International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 8; Issue 03 (E); March 2019; Page No.17854-17857
DOI: http://dx.doi.org/10.24327/ijcar.2019.17857.3401

Research Article

EVALUATION OF ANTIOXIDANT POTENTIAL IN DIFFERENT PEEL EXTRACTS OF YELLOW CITRUS LIMON

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A R T I C L E I N F O

Article History:
Received 4th December, 2018
Received in revised form 25th January, 2019
Accepted 18th February, 2019
Published online 28th March, 2019

Key words:

Citrus limon, peel extracts, DPPH, antioxidants, pharmaceutical efficacy.

A B S T R A C T

Antioxidants play a vital role in the prevention and treatment of various diseases. The main objective of the present study was to access the antioxidant potential of specifically yellow colored, mature *Citrus limon* peel extracts in relation to its pharmacological importance. The determination of antioxidant activity was done using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, Reducing power assay and Superoxide dismutase (SOD) scavenging assay using ascorbic acid as reference standard. Extraction of lemon peels was carried using solvents of different polarity which includes methanol, ethyl acetate, chloroform and petroleum ether. Antioxidant assays revealed the strong antioxidant potential of the peel extracts as their IC50 values was found comparable with the ascorbic acid standard. DPPH antioxidant assay revealed that highest free radical quenching capacity was found in ethyl acetate extract with IC50 value 115.63 µg/ml AAE. Highest quenching capability (SOD assay) of ethyl acetate peel extract was reported to be 100.80 µg/ml AAE along with highest reducing capacity followed by methanol peel extract. The results of preliminary quantitative estimation indicated the presence of appreciable amounts of antioxidants which suggests that lemon peels may be proposed as a therapeutic approach for drug development against diseases and its pharmaceutical efficacy can be further studied.

INTRODUCTION

Metabolic processes in our body leads to the constant production of free radicals which includes a class of reactive oxygen species such as hydroxyl radical, superoxide radical, hydroperoxyl radical, peroxyl radical, hypochloride radicals and reactive nitrogen species such as nitric oxide radical and peroxynitrite anion as well (Halliwell, 1995). These radical species on oxidation leads to damage of cellular tissues causing various diseases such as neurodegenerative disorders, ageing, cancer etc. (Valko, 2007). Antioxidants are compounds responsible to inhibit the oxidation process as well as involved in defense mechanism associated with the attack of free radicals. Cellular antioxidants may be enzymatic (superoxide dismutase, catalase, glutathione peroxidase) or non-enzymatic (vitamin C, vitamin E, glutathione, carotenoids, thiols, polyphenols and flavonoids). Phenolic compounds are commonly found in plants and they possess several biological properties including antibacterial and antioxidant activities (Mokbel and Hashinaga, 2005; Win *et al*., 2011). Antioxidants have gained considerable attention in relevance to radicals and oxidative stress (Kalcher, 2009).

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Citrus species possess antioxidant potential which may serve in the treatment of various diseases (Fidrianny, 2016) and can be widely explored for the quest of antioxidants in relation to its pharmacological importance. Therefore, there has been an increasing interest in phytochemicals along with their antioxidant properties which serve as potential disease preventing agents (Jayaprakasam *et al*., 2010).

MATERIALS AND METHODS

Collection of Plant Material

Lemon peels were collected from the local market as well as fruit juice shops of Bhopal, M.P., India. Extra pulp from the lemon peels was removed and then they were properly washed with normal water and then with distilled water. Peels were shade dried and grinded using a mechanical blender.

Extract Preparation

The plant extracts were prepared using maceration technique in which the coarsely grinded peel powder was kept in different solvents based on 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.

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), NBT solution (1 mg/ml), plain DMSO (dimethyl sulfoxide), alkaline DMSO, distilled water etc.

Preliminary Phytochemical Screening (Kokate et al., 1993)

Qualitative phytochemical screening 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 (Ainsworth and Gillespie, 2007; Alhakmani *et al*., 2013) 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 (Zhiesen, 1999) and results were expressed as Rutin equivalent (RE), mg RE/g extract or µg RE/mg extract.

Quantitative Analysis

The quantitative analysis of the four different peel extracts of yellow *Citrus limon* for determination of antioxidant potential was done using three antioxidant assays: DPPH assay, Reducing power assay and Superoxide dismutase (SOD) assay. 1,1- diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay (Gulcin *et al*., 2006)

Assessment of the antioxidant potential of the peel extracts was done on the basis of free radical scavenging activity of the stable radical 1,1- diphenyl-2-picrylhydrazyl (DPPH) using ascorbic acid (100 µg/ml) as standard. Control solution was prepared using methanol and DPPH solution in 1:2 ratio. 0.1 mM DPPH solution and different concentrations of extract samples was prepared in methanol. 2 ml of test sample solution of each extract was mixed with freshly prepared 2 ml DPPH solution in different concentration ratios varying from 10-100 µg/ml. Mixture was then incubated in dark conditions at room temperature for 10 minutes and absorbance was recorded spectrophotometrically at wavelength of 515 nm against blank (methanol and DPPH in ratio 2:2). % 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. A curve between % inhibition and concentration was plotted and then IC50 value was calculated using line of regression. IC50 value indicates that concentration of test sample which reduces the concentration of radicals to 50% and expressed as Ascorbic Acid Equivalents (AAE).

Reducing Power assay (Jain and Jain, 2011)

Prepare test samples of different concentrations in methanol. 0.5 ml of this test sample was taken and 0.5 ml of phosphate buffer (0.2 M, pH 6.6), 0.5 ml of potassium ferricyanide was added to it. Reaction mixture was incubated at 50°C for duration of 20 minutes and allowed to cool. After cooling, 1.5 ml of trichloroacetic acid solution (10% W/V) was added so as to terminate the reaction. Thenafter, 0.5 ml of freshly prepared ferric chloride (0.1% W/V) was added and absorbance was measured at 700 nm wavelength spectrophotometrically 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.

Superoxide Scavenging Assay (SOD) (Patil et al., 2009)

Prepare test samples of different concentrations in DMSO. 0.3 ml from each extract solution and of different concentrations was taken and 1 ml alkaline DMSO followed by 0.1 ml NBT solution was added so as to obtain the final volume of 1.4 ml. For preparing control solution add 0.3 ml plain DMSO, 1 ml alkaline DMSO followed by 0.1 ml NBT solution. Absorbance of the test series and control was recorded at 560 nm wavelength using plain DMSO as blank. % inhibition of the extracts was calculated by using formula: % Inhibition of NBT = (Control-Sample) / Control × 100. Graph was plotted between absorbance versus concentration, and then IC50 values was estimated with the help of line of regression in terms of Ascorbic Acid Equivalents (AAE).

RESULTS

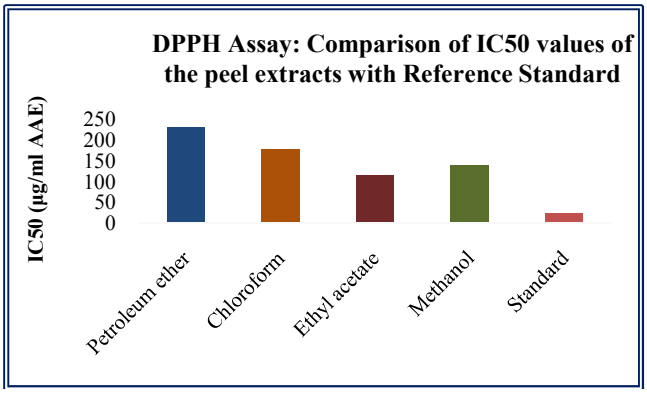
Phytochemical Screening: Phytochemical screening of different extracts showed the presence of carbohydrates, flavonoids, phenolics, tannins, alkaloids, fats and oils, saponins, etc.

Total Phenolic Content and Total Flavonoid Content

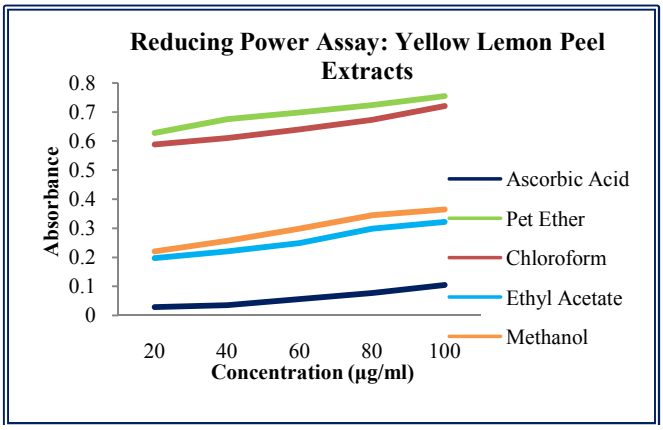
TPC ranged from 90.35 ± 0.5 to 111.2 ± 1.18 mg GAE/g extract in yellow lemon peel. Lower phenolic content was found in chloroform peel extract (90.35 ± 0.5 mg GAE/g extract) whereas TPC reported in methanol peel extract of yellow lemon is 111.2 ± 1.18 mg GAE/g extract which is highest among tested different extracts. TFC ranged from 20.5 ± 0.57 to 259.25 ± 0.95 mg RE/g extract in yellow lemon peel extracts. The highest flavonoid content was reported in ethyl acetate extract (259.25 ± 0.95 mg RE/g extract), while the chloroform extract (20.5 ± 0.57 mg RE/g extract) was found to contain the lowest flavonoid content.

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

S.No	Plant Extract	IC50 (µg/ml AAE)
1.	Petroleum ether	231.13
2.	Chloroform	176.56
3.	Ethyl acetate	115.63
4.	Methanol	140.24
5.	Standard	24.11



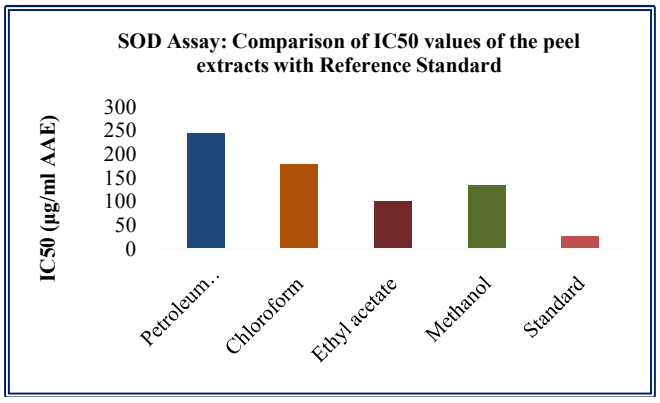
Graph 1 DPPH assay: Comparison of IC50 values of different peel extracts of lemon with standard



Graph 2 Comparison of Reducing capabilities of different peel extracts of Yellow lemon with standard

Table 2 Superoxide radical scavenging activity of different peel extracts of *Citrus limon* in terms of their IC50 values

S.No	Plant Extract	IC50 (µg/ml AAE)
1.	Petroleum ether	245.48
2.	Chloroform	178.31
3.	Ethyl acetate	100.80
4.	Methanol	134.23
5.	Standard	26.97



Graph 3 SOD assay: Comparison of IC50 values of different peel extracts of *Citrus limon* with standard

DISCUSSION

Phytochemical constituents acts as natural antioxidants, free radical scavengers and quenchers of singlet oxygen. The lemon peels which are highly wasted can serve in the therapeutic designing of natural drugs as an alternative treatment of various diseases. The yellow lemon peel extracts of methanol, ethyl acetate, chloroform and petroleum ether are found to contain phytoconstituents which were further tested for their radical scavenging activities. DPPH assay used for the quantification of novel antioxidants is a sensitive, reliable and fast method. It is a rapid analytical technique for the determination of preliminary details about the radical scavenging activities. The peel extracts of different polarities were investigated for their quenching capacities using ascorbic acid as reference standard. Results reveal that ethyl acetate extract of the *Citrus limon* peel was found with highest free radical quenching abilities among all the peel extracts. Ethyl acetate peel extract was reported the highest quenching capacity with IC50 value 115.63 µg/ml AAE, whereas pet ether was found with a minimum quenching capacity with IC50 value of 231.13 µg/ml AAE (Table 1). Results revealed that ethyl acetate peel extract of *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 > Pet ether (Graph 1).

The reducing ability of different 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 reduction 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 capabilities of different lemon peel extracts are shown (Graph 2) indicating that ethyl acetate possess highest antioxidant activity followed by methanol, chloroform and petroleum ether peel extract.

The principle of SOD scavenging assay is based on the inhibition of NBT reduction. Formation of superoxide radical takes place when one electron is gained by oxygen molecule from any other substance. A variety of enzyme complex are involved in the univalent reduction of molecular oxygen to superoxide. Two molecules of superoxide dismutase rapidly to molecular oxygen and hydrogen peroxide and this reaction is further accelerated by superoxide dismutase (SOD). Antioxidants acts as superoxide scavengers as they compete with NBT to react with superoxide. The reduction of NBT by 0_2^- radicals is indicated by blue color which is measured spectrophotometrically. Thus, percent inhibition of NBT reduction along with their IC50 values can be used to quantify scavenging of superoxide radical (Table 2). Ethyl acetate extract showed the highest quenching capacity with IC50 value 100.80 µg/ml while the petroleum ether peel extract showed the lowest quenching capacity with IC50 value 245.48 µg/ml AAE (Graph 3).

CONCLUSION

Current research is focused on finding an alternative source of treatment from natural resources. There exists lack of scientific evidence for the bioactivities of natural plant sources. Citrus species are rich in tannins, phenolic and flavonoid components. The presence of appreciable amounts of antioxidant activities may prove its efficacy as potential drug which may be explored for further studies. 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 an alternative non toxic agent in treating diseases.

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How to cite this article:

Nidhi Chauhan, Jyoti Saxena and Balvinder Kaur (2019) 'Evaluation of Antioxidant Potential in Different Peel Extracts of Yellow Citrus Limon', *International Journal of Current Advanced Research,* 08(03), pp. 17854-17857.
DOI: http://dx.doi.org/10.24327/ijcar.2019.17857.3401

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