Original Article

In Vitro Analysis Of Antioxidant And Antimicrobial Activity Of Lemon Peel Powder

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Abstract

Traditionally lemon has been used for various activities because of its citrus activity. It acts as a perfect antioxidant. It has lot of commercial uses and it has lot of medicinal properties also. Various byproducts have been obtained from this citrus fruit. This research involves the biochemical characterization of lemon peel. The phytochemical analysis has led to know what kind of secondary metabolites present in the lemon peel. Phenol and flavonoid were quantified using garlic acid quercetin as a standard respectively. Carbohydrates are estimated using phenol sulphuric method, and proteins by Lowry's method. The nutritional value was analyzed for carbohydrates, proteins, and vitamins. The lemon peel has good estimation of nutritional values. Antioxidant activity was measured by DPPH radical scavenging activity. Antibiogram test was performed to test anti-microbial activity.

Keywords: Biochemical characterization, phytochemicals, phenol flavonoid, DPPH, Lowry's method.

INTRODUCTION

The lemon, *Citrus limon* (L.) is species of small evergreen plant in flowering plant family Rutaceae, widely grown in South Asia. Lemon fruits are typically yellowish and spherical in shape as shown in Figure 1.

Fig 1: Lemon

Citrus fruits find extensive usage in food industries for the production of fresh juices and preservatives. Citrus waste primarily comprises of lemon peels that constitute approximately 50% of mass of fruit. The peels possess rich composition of bioactive molecules including, natural antioxidants, phenolic acids, and flavonoids. [6]. The products containing such ingredients help in prevention of chronic diseases and are widely used in healthcare formulations. Thus, commercial demand of such value-added products is on the rise [7]. The categorization on the basis of biogenesis or biosynthetic origin leads to further classification and respective applications [8].

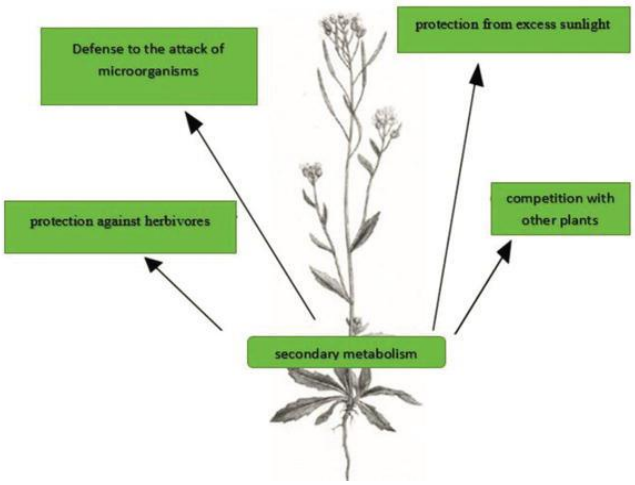


Fig 2: Production of secondary metabolites

In the present study, antioxidant and antimicrobial activities were carried out for the lemon peel powder.

MATERIALS AND METHODS

MATERIALS:

CHEMICALS

Ethanol, Hexane, Potato Dextrose Agar, Chloroform, Concentrated Sulphuric acid, DMSO (Dimethyl sulphoxide), Sodium Hydroxide, Ammonia, Glacial acetic acid, Ferric chloride, Sodium Carbonate, Hydrochloric Acid. Molisch's reagent, Mayer's reagent, Folin-Ciocalteu phenol reagent were used in the studies.

INSTRUMENT USED:

UV spectrophotometer, laminar air flow, hot air oven, autoclave, weighing balance, and incubator were used for various experimental studies and analysis.

SAMPLE COLLECTION AND PREPARATION:

Lemon (*Citrus limon*) sample was collected from the local market. Then the lemon zest was peeled off and dried in a hot air oven. The collected biomass was finely converted in to a powder form. Among that 30 grams of lemon zest powder was taken for the extraction process. Hexane, the polar solvent was used for the extraction of phytochemical components from the lemon zest. 100 ml of hexane was added to lemon zest, mixed for a regular time interval of about 2 to 4 hrs and kept undisturbed for 18 to 24 hrs.

QUALITATIVE ANALYSIS OF LEMON ZEST:

- Tests for carbohydrates, tannins, saponins, flavonoids, alkaloids, quinine, glycosides were performed using standard protocols.
- Cardiac glycosides, terpenoids, phenols, coumarins, steroids, phytosteroids, phlobatannins, and anthraquinones were estimated by standard methods

QUANTIFICATION ANALYSIS:

PHENOLS:

Various aliquots of sample and gallic acid (0.2 to 1.0ml) were pipetted out into test tubes. Distilled water was added to each of test tubes by making the solution to 3ml. 0.5ml of Folin's-Ciocalteau reagent was subsequently added. After 3 min, 2 ml of 35% of Na_2CO_3 solution was added to test tubes and mixed methodically. The mixture was kept in boiling water bath for exactly one min, cooled and absorbance was determined at 650 nm against the blank reagent. Standard curve was prepared using different concentrations of Gallic acid.

FLAVONOIDS:

0.5ml sample was added to test tube containing 1.25 ml

of distilled water. Then 0.075ml 0f 5 % sodium nitrite solution was added and allowed to stand for 5 mins. This was followed by adding 0.15 ml of 10% aluminum chloride. After 6 min, 0.5 ml of 1M NaOH was added and mixture was diluted with another 0.275 ml of distilled water. The absorbance of mixture at 510 nm was measured immediately. The flavonoids content was expressed as milligrams of quercetin equivalents/g sample.

ANALYSIS OF NUTRITIONAL VALUES

ESTIMATION OF PROTEIN BY LOWRY METHOD:

ESTIMATION OF CARBOHYDRATES BY PHENOL SHULPHURIC ACID

0.1ml and 0.2 ml of the sample solution was pipetted out in two separate test tubes. The volume was made up in each tube to 1ml with water. 1ml of water served as blank. 1 ml of phenol solution was added to each tube. 5ml of 96% sulphuric acid was added to each tube and agitated thoroughly. After 10mins the contents in the tubes were placed in a water bath at 25-30 °C for 20 mins. The absorbance was measured at 440 nm. The amount of total carbohydrates present in the sample solution was calculated using the standard graph.

ESTIMATION OF VITAMINS

Vitamin A Test, Vitamin D Test, Vitamin E Test, Vitamin B1 Test, Vitamin B2 Test, Vitamin B6 Test, Vitamin C Test were performed by using the standard procedure.

ANTIOXIDANT ACTIVITY BY DPPH RADICAL SCAVENGING:

The percentage of DPPH was calculated

% Inhibition = $((A_C-A_A)/A_C)$ X 100

Where, A_C- Absorbance of control sample,

A_A -Absorbance of test sample.

Later graph was plotted with %inhibition v/s concentration.

ANTIBACTERIAL ACTIVITY:

Test bacteria:

Antibacterial activity of lemon was assessed against two bacteria species: Staphylococcus aureus and Escherichia coli; Then each bacterium was inoculated by using inoculation loop in 3ml of broth. Then the cultures were allowed to kept in incubator for 18 to 24 hours at 36 °C.

Agar diffusion method After spreading the bacteria in the agar plates then different concentration of lemon extract was added to each of the well. The plates were incubated for 24 hrs at 36 °C under aerobic conditions. After incubation, confluent bacterial growth was observed.

RESULT AND DISCUSSION

SAMPLE COLLECTION AND PREPARATION:

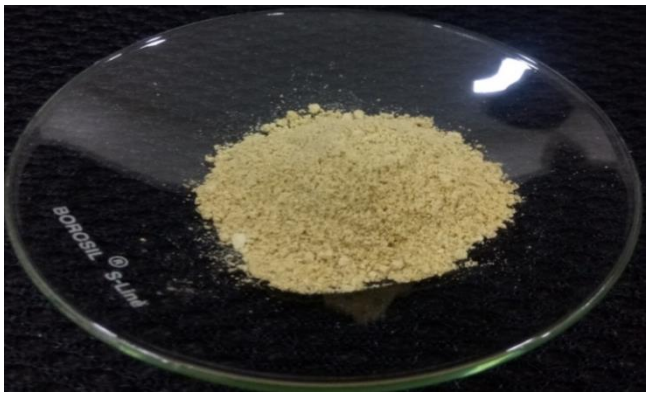


Figure 5. Finely grinned lemon zest powder.

The finely grinded lemon zest powder had a strong essence of lemon flavor. About 64 grams of lemon zest powder was obtained after grinding. The ground sample was allowed to incubate for about 24 hrs after pouring hexane such that most of the components from the zest were extracted into the solvent.

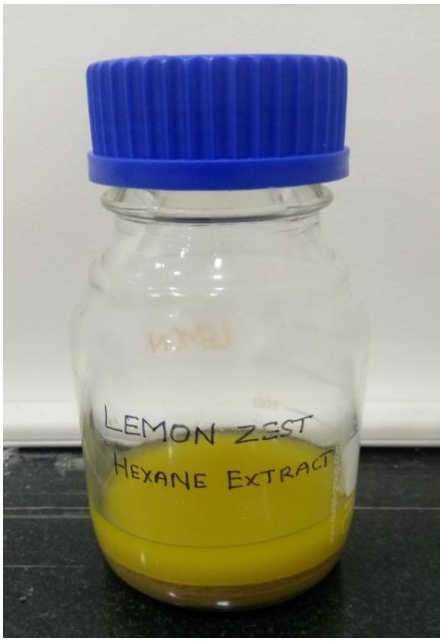


Figure 6. Cold hexane lemon zest extract.

After the extraction process was completed, the extract was filtered with Whatman filter paper [125mm]. The extract was allowed to dry in the hot air oven, then scratched and weighed. It was found to be about 100 mg. The scraped sample was added in the falcon tube and 1 ml of DMSO was added. The final concentration of sample was about 100 mg per ml. The prepared solution served as the stock solution and working standard was prepared from the stock solution for the qualitative and quantitative analysis.

QUALITATIVE ANALYSIS OF LEMON ZEST:

The qualitative analysis of cold hexane lemon zest shows that the positive results to several components, namely tannins, flavonoids, cardiac glycosides, terpenoids, and phenols.

Table.4. 1. Qualitative analysis of lemon zest using Hexane extract

TEST	OBSERVATION (color)	RESULT
Carbohydrates	Brown color	-
Tannins	Dark blue	+
Saponins	No foam	-
Flavonoids	Pale yellow	+

Alkaloids	Yellow color	-
Quinine	Pale yellow color	-
Glycosides	Double layer formed	-
Cardiac Glycosides	Brown ring formation	+
Terpenoids	Light red brown color	+
Phenols	Pale yellow	+
Coumarins	Pale yellowish white	-
Steroids and Phytosteroids	Two layer of white	-
Phlobatannins	Pale yellow	-
Anthraquinones	Pale yellow	-

Figure 7. The qualitative analysis test of lemon zest hexane extracted sample.

QUANTIFICATION ANALYSIS:

PHENOL:

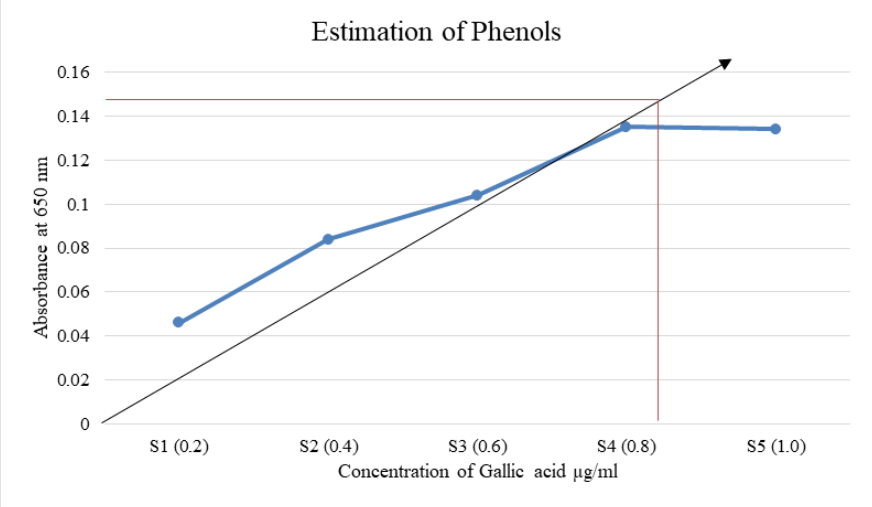
Concentration and absorbance of Gallic acid for standard:

Sample	Concentration (µg/ml)	Absorbance (650nm)
1	0.2	0.046
2	0.4	0.084
3	0.6	0.104
4	0.8	0.135
5	1.0	0.134

Concentration and absorbance of lemon zest:

Concentration (µg/ml)	Absorbance (650nm)
0.2	0.149
0.4	0.325

Phenolic Estimation



CALCULATION:

0.2 mg/ml of sample contains = 8.6 µg of phenol content

For 0.1 mg of sample = 4.3 µg of phenol

1000 µg of sample contains = 43 µg of phenol.

1mg of lemon zest contains 43 µg of phenol.

FLAVONOIDS:

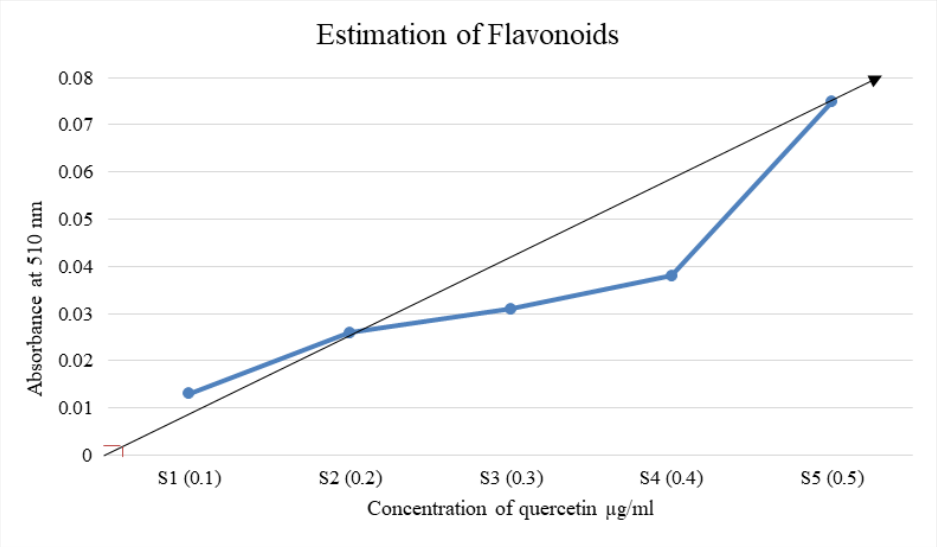
Concentration and absorbance of Quercetin for standard:

Sample	Concentration (µg/ml)	Absorbance (510nm)
S1	0.2	0.013
S2	0.4	0.026
S3	0.6	0.031
S4	0.8	0.038
S5	1.0	0.075

Concentration and absorbance of lemon zest:

Concentration (µg/ml)	Absorbance (510nm)
0.2	0.002
0.4	0.001

Flavonoids estimation:



CALCULATION:

1µg of lemon zest sample contains 0.2 µg of flavonoids

1 µg = 0.2 µg

1mg = 200µg of flavonoids

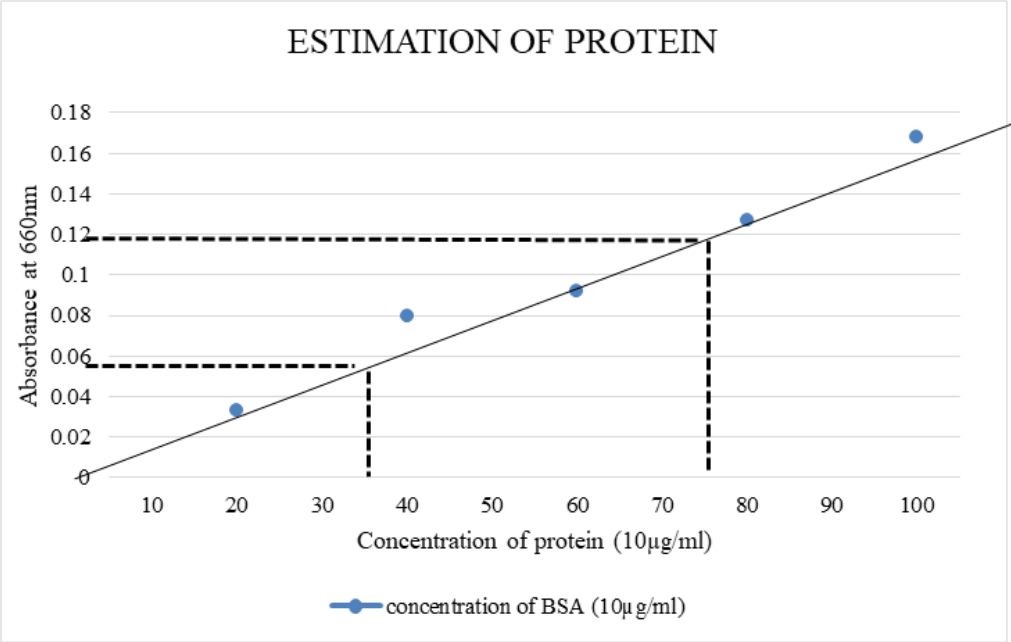
1mg of lemon zest contains 200 µg of flavonoids

ESTIMATION OF PROTEINS

Protein estimation was done by Lowry's method the absorbance of the reaction mixture was plotted in the graph below. It shows that the quantity of protein increases with respect to concentration.

S.NO	CONCENTRATION	OD VALUE
1.	0.2 ml	0.033
2.	0.4 ml	0.080
3.	0.6 ml	0.092
4.	0.8 ml	0.127
5.	1 ml	0.168

S.NO	CONCENTRATION	SAMPLE 1
1.	0.1 ml	0.052
2.	0.2 ml	0.113



CALCULATION:

0.1mg\ml of sample contains= 33µg of protein

1mg\ml of sample contains= 330µg of protein

0.2mg\ml of sample contains=72µg of protein

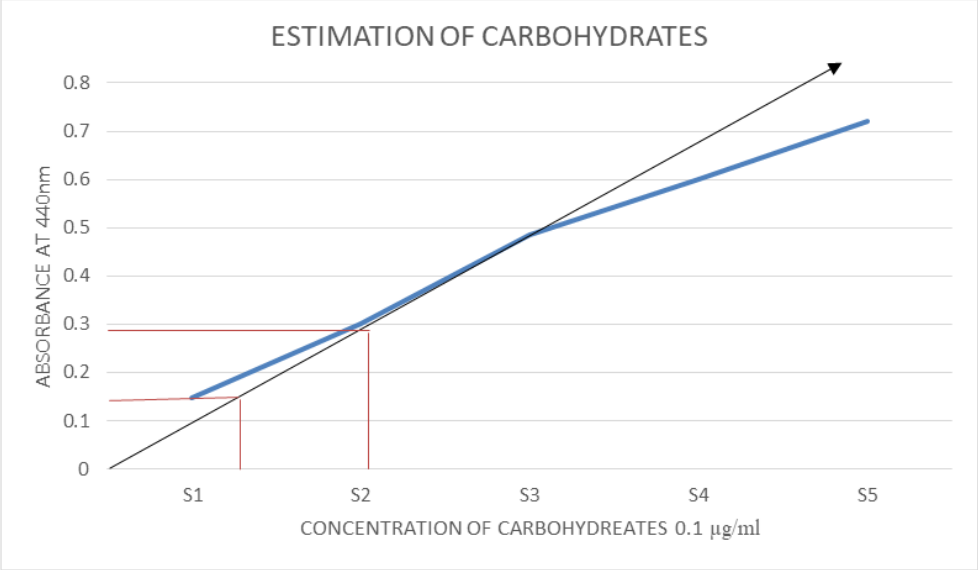
2mg\ml of sample contains=720µg of protein

1ml of sample contains=360µg of protein

Mean= (330+360)/2=345µgof protein

Approximately 345µg of protein present in 1mg\ml of sample.

ESTIMATION OF CARBOHYDRATES:



CALULATION:

0.2mg/ml of sample contains 0.014µg of carbohydrates.

0.1mg/ml of sample contains 0.07µg of carbohydrates.

100µg/ml of sample contains 7µg of carbohydrates.

ESTIMATION OF VITAMINS:

The different vitamin tests showed the type of vitamin present in lemon peel. In lemon peel, vitamin A, B6, and C are present.

S.NO	IDENTIFICATION	TREATMENT	OBSERVATION	RESULT
01	Vitamin A	Sample + Carr-Price reagent	Yellow solution and precipitation	+
02	Vitamin D	Sample + H_2O_2 + Heat + Carr-Price reagent	Yellow solution and white precipitation	-
03	Vitamin E	Sample + ethanol + conc. HNO_3	Formed a small explosion and yield yellow brownish gas. Then the solution form 2 layers. Above is orange, and below is yellow.	-
04	Vitamin B1	Sample+ NaOH + $K(Fe(CN)_6)_3$ + isobutanol.	Formed 2 layers. Above is cloudy white, and below is clear	-
05	Vitamin B2	Sample+ ethanol	Yield clear yellow like the color of stabilIo (There is fluorescence).	-
06	Vitamin B6	Sample+ $FeCl_3$	Blue pigment is formed	+
07	Vitamin C	Sample+ Fehling A and B Reagent	Before heated: blue greenish After heated: Dark blue and formed red brick precipitate red	+

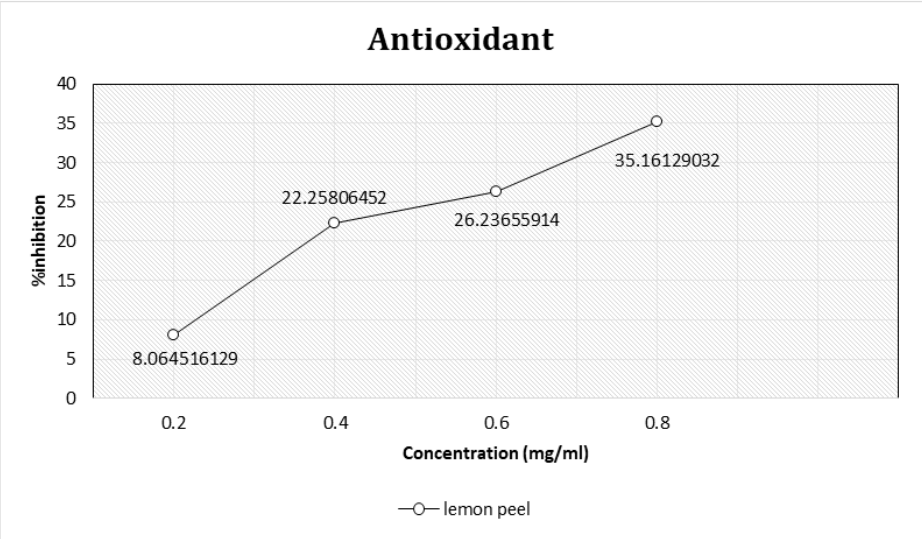
ANTIOXIDANT ACTIVITY

Antioxidant activity of lemon peel was measured using DPPH method. The OD value and the % inhibition has shown in table. It depicts that comparatively *M.pudica* has more antioxidant activity than *P.hysterophorus.*

Table: OD value and % inhibition of DPPH assay

	Lemon peel	
SAMPLE	OD VALUE	%INHIBITION
200µg/ml	0.755	18.81
400 µg/ml	0.623	33.01
600 µg/ml	0.586	26.98
800 µg/ml	0.603	35.16

Control = 0.930



Graph: % inhibition for antioxidant assay

ANTIBACTERIAL ACTIVITY:

SERIAL DILUTION:

Lemon peels were taken and crushed using motar and pestle. This was taken as sample. Eight test tubes were taken and marked as 10^{-1} to 10^{-8}. 1ml of tomato crushed sample was taken and diluted with 9 ml of autoclaved distilled water and mixed well. This was denoted as first dilution (10^{-1}). The same procedure was repeated till 10^{-8} dilution.

ANTIBACTERIAL TEST:

The pathogenic bacteria are isolated from the spoiled fruit or vegetables and serially diluted and isolated. The isolated bacteria were cultured by mat culturing technique and the wells are punched in agar plates and the plant extract of different concentration was poured in the wells for zone of inhibition.

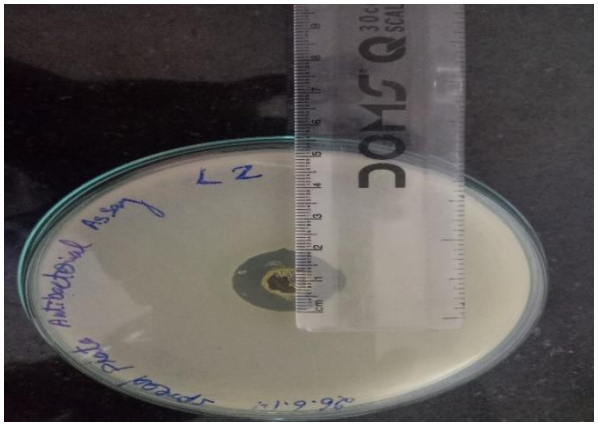


Fig 8: well diffusion method

This indicates that lemon zest possesses the antibacterial activity. The ethanolic lemon zest extract had a strong antibacterial activity against bacteria like Pseudomonas and Ervinia and some fungal like Rhizopus, Fusarium etc. The antibacterial activity of lemon zest over the bacteria was 20mm average zone of inhibition.

CONCLUSION

Lemon is a familiar fruit which have been consumed for several centuries. Lemon is rich in nutrition and antioxidant where this research involves the biochemical characterization of lemon peel. The phytochemical analysis has led to know what kind of secondary metabolites present in the lemon peel. Phenol and flavonoid were quantified using gallic acid quercetin as a standard respectively. Carbohydrates are estimated using phenol sulphuric method, and proteins by Lowry's method. The nutritional value was analyzed for carbohydrates, proteins, and vitamins. The qualitative analysis of cold hexane lemon zest shows that the positive results to several components, namely Tannins, Flavonoids, Cardiac Glycosides, Terpenoids, and Phenols. Further estimation was done like quantification for phenol and flavonoids were phenol contain 1mg of lemon zest contains 43 µg of phenol, 1 mg of lemon zest contains 200 µg of flavonoids. In nutrition value analysis estimation of protein by Lowry's method, estimation of carbohydrate by phenol sulfuric acid and estimation of vitamin were done. Result for estimation of protein is Approximately 345µg of protein present in 1mg\ml of sample. Estimation of Carbohydrate is 100µg/ml of sample contains 7µg of carbohydrates. Estimation for vitamin different vitamin tests showed the type of vitamin present in lemon peel. In lemon peel vitamin A, B6, and C is present. The antioxidant activity was estimated by DPPH assay highest inhibition was found at 800 µg/ml is 35.16%. Antibacterial activity was done result indicates that lemon zest contains the antibacterial activity. The antibacterial activity of the ethanolic lemon zest extract had a strong antibacterial activity against bacteria like Pseudomonas and Ervinia and some fungal like Rhizopus, Fusarium etc. the antibacterial activity of lemon zest over the bacteria were 20.00 mm average zone of inhibition. The lemon peel has good estimation of nutritional values. It has better antioxidant activity which was measured by DPPH radical scavenging activity. Antibiogram test was performed to test anti-microbial activity.

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