ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 10, Issue, 03(F), pp. 31562-31564, March, 2019

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

EVALUATION OF ANTIOXIDANT POTENTIAL IN DIFFERENT PEEL EXTRACTS OF GREEN *CITRUS LIMON*

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DOI: http://dx.doi.org/10.24327/ijrsr.2019.1003.3286

ARTICLE INFO

Article History:
Received 10th December, 2018
Received in revised form 2nd January, 2019
Accepted 26th February, 2019
Published online 28thMarch, 2019

Key Words:

Antioxidants, *Citrus limon*, green peels, radical scavenging, pharmacological importance.

ABSTRACT

The present study aims to evaluate the antioxidant potential of the green colored peel extracts of *Citrus limon* belonging to the family Rutaceae. The peel extracts in different solvents were investigated for their antioxidant activity using *in vitro* assays and the results were compared with the standard ascorbic acid. Determination of total phenolic and total flavonoid contents in these extracts were done using gallic acid and rutin/quercetin equivalents respectively. Radical scavenging activities were determined using DPPH assay and reducing power assay. The free radical scavenging and antioxidant activities may be attributed to the presence of phenolic and flavonoid compounds present in the extracts. The results in the present study indicated that *Citrus limon* peels serve as potential source of natural antioxidants which may suggested for the polyherbal formulations and drug designing for the treatment of various diseases and can be studied further.

INTRODUCTION

Free radicals are produced during the various Metabolic processes in our body. Their excessive production cause cell death or cell dysfunction as they are capable of attacking the healthy cells, leading to structure deformation and creating the conditions of oxidative stress. Thus, they ultimately are the major contributor to the process of aging and other degenerative diseases such as cancer, cardiovascular disease, declination of immune system and brain dysfunction[1]. Antioxidants play a vital role in the prevention of oxidative stress caused by reactive oxygen species (ROS) such as superoxide anion radical, singlet oxygen, hydrogen peroxide, nitric oxide radical, other lipid peroxides[2] and treatment of various diseases. Natural plants containing phytonutrients have been explored for assessing their antioxidant potential. Antioxidants participate in oxidative stress defense and acts as free radical stabilizers which significantly helps in treating various diseases like cardiovascular, cancer and other neurodegenerative disorders[3,4]. Natural medicinal plants including Citrus species possess antioxidants such as phenolic and flavonoid compounds[5,6,7]. Literature study suggests that lemon peels possess cytotoxic and antimicrobial activities[8,9] and lemon fruit posses anti oxidative stress[10] and therefore lemon peels was selected for the present study. The main objective of the study is to access the antioxidant potential of *Citrus limon* specifically green peel extracts in reference to its pharmacological importance which can be studied further.

MATERIALS AND METHODS

Collection of Plant Material

Plant material was collected from the local market and fruit juice shops of Bhopal, M.P. India, which consists of shade dried powdered peel of green *Citrus limon* belonging to the family Rutaceae.

Extract Preparation

Peels were first shade dried and mechanically powdered and then were coarsely grinded using grinder. The finely powdered peels were kept in an airtight container until use. The plant extracts were prepared using maceration technique where the coarsely grinded peel powder was kept in different solvents on the basis of increasing polarity from petroleum ether (pet ether), chloroform, ethyl acetate to methanol for 48 hours and then filtered. The solvents were then subsequently vapourized at 40°C using soxhlet apparatus and the extracts obtained were stored in refrigerator for further investigations.

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Chemicals & Reagents

Antioxidant assays were performed using chemicals of analytical grade like Methanol, Ethyl acetate, Chloroform, Petroleum ether, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Potassium ferricyanide (1% W/V), Phosphate buffer (0.2 M, pH 6.6), Trichloroacetic acid solution (10% W/V), Ferric chloride (0.1% W/V), etc.

Preliminary Phytochemical Screening[11]

Qualitative phytochemical testing of different peel extracts was performed for presence of various phytochemical constituents using standard tests.

Quantitative Estimation of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

TPC of the peel extracts was determined by Folin-Ciocalteu method[12-13] using gallic acid as standard reference and results were obtained from the calibration curve of gallic acid and expressed as mg gallic acid equivalent (GAE)/g extract or µg GAE/mg extract. The total flavonoid content was determined with colorimetric assay using rutin as reference standard[14] and results were expressed as Rutin equivalent (RE), mg RE/g extract or µg RE/mg extract.

Quantitative Analysis (Antioxidant activity)

The quantitative analysis of the different peel extracts of green *Citrus limon* was done using antioxidant assays such as 1,1-dipheny l-2-picrylhydrazyl (DPPH) radical scavenging activity assay and reducing power assay.

1,1-Diphenyl-2-Picrylhydrazyl (DPPH)Radical Scavenging Activity Assay [15]

The hydrogen donating ability of the peel extracts for the assessment of their antioxidant potential was examined in the presence of 1,1- diphenyl-2-picrylhydrazyl (DPPH) stable radical. Ascorbic acid was used as reference standard. Methanol and DPPH solution in 1:2 ratio was used to prepare control. 0.1 mM DPPH solution and different concentrations of test samples was prepared using methanol. 2 ml of test sample solution of each extract was mixed with 2 ml DPPH solution in various concentration ratios (10-100 µg/ml). Reaction mixture was incubated in dark at room temperature for 10 minutes and absorbance was measured spectrophotometrically at 515 nm against blank (methanol and DPPH in ratio 2:2). Percentage inhibition (% inhibition) was calculated using the formula: $\% I = [(A_C - A_S / A_C) X 100]$

where, A_C = Absorbance of control and A_S = Absorbance of sample.

Reducing Power Assay [16]

It was measured by mixing 0.5 ml test samples of different concentrations with 0.5 ml of phosphate buffer and 0.5 ml of potassium ferricyanide. Reaction mixture was then incubated at 50°C for 20 minutes and allowed to cool. After cooling process, 1.5 ml of trichloroacetic acid solution was added to terminate the reaction. Thenafter, 0.5 ml of freshly prepared ferric chloride was added and absorbance was recorded at 700 nm using ascorbic acid as reference standard. A curve for absorbance versus concentration was then plotted. The increase in reducing power was studied with the increased absorbance of the reaction mixture.

RESULTS AND DISCUSSION

Phytochemical Screening: Phytochemical screening of different extracts showed the presence of carbohydrates, flavonoids, phenolics, tannins, alkaloids, fats, saponins, etc.

Total Phenolic Content and Total Flavonoid Content

TPC ranged from 81.3 ± 0.11 to 140.9 ± 1.50 mg GAE/g extract in green lemon peel. Lower phenolic content was found in chloroform peel extract whereas TPC reported in methanol peel extract of green lemon is 140.9 ± 1.50 mg GAE/g extract which is highest among tested different extracts. TFC ranged from 46 ± 0.81 to 256.25 ± 0.95 mg RE/g extract in green lemon. The highest flavonoid content was reported in ethyl acetate extract i.e. 256.25 ± 0.95 mg RE/g extract, while the chloroform extract was found to contain the lowest flavonoid content (46 ± 0.81 mg RE/g extract).

Antioxidant Activity Assays

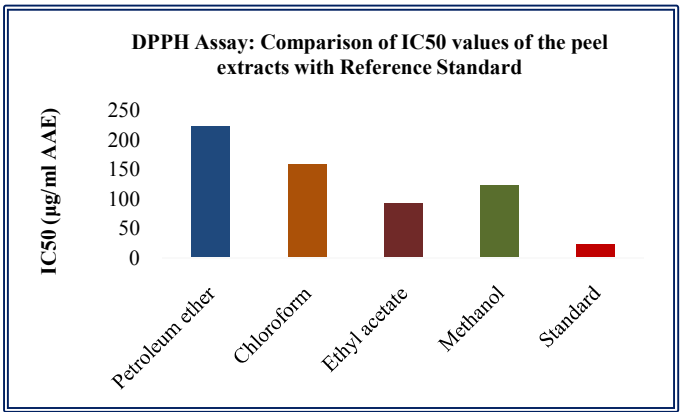
Antioxidants acts as free radical scavengers and participates in the antioxidant defense mechanisms. One of the most sensitive and widely used method to access the antioxidant potential is DPPH assay[17]. The ability of lemon peel extracts to bleach DPPH radical was quantified using DPPH assay. DPPH is nitrogen centered stable free radical which change its color from violet to yellow due to the formation of diphenylpicryl hydrazine. The peel extracts of different polarities were investigated for their quenching capacities using ascorbic acid as reference standard. Results indicated that ethyl acetate extract of the *Citrus limon* green peel was found to contain highest free radical quenching abilities among all the peel extracts as it was comparable with the standard. Ethyl acetate peel extract possess the highest quenching capacity with IC50 value 94.00 µg/ml AAE, whereas pet ether was found with a minimum quenching capacity and reported with IC50 value of 223.48 µg/ml AAE (Table 1). Results reveal that ethyl acetate peel extract of green *Citrus limon* possess appreciable amounts of antioxidant capabilities. Order of antioxidant capabilities reported in different peel extracts on the basis of DPPH assay is: Ethyl acetate > Methanol > Chloroform > Petroleum ether (Graph 1).

The reducing power ability of different green peel extracts of *Citrus limon* were determined by the ferric chloride method. In this assay, the antioxidants present in plant extracts are responsible for the transformation of Fe (III) in ferric chloride to Fe (II) with a color change from yellow to blue-green. The intensity of color depends on the reducing potential of the bioactive components present. The reducing capacity of any component or antioxidants depends on their hydrogen atom donating ability which terminates the free radical reactions[18]. The reducing capabilities of different lemon peel extracts are shown (Graph 2) indicating that ethyl acetate possess highest antioxidant activity followed by methanol, chloroform and pet ether peel extract.

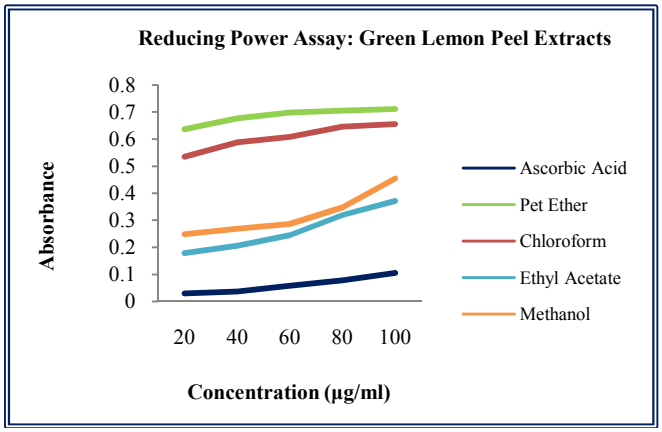
Table 1 DPPH scavenging activity (IC50 values) of peel extracts of *Citrus limon*

S.No	Plant Extract	IC50 (µg/ml AAE)
1.	Petroleum ether	223.48
2.	Chloroform	159.12

3.	Ethyl acetate	94.00
4.	Methanol	123.84
5.	Standard	24.11



Graph 1 DPPH assay: Comparison of IC50 values of peel extracts of green lemon with standard



Graph 2 Comparison of Reducing capabilities of peel extracts of green lemon with standard

CONCLUSION

The present study demonstrates that peel extracts of green lemon possess effective antioxidant potential. The presence of appreciable amounts of phenolic and flavonoid content in the peel extracts of green lemon peel has directly contributed in the antioxidant activity by neutralising free radicals. Citrus species are are found to contain tannins, phenolics and flavonoid components which may possess a wide range of therapeutic uses such as anticarcinogenic, antineurological disorders, cardiovascular impairments, radical scavenging capabilities and antioxidant activities. Current research focus on finding an alternative pathway for treating diseases from natural resources. The presence of appreciable amounts of antioxidant activities may prove its efficacy as potential drug, therefore further investigations are necessary to isolate the bioactive components of the peel extracts so as to reveal its pharmacological importance. Thus, the present study ascertains the evaluation of *Citrus limon* peels as potential antioxidants which could be of considerable interest in the progression of new herb formulation as a therapeutic drug.

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