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Observation of Phytochemicals, Total Phenolic, Total Flavonoid and DPPH content assay *Lemon (Citrus*) Peel for Importance in medicinal uses

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ABSTRACT

Background

In nanocarriers, the herbal medicine is molded to fuel growing demand in a medicinal age for different fields to amplify therapeutic benefit. The goal of this analysis was to screen citrus lemon waste peel hydro alcoholic extract to show potent phytochemicals, complete phenolic and flavonoid antioxidant DPPH content to find current sources for potential novels.

Methods

Various fractions of lemon waste peel prepared on the basis of their polarity and screened for phytochemical groups by solvent- solvent extraction method, total phenolic (TPC), flavonoid (TFC) material. The in vitro DPPH assay has demonstrated the antioxidant effects of the extracts.

Results

The existence of proteins, amino acids, sugars, alkaloids, flavonoids, phenolic etc useful phytochemicals was assured by tests screening separate fractions of lemon extract. Quantitative analyses found that aqueous fractio ns showed impressive results for TPC (138.2436003±0.996), TFC (56.28301384±1.384) and DPPH assay amo unts (62.18).

Conclusion

The existence of different phytochemicals was suggested by our observations. The quantitative DPPH assay shows that the extract of the plant has a strong antioxidant activity that can be an excellent biological and chemical analysis choice.

Key words – *citrus* (*Lemon);* phytochemical's; TPC; TFC; Antioxidant.

Abbreviation-

TPC- Total phenolic content

TFC- Total flavonoids content

D/W- Dilution water

D.F- Dilution factor

M- Mole

ml- Milliliter

ppt- Precipitate

UV- Ultraviolet

Cons. Constant

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1. INTRODUCTION

Medicinal plant ingestion prevents and cures a variety of illnesses, and in ancient times they were the primary treatment therapy until the invention of prescription medicines in the 19th century (Rao et al. 2014). This identification of natural products that are biologically active played an important part in the discovery of new chemical entities (NCEs). India is the largest producer of medicinal herbs and is properly referred to as the global botanical garden (Sharrif et al. 2006). For decades, citrus fruits have been used as traditional Asian medicine because they are considered a rich source of secondary metabolites that have a wide variety of biological activities (Kalpa et al. 2012).

In the food industry, citrus fruits such as orange, lemon and lime are primarily used for the manufacture of fresh juice or beverages based on citrus. As a consequence, citrus fruit peels are produced as waste, although they contain a lot of flavonoids (Pfaltzgraff et al. 2013). Lemon is one of the world's most popular cultivated commercial citrus fruit varieties. This is a perennial evergreen subtropical plant of the citrus genus of the rutaceae family and the aurantioideae subfamily, comprising 130 genera in seven subfamilies. It is a rich vitamin C source and has peels that are uniquely aromatic. Its peel extracts are high in bioactive additives and are potential antioxidants (Karimi et al. 2012). A broad range of antioxidant, antibacterial (Suja. et al. 2017), antifungal (Johann et al. 2007), antimicrobial (Rana et al. 2017) and anticancer (Dhanavade. et al. 2011) activities have been found in their peel extracts.

In the current scenario, attention has been focused on industrial waste, in particular those containing residual phenols from the raw material of the plant used. In view of the medicinal and therapeutic values of Citrus limon, the present study was carried out in an attempt to make use of the peel that is usually thrown out as waste in a beneficial way. This paper presents a detailed phytochemical analysis of the lemon peel, Total phenolic content (TPC), Total flavonoid content (TFC) and antioxidant DPPH.

2. MATERIALS AND METHODS

2.1 Collection of samples

Lemon peels from the local market and lemon juice shops of Bhopal, M.P., India were collected in the summer season. The peels were properly cleaned with natural water and then properly removed with purified water and then with extra pulp. The peels were then dried for 12-15 days at an ambient temperature for shade. Second, dried lemon peels were coarsely used with a mortar and pestle and then a mechanical blender was used to ground them further.

2.2 PREPARATION OF PLANT EXTRACTS

Thirty gm. 340 ml of organic solutions of ethanol & D.W. were collected from the sample (lemon peel dry powder). Extraction at Soxhlet. The extraction was completed in 3 days at 65 C. In order to form a paste, extracts were then evaporated at 45 C and further transferred to sterile and refrigerated once used.

3. Qualitative analysis Of Plant extract

The existence of numerous phytoconstituents such as carbohydrates, proteins, flavonoids, glycosides, hormones, alkaloids, tannin and phenolic compounds was qualitatively studied in the Hydro-Alcoholic lemon peel extracts. The qualitative phytochemical screening tests (Kokate et al. 1993), (Trease & Evans. 1989). They performed the following qualitative tests:

3.1 Test for carbohydrate:

Molisch’s reagent was added to 2-3 ml of aqueous plant extract. For 2-3 ml of aqueous plant extract, Molisch's reagent was added. Naphthol solution alcohol was added to a limited volume and the mixture was shook well, then added cons. On the sides of the sloping test tube, sulphuric acid (H2SO4) is applied to allow it to stand for 3-5 minutes. Violet ring is located at the junction of 2 solution suggests the presence of carbohydrate.

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Naphthol solution alcohol was added to a limited volume and the mixture was shook well, then added cons. On the sides of the sloping test tube, sulphuric acid (H2SO4) is applied to allow it to stand for 3-5 minutes. At the intersection of 2 solutions, the violet ring shows the presence of carbohydrate.

3.2 **TEST FOR ON REDUCING POLY SACCHROIDES**

(a) Iodine test: 2-3 ml of assay solution and a few drops of iodine solution is dissolved. The presence of iodine is suggested by the blue precipitate pigment.

(b) Tannic acid test for starch: Slowly apply 20% tannic acid to the 2-3ml test solution and a few drops combined. If the PPT in the tube is shown, it means that the test is positive.

3.3 TEST FOR PROTEINS:

(a) Biuret Test: The test solution was treated with 4% NaoH solution and 2-3 drops of 1% Cuso4 and observed violet or pink color formation.

(b) Million's test: Add 1 to 2 ml of concentrate, and a few drops of reagents from Million. The existence of protein is suggested by White ppt.

3.4 TEST FOR AMINO ACID

(a) Cysteine test: 5ml test solution, a few drops of 40 percent NaoH and 10 percent lead acetate solution are placed in the test tube and then further heated for 10-15 minutes in boiling water in 40-45 C if the presence of amino acid is shown after 10-15 minutes when black ppt is seen.

3.5 TEST FOR STEROID

(a) Salkowski test: TO1-2 ml of extract, 2 ml of chloroform and 2 ml of Contras. Then shake well with H2SO4. If the chloroform coating is red and the layered sulfuric acid displays greenish yellow fluorescence, the presence of steroids is shown.

3.6 TEST FOR GLYCOSIDES:

The compound formed in the sugar molecule by removing the hydroxyl group. -glycosides

(a)Killer killani test: Added 3 ml of test solution extract in a test tube and added 1.5 ml of glacial acetic acid, then mixed 5 percent fecl3 and kept for some time after adding 1 ml of Cons. From H2So4. If the reddish brown colour appears at the junction of the 2 liquid layers and the bluish green rings appear at the top layer, the presence of de-oxy sugar is indicated.

3.7 TEST FOR ANTHRAQUINONE GLYCOSIDES

(a) Borntrager test: Add 5 ml of test solution and dilute H2SO4 to boil for 10-12 minutes in a water bath and filter. Add equal amount of chloroform shake after filtrate well then separate added ammonia. If the ammonia layer turns pink and red, this means the presence of anthraquinone glycosides.

3.8 TEST FOR COUMARINS GLYCOSIDES: 2 ml of 10 percent sodium hydroxide was added to the 1-2 ml test solution. The presence of coumarins is shown by the formation of a yellow color that fluoresces under ultra violet light (Sangeeta et al., 2015)

3.9 TEST FOR FLAVONOIDES:

(a) Shinoda test: Add 2-3 ml of test solution to the test tube and 1 ml of CONS. A few HCL drops. Add a 4 to 5 Mg ribbon after 5 minutes. Orange, red pink, or purple color formation indicates the presence of flavonoids.

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3.10 ALKAOIDES TEST:

(a) Wagner's test: Wagner's reagents added to 1-2 ml filtrate solution and a few drops if seen radish brown Precipitate confirms the test as positive.

3.11 TEST FOR TANNIN COMPOUND:

(a) Acetic acid test: 2 ml of test solution with the addition of acetic acid solution. The red color formation indicated the presence of tannins.

(b) Lead acetate test: 2 ml evaluation solution and applied a solution of lead acetate. White ppt formation suggests the presence of tannins.

3.12 TEST FOR PHENOLIK COMPOUND:

(a) Iodine test: 2 ml of test solution and iodine solution in the test tube is applied. The presence of phenol was suggested after 2-4 minutes of red colour formation.

3.13 TEST FOR ORGANIC ACID

(1) Confirmatory Oxalic Acid Test:

(a) Lead acetate test:
A 2 ml test solution and a few drops of 5 percent lead acetate were added. The presence of oxalic acid was ind icated in the formation of white ppt.

(2) The Malic Acid Confirmatory Exam:

(a) Ferric chloride test: 2 ml of test solution and a few drops of 40 percent Fecl3 solution were added. The yellowish colour formation indicated the presence of malic acid.

3.14 TEST FOR INORGONIC ACID

(a)Test for sulphate: Add 10 percent white lead acetate reagents to 2 ml test solution, then add 0.50 ml NaoH solution to white ppt indicated sulphate presence formation after 5 to 7 minutes.

3.15 TEST FOR CHLORIDE

Add 3 ml of lead acetate solution to 5 ml of filtrate test solution in the test tube and heat for 5 minutes at 60 C in the water bath. White ppt indicated the presence of chloride after refrigerated formation.

3.16 TESTFOR CORBONET:

(a) With dilute acid: 3ml test solution and added dilution acid to the test tube (HCL). CO2 bubbles are provided as soon as we mix dilute acids. That is shown by the presence of carbonate.

3.17 TEST FOR NITRATE:

The brown ring test: With test solution of ferrous sulphate yield no brown colour but if we are slowly added sulphuric acid. A brown ring will form at the junction of the two layers after 5 -10 minutes, indicating the presence of nitrate ions.

4. Quantitative analysis Of Plant extract

4.1 TOTAL PHENOLIC CONTENT ASSAY:

Total phenolic content determination (TPC) The total phenolic content was determined using the Folin-Ciocalteu reagent method, based on colorimetric reduction (Singleton et al.1965). Test samples are 0.05 mg in

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50 ml D/W, 0.05 mg tannic acid in 50 ml, 1 ml FCR in 9 ml D/W or 1:9 and 7 gm 35 percent sodium carbonate in 20 ml D/W. Then prepare 3 different types of solutions in 7 different test tubes (5 Normal, 1 blank and 1 sample) with the first Standard solution, 0.2 ml of tannic acid, 0.8 ml of D/W and 1 ml of FCR, 1 ml of sodium carbonate added after a few minutes. 1 ml FCR for 1 ml D/W null, and 1 ml Na2co3 added after 3-4 minutes. 0.6 ml tannic acid solution, 0.4 ml D/W, 1 ml FCR and 1 ml sodium carbonate were applied to the last sample after a few minutes. The entire mixture was permitted to stand for 90 minutes in the dark. The blue color solution absorbance was read 765 nm against the blank on a UV visible spectrophotometer. In triplicates, the overall phenolic concentration (mg/ml) of the sample was measured and the normal curve extrapolated.

• Reagents

1. Sodium carbonate solution (35%): 35 gm of sodium carbonate was dissolved in 100 ml of water and then purified after allowing it to stand overnight at room temperature.

2. Tannic acid stock solution 0.05 mg Dissolve tannic acid in 50 ml of distilled water.

4.2 TOTAL FLAVONOID CONTENT ASSAY

The cumulative flavonoid content of all extracts estimated in this process is as follows, based on the aluminium chloride method. For the calculation of the overall flavonoid content of sample samples, the aluminium chloride process (Change et al., 2002) is used. Aliquots of extract solutions are taken and up to 3 ml of D/W are made up of the volume. Then 0.1 ml of aluminum chloride (10 percent), 0.1 ml of sodium hydroxide (1 M) and 2.8 ml of purified water were sequentially added. Then prepare different solutions of 3 forms in various 7 test tubes (5 Standard, 1 blank and 1 sample). Prepare a distinct catecholine concentration norm curve (20ul, 40ul, 60ul, 80ul, 100ul). Following 40-50 minutes of dark room temperature incubation, the test solution is vigorously shook. Absorbance at 510 nm is reported by UV spectrophotometer triplicate tests for each sample. The flavonoid concentrations determined from the calibration plot in the test samples are expressed as mg rutin equivalent/g of the sample. (In 2013, Hajimahmoodi M, et al.). By using the following formula, TFC was computed:

$$TFC = (R \times D.F \times V \times 100) / W$$

D.F-dilution factor, V-volume of stock solution, 100-for 100 gm dry product, W-weight of plants used in the procedure, when- R- outcome obtained from the standard curve.

Reagents

1. 10g Chloride aluminium was dissolved in 100 ml of D/W.

2. Dissolve 0.4gm with 10 ml of distilled water.

3. Standard rutin solution (1mg/ml): Rutin 10 mg dissolved in 10 ml of D/W

5. DPPH Scavenging Assay-

The DPPH test was conducted as stated by (Hsu et al. 2007). A volume of 2 ml of 0.1 mmol/L DPPH solution was mixed with 1.5 ml of varying lemon peel extract concentrations (20 to 100 μg/ml). The mixture was shook vigorously and incubated for 30 minutes in the dark at room temperature. A spectrophotometer measured the reduction of the free radical DPPH by reading the absorbance at 517 nm. The solution was used as a control with no extract and with DPPH and methanol. In 3 independent assays, the experiment was repeated. Positive controls were used with ascorbic acid. The formula was determined for the inhibition of DPPH free radical in percentage.

$$\text{DPPH Scavenging activity (\%)} = A_{\circ} A_1/A \times 100$$

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Where, $A_\circ$ = Absorbance of control

A_1 = Absorbance of sample/standard

6. **RESULTS AND DISCUSSION**

Hydro-Alcoholic extract has revealed Citrus lemon peels include proteins, carbohydrates, amino acid, glycosides, flavonoids, phenolics, etc. Table number 1 shows the outcome of the preliminary phytochemical analysis.

Table number 1: Preliminary phytochemical analysis of Lemon fruit peels

S.NO.	Phytochemicals	Test performed	Results
1	Carbohydrates	Molisch's test	+
2	Reducing polysaccharides (Starch)	Iodine test Tannic acid	- +
3	Proteins test	Biuret test Million's test	+ +
4	Amino Acids test	Cysteine test	+
5	Steroids test	Salkowski test	+
6	Glycosides	Killer Killani test	+
7	Anthraquinone Glycosides	Borntrager's test	+
8	Coumarins Glycosides	With NaoH	+
9	Flavonoids	Shinoda test	+
10	Alkaloids	Wagner test	+
11	Tannins compounds	With Lead acetate Acetic Acid	- +
12	Phenolic compounds	Iodine test	+
13	Organic Acid	Oxalic Acid test Malic Acid test	+ -
14	Inorganic Acid	Sulphate test	+
15	Chloride	Chloride test	+
16	Carbonate	Acid liberate test Mercuric chloride test	- -
17	Nitrate	Brown ring test	+

+ = Indicates presence of phytochemicals

- = Indicates absence of phytochemicals.

Total phenolic content (TPC) assay:

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Total phenolic content (TPC) determination The total phenolic content was determined on the basis of a colorimetric reduction based method using the Folin-Ciocalteu reagent. The TPC of the extracts was resolved by extrapolation from the calibration curve (Y = 0.007x+0.0211; R2 = 0.9932) prepared from the concentration of tannic acid (Figure.6.1.1) and expressed in mg per gram of tannic acid equivalence (TAE). The results are shown below as table nu.2, graph nu.6.1.1 and table 3, respectively.

Concentration µg/ml	Absorbance (765 nm)
20	0.181
40	0.293
60	0.422
80	0.574
100	0.743

TPC: Table number- 2

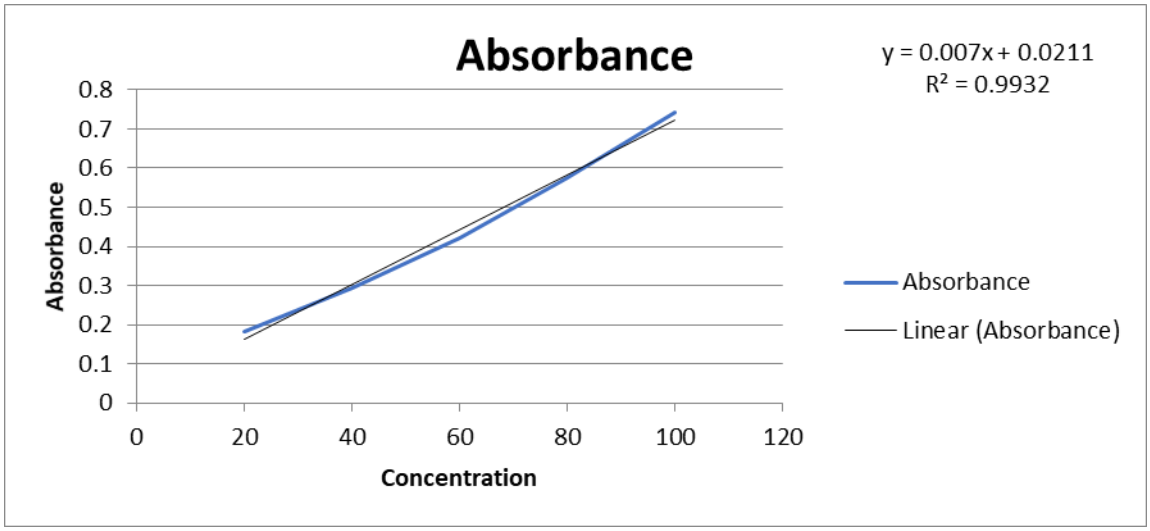

Fig.6.1.1: Standard Curve of Tannic acid

QUANTITATIVE ANALYSIS	***lemon Plant***
TOTAL PHENOLS (µg of TAE/serving)	138.2436003±0.996

Table number. 3- Total Phenolic content of lemon peels Hydro alcoholic extract

Total flavonoids content (TFC) assay: Total flavonoid extract content, estimated according to the method of aluminum chloride. For the determination of the total flavonoid content of the extracts, the aluminium chloride method (Chang et al., 2002) is used. The extract TFC was resolved by extrapolation from the calibration curve (Y = 0.0122x+0.6975; R2 = 0.9905) prepared from the concentration of quercetin (Figure 6.2.1) and expressed in mg of the equivalence of quercetin (QE) per gram. Below, the results are shown as table nu.4, graph 6.2.1 and table nu. 5.

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TFC:

Concentration µg/ml	Absorbance (765 nm)
20	0.9
40	1.207
60	1.477
80	1.682
100	1.883

Table number- 4

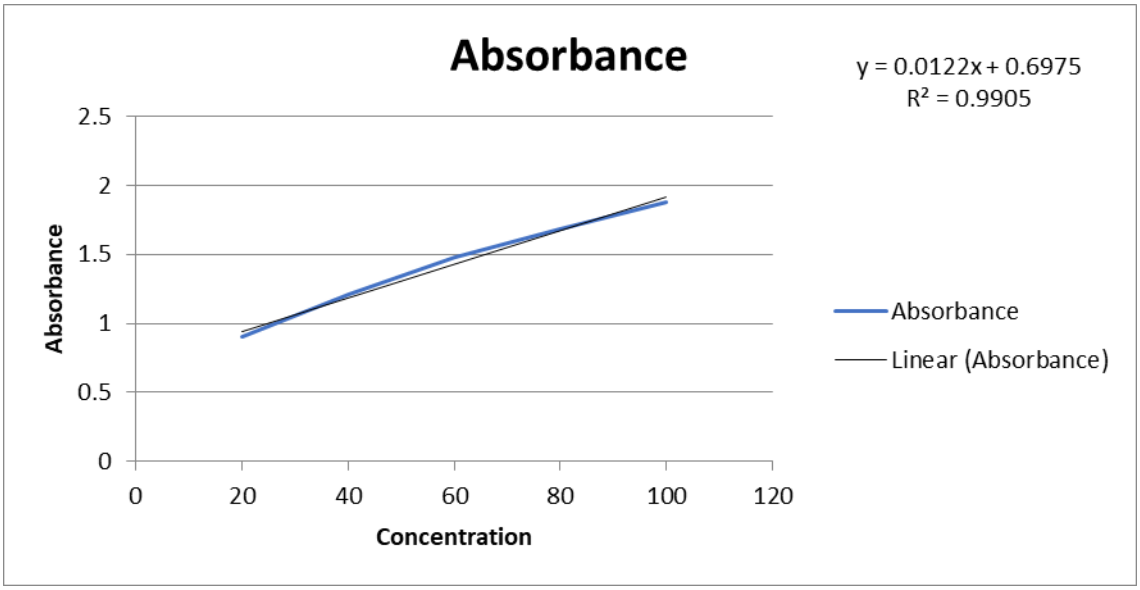

Fig 4.2.1: Standard Curve of Quercetin

QUANTITATIVE ANALYSIS	***lemon Plant***
TOTAL FLAVONOIDS (mg of QE /serving)	56.28301384±1.384

Table number: 5. Total Flavonoid in lemon peels Hydro- alcoholic extract

DPPH radical scavenging activity: DPPH can be used to determine the activity of free radical scavenging as it forms a stable molecule on acceptance of an electron or atom of hydrogen (Jun M et al. 2004). Due to the scavenging effect of plant extract, there was a decrease in DPPH concentration. Citrus (Lemon) peel extracts and standard H-donor activity were shown among the extracts tested. The extract and the standard reduced the concentration-dependent DPPH to the yellow coloured product. The extract's DPPH scavenging activity was good then and its IC50 value was shown in graph number 6.3.1 and table number 6 as results.

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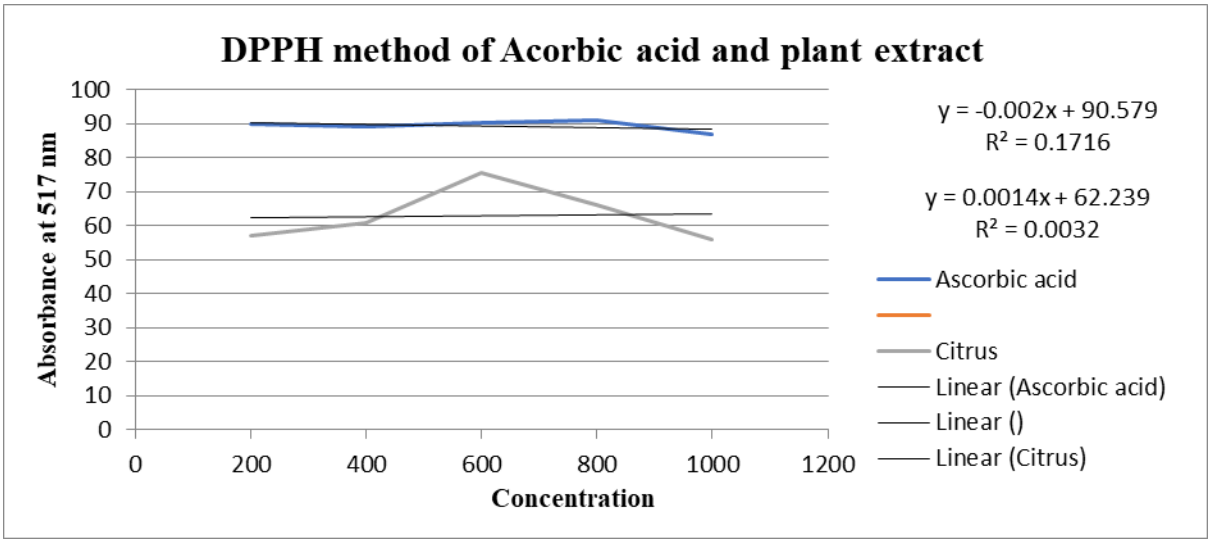

Fig.6.3.1- D DPPH of *Citrus (Lemon*) Extract

Sample	IC 50 Value
Ascorbic Acid	21.03
Citrus	62.18

Table number – 6

7. CONCLUSION

Natural plants are widely used for research purposes these days, and they have more than one chemical entity, so they have been widely used for research investigations. The objective of this study was to test the effective dosage response and minimal side effects of lemon (citrus) peels used for base compounds compared to synthetic compounds. It is seen as a beneficial medicinal plant with various medicinal properties by the robust linear associations found between the phenolic, flavonoid and antioxidant potential calculated by the DPPH assay and phytochemical screening of lemon peel extract. Since there are more constituents in the Hydro Alcoholic extract of lemon peels, it may be considered beneficial for further investigation. For its better therapeutic and commercial utilization, a typical research and developmental job needs to be carried out.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest associated with this manuscript**.**

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8.REFRENSES

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