatoes contain a great deal of Vitamin A and Vitamin C. This is primarily because these vitamins and beta-carotene work as antioxidants to neutralize harmful free radicals in the blood. Free radicals in the blood stream are dangerous because it may lead to cell damage. Remember, the redder the tomato you eat is, the more beta-carotene it contains. In addition, you also want to Keep in mind that cooking destroys the Vitamin C, so for these benefits, the tomatoes need to be eaten raw.

Reactive oxygen species (ROS) contribute to a great variety of diseases. ROS including hydrogen peroxide, super oxide radical anion, nitric oxide and singlet oxygen react with biological molecules leading to cell and tissue injury . Plants exhibit efficient antioxidant activity owing to their phenolic constituent. It is believed that lycopene is a powerful antioxidant, a compound that blocks the action of activated oxygen molecules, known as free radicals that can damage cells. It is reported in the literature the antioxidant activity of animal models. These results are important because lycopene is at least twice as great as beta carotene, *S. lycopersicum* (tomato) is one of the important another carotenoid that is also thought to be an effective vegetable used in Indian diets[35].

From above graphs and tables the Free radical scavenging activity of aqueous extract of Lemon, tomato and Amla when compared to the ascorbic acid the values are observed as all the extracts are posses the antioxidant activity in both DPPH and H_2O_2 assay. From the above graphs with combination of aqueous extracts of lemon plus amla and tomato plus lemon shows potent antioxidant activity than individual use of extracts of amla, tomato and lemon.

Recent reports indicate that increased dietary intake of antioxidant-rich foods decreases the incidence of human diseases. However, synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) that have been widely used as antioxidants in the food industry may be responsible for liver damage and carcinogenesis. For this reason, the use of natural antioxidants with lesser side effects, are preferred.

Conclusion

Now a days, research on Indian traditional medicinal plants has gained a new recommence. Although, the other systems of medicine are effective they come with a number of undesired effects that often lead to serious complications. Being natural, herbal medicine alleviates all these problems. *Emblica officinalis*, *Citrus limon* and *Solanum lycopersicum* has an important position in Ayurveda- an Indian indigenous system of medicine. *Emblica officinalis*, *Citrus limon* and *Solanum lycopersicum* due to its strong antioxidant and biological properties prevent innumerable health disorders as it contains essential nutrients and highest amount of vitamin C, lycopene, flavonoids, tannins and polyphenols. It can be used as a possible food additive or in nutraceuticals and biopharmaceutical industries.

This study suggests that the antioxidant individual and combination of these *Emblica officinalis*, *Citrus limon* and *Solanum lycopersicum* extracts were 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. These finding suggest that these plants are potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of ageing and age associated oxidative stress related degenerative. Further studies are required for the isolation and

Characterization of antioxidant components and also in vivo studies are needed for understanding their mechanism of action as an antioxidant better.

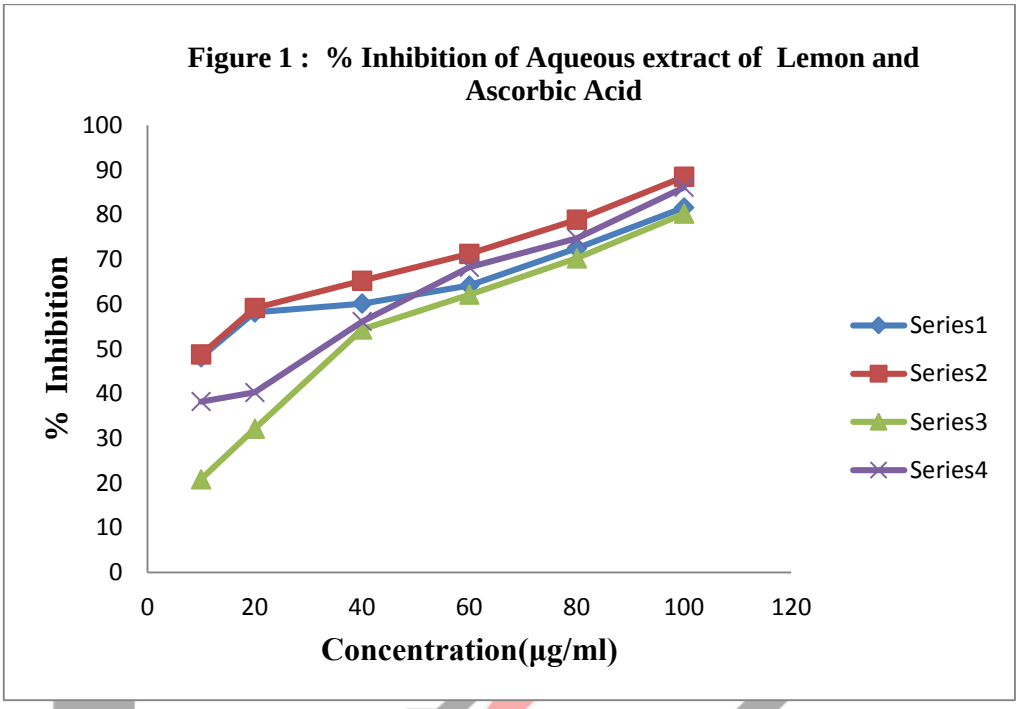
References

[1] Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, and Morelli I. Antioxidant principles from Bauhinia. Terapotensis. Journal of Natural Products 64, 2001, 892–895.
[2] Nirmala Ravimannan and Asiri Nisansala , Study on antioxidant activity in fruits and vegetables – A Review Int. J. Adv. Res. Biol. Sci. (2017). 4(3): 93-101.
[3] Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected Indian medicinal flora. Turk J Biol. 2005; 29: 41-47.
[4] Owolabi J, Omogbai EKI, Obasuyi O. Antifungal and antibacterial activities of the ethanolic and aqueous extract of Kigelia africana (Bignoniaceae) stem bark. Afr. J. Biotechnol. 2007; 6 (14): 882-885.
[5] Gislene GF, Nascimento, Juliana Locatelli, Paulo C. Freitas, Giuliana Silva, Antibacterial activity of Plant extracts and Phytochemicals on Antibiotic Resistant bacteria. Brazilian Journal of Microbiology. 2000; 31: 247-256.

[6] Tachakittirungrod, Siriporn O, Sombat C. Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract. Food Chemistry. 2007; 103: 381–388.
[7] Shahidi F. Natural antioxidants. An overview: Natural antioxidants, chemistry, health effects and applications. Champaign, IL.1997; AOCS Press: pp. 1–11.
[8] Valentao P, Fernandes E, Carvalho F, Andrade PB, Seabra RM, Bastos M. Antioxidative properties of cardoon (Cynara cardunculus L.) infusion against superoxide radical, hydroxyl radical and hypochlorous acid. Journal of Agricultural and Food Chemistry, 2002; 50: 4989–4993.
[9] HLSharma,KKSharma Principles of pharmacology 2nd Ed,p667-670
[10] What Is Oxidative Stress? JMAJ 45(7): 271–276, 2002
[11] DM Vasudevan, Text Book of Biochemistry 7th Edition p436-438
[12] Free radicals, oxidative stress, and diseases Enrique cadenzas psc 616 page 3-4
[13] Langseth L. Oxidants,Anti-oxidants, and disease prevention. Belgium, International life science institute,1996
[14] journal of Indian academy of oral medicine and radiology, July-September 2011;23(3):S351-353
[15] Sies, Helmut (1997). Oxidative stress: Oxidants and antioxidants Experimental physiology 82 (2): 291–5.
[16] Herrera, E.; Barbas, C. (2001). "Vitamin E: Action, metabolism and perspectives".Journal of Physiology and Biochemistry 57 (2): 43–56.
[17] Packer, Lester; Weber, Stefan U.; Rimbach, Gerald (2001). Molecular aspects of alpha tocotrienol antioxidant action and cell signalling. The Journal of nutrition 131 (2): 369S–73S.
[18] Traber, Maret G.; Atkinson, Jeffrey (2007).Vitamin E, antioxidant and nothing more .Free Radical Biology and Medicine 43 (1): 4–15.
[19] Stanner SA, Hughes J, Kelly CN, Buttriss J (2004). "A review of the epidemiological evidence for the 'antioxidant hypothesis'". Public Health Nutr 7 (3): 407–22.
[20] Food, Nutrition, Physical Activity, and prevention of cancer: a global perspective world cancer research Fund (2007).
[21] Bhandari PR, Kamdod MA. Emblica officinalis (Amla): A review of potential therapeutic applications. International Journal of Green Pharmacy (IJGP). 2012;6(4).
[22] Silalahi J. Anticancer and health protective properties of citrus fruit components. Asia Pac J Clin Nutr 2002;11:79-84.
[23] Bertuzzi G, Tirillini B, Angelini P, Venanzoni R. Antioxidative action of Citrus limonum essential Oil on skin. Eur J Med Plants 2013;3:1-9
[24] Hiroyuki. T, Tetsuro.K, Masayuki, Afework.K, Fusao.O..Antimicrobial activity of citrus fruits juice against vibrio species, 2006; 52(2): 157-160.
[25] Silaste ML, Alfthan G, Aro A et al. Tomato juice decreases LDL cholesterol levels and increases LDL resistence ot oxidation. Br J Nutr 2007; 98: 1251-1258.
[26] Blum A, Merei M, Karem A et al. Effects of tomatoes on the lipid profile. Clin Invest Med 2006;29:298-300.
[27] Blum A, Monir M, Wirsansky I, et al. The beneficial effects of tomatoes. Eur J Intern Med 2005; 16: 402-404.
[28] Periago MJ, Jacob K, Boehm V et al. Influence of lycopene and vitamin C from tomato juice on biomarker of oxidative stress and inflammation. Br J Nutr 2008; 99:137-146.
[29] Jose JK, KUTTAN R. Antioxidant activity of Emblica officinalis. Journal of clinical biochemistry and nutrition. 1995;19(2):63-70.
[30] Hindi NK, Chabuck ZA. Antimicrobial activity of different aqueous lemon extracts. Journal of Applied Pharmaceutical Science. 2013 Jun 1;3(6):74.
[31] Rashmi Dahima et al Der In-vitro antioxidant and reducing potential activity of extracts of Citrus Limon and Solanum Lycopersicum Pharmacia Lettre, 2016, 8 (10):258-269.
[32] Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharmaceutical Journal, 21(2), 143-152.
[33] Dewanjee S, Maiti A, Majumder R, Majumder A. Evaluation of antimicrobial activity of hydroalcohalic extract of Schma wallichii bark. Pharmcolologyonline. 2008; 1: 523-528.
[34] Pandey, Govind, Some important anti-cancer herbs: A review, International Research Journal of Pharmacy, 2(7), 2011, 45-52.
[35] Balaswamy K, at al., (2015) Antioxidant Activity of Tomato (Lycopersicon esculentum L.) of Low Soluble Solids and Development of a Shelf Stable Spread. Int J Food Sci Nutr Diet. 04(4), 202-207.

Table1: Free radical scavenging activity of aqueous extract of Lemon and ascorbic acid

S.No	Concentration (µg/ml)	DPPH (% Inhibition)		Hydrogen peroxide (% Inhibition)	
		Aqueous extract	Ascorbic acid	Aqueous extract	Ascorbic acid
1	10	48.12	48.72	20.84	38.21
2	20	58.14	59.12	32.12	40.24
3	40	60.12	65.24	54.36	56.12
4	60	64.16	71.26	62.12	68.24
5	80	72.46	78.86	70.24	74.68
6	100	81.58	88.46	80.24	86.12



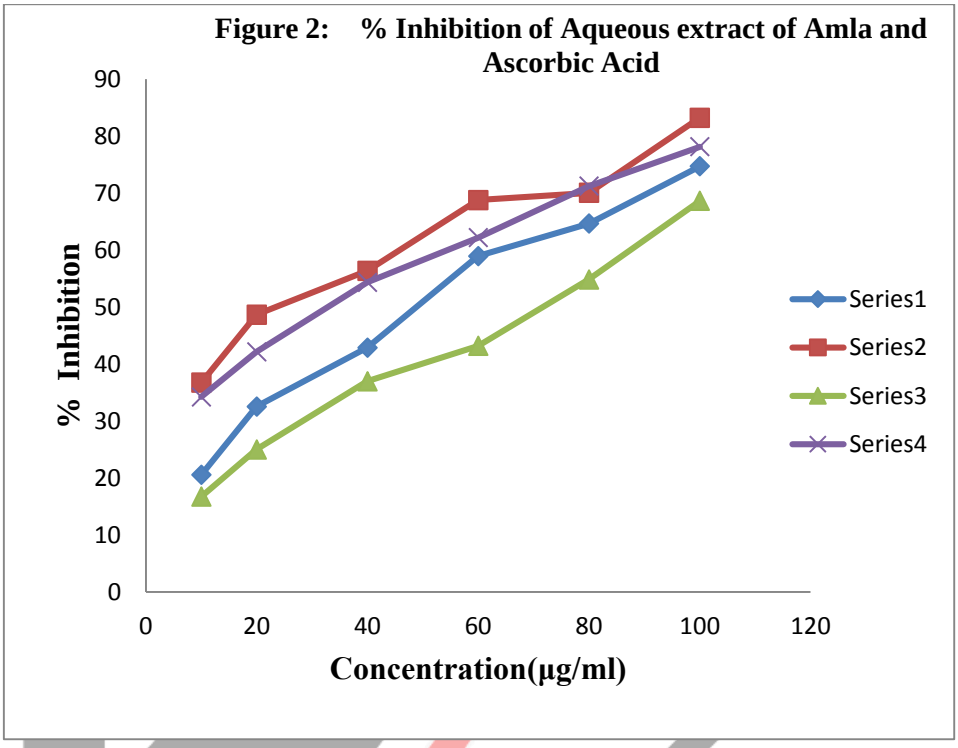
Series 1 % inhibition of Test by DPPH **Series 3** % inhibition of Test by H_2O_2

Series 2 % inhibition of Standard by DPPH **Series 4** % inhibition of Standard by H_2O_2

Table2: Free radical scavenging activity of aqueous extract of Amla and ascorbic acid

S.No	Concentration (µg/ml)	DPPH (% Inhibition)		Hydrogen peroxide (% Inhibition)	
		Aqueous extract	Ascorbic acid	Aqueous extract	Ascorbic acid
1	10	20.52	36.70	16.70	34.21
2	20	32.53	48.66	24.99	42.13
3	40	42.87	56.35	36.97	54.34
4	60	58.94	68.77	43.17	62.18
5	80	64.64	70.05	54.83	71.23
6	100	74.71	83.21	68.63	78.12

Figure 2: % Inhibition of Aqueous extract of Amla and Ascorbic Acid



Series 1 % inhibition of Test by DPPH **Series 3** % inhibition of Test by H_2O_2

Series 2 % inhibition of Standard by DPPH **Series 4** % inhibition of Standard by H_2O_2

Table3: Free radical scavenging activity of aqueous extract of Tomato and ascorbic acid

S.No	Concentration (µg/ml)	DPPH (% Inhibition)		Hydrogen peroxide (% Inhibition)	
		Aqueous extract	Ascorbic acid	Aqueous extract	Ascorbic acid
1	10	30.52	48.70	26.70	31.21
2	20	38.53	51.66	28.99	38.13
3	40	52.87	55.35	33.97	45.34
4	60	63.94	64.77	38.17	52.18
5	80	69.64	70.05	45.83	64.23
6	100	78.71	81.21	62.63	70.12

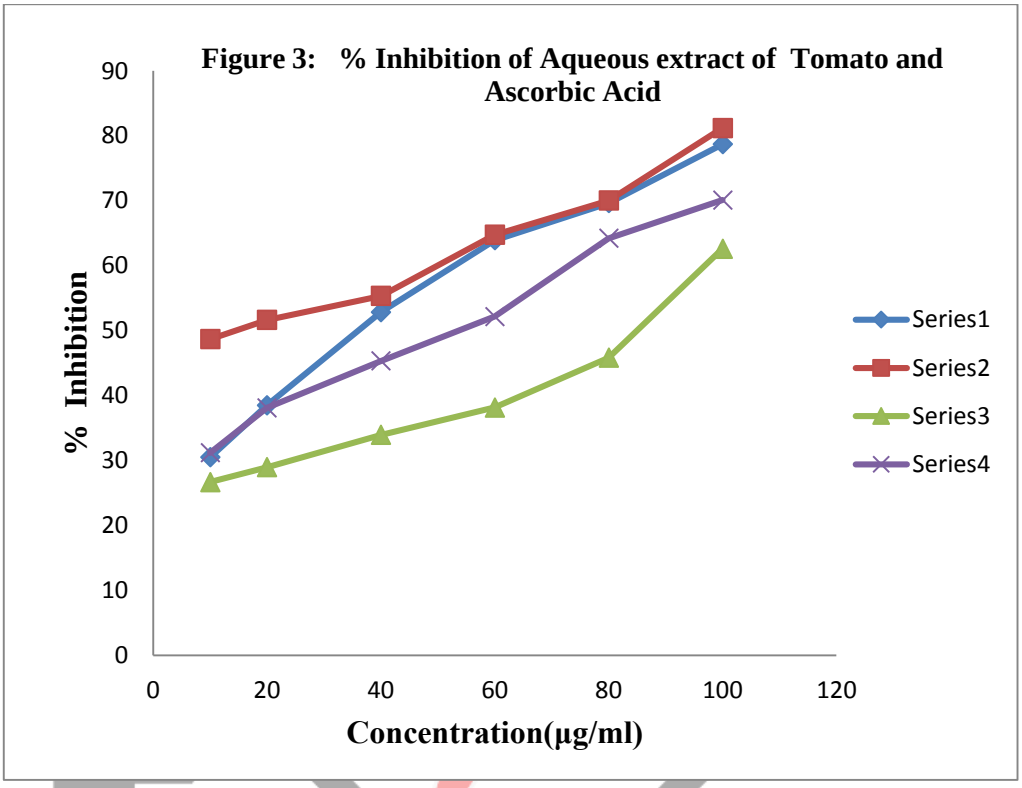


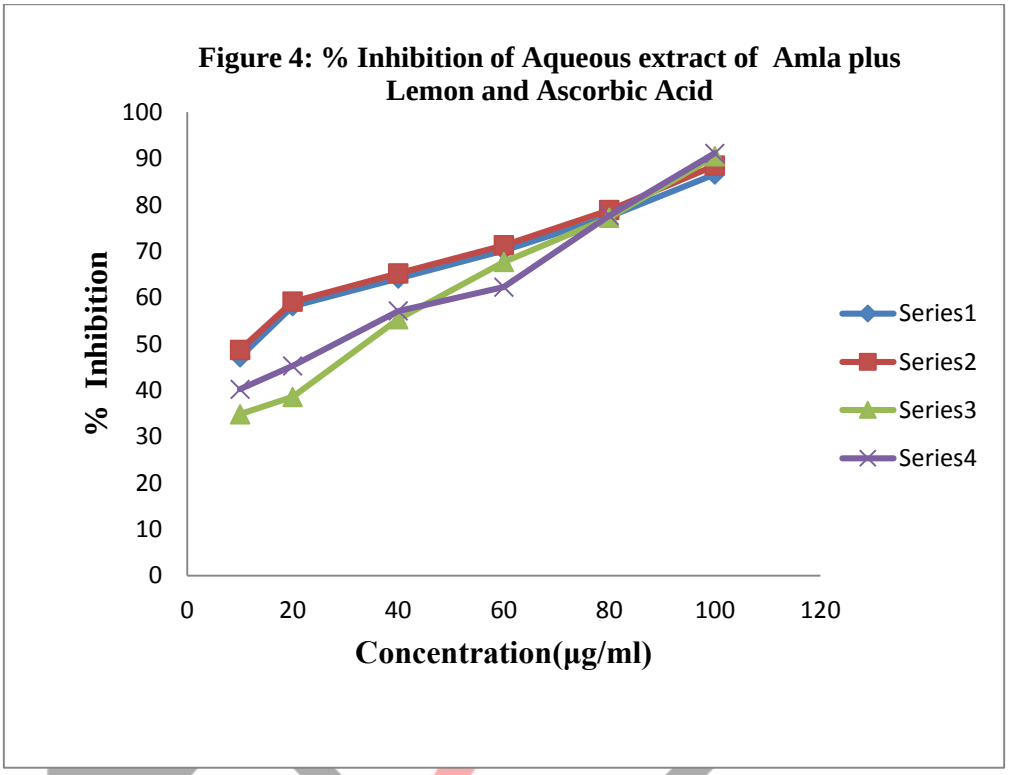
Figure 3: % Inhibition of Aqueous extract of Tomato and Ascorbic Acid

Series 1 **% inhibition of Test by DPPH** **Series 3** **% inhibition of Test by H_2O_2**

Series 2 **% inhibition of Standard by DPPH** **Series 4** **% inhibition of Standard by H_2O_2**

Table 4: Free radical scavenging activity of aqueous extract of Amla Plus Lemon and ascorbic acid

S.No	Concentration (50:50 µg/ml)	DPPH (% Inhibition)		Hydrogen peroxide (% Inhibition)	
		Aqueous extract	Ascorbic acid	Aqueous extract	Ascorbic acid
1	10	47.12	48.70	34.84	40.21
2	20	58.14	59.12	38.56	45.24
3	40	64.12	65.24	55.36	57.12
4	60	70.16	71.26	67.68	62.24
5	80	77.46	78.86	77.24	77.68
6	100	86.58	88.46	90.46	91.12

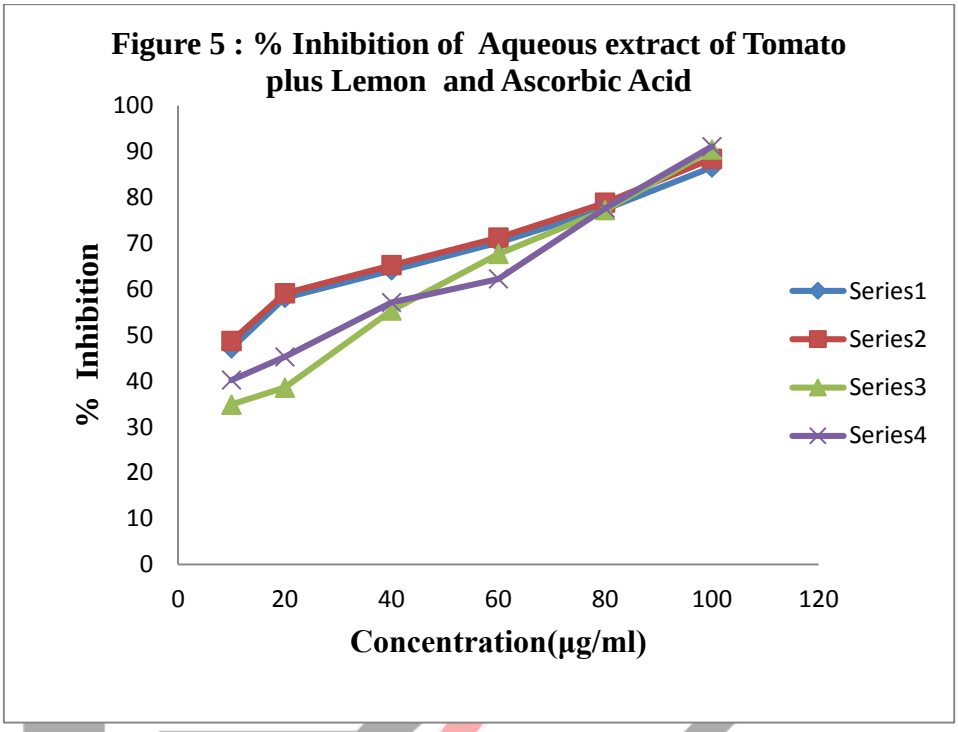


Series 1 % inhibition of Test by DPPH **Series 3** % inhibition of Test by H_2O_2

Series 2 % inhibition of Standard by DPPH **Series 4** % inhibition of Standard by H_2O_2

Table 5: Free radical scavenging activity of aqueous extract of Tomato Plus Lemon and ascorbic acid

S.No	Concentration (50:50 µg/ml)	DPPH (% Inhibition)		Hydrogen peroxide (% Inhibition)	
		Aqueous extract	Ascorbic acid	Aqueous extract	Ascorbic acid
1	10	29.26	30.21	36.84	37.21
2	20	41.46	42.84	44.12	45.24
3	40	53.68	54.24	57.36	58.12
4	60	65.16	68.64	67.68	68.24
5	80	73.46	74.86	73.24	74.68
6	100	85.58	86.24	83.46	84.12



Series 1 % inhibition of Test by DPPH **Series 3** % inhibition of Test by H_2O_2

Series 2 % inhibition of Standard by DPPH **Series 4** % inhibition of Standard by H_2O_2