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Quantitative Estimation Of Total Phenolic Content And Total Flavonoid Content Of Yellow And Green *Citrus Limon* Peel Extracts

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Abstract: The lemon peel is known for its wider aspect in medico-pharmacology. The production of citrus juice leads to a large quantity of peel and seeds waste which possess high contents of bioactive components. These components can be developed into useful and potential products after their value addition and may be useful as health enhancing drugs. The present study aims to estimate quantitatively the total phenolic and flavonoid content in different peel extracts of yellow and green *Citrus limon*. The extracts were investigated for the presence of phytochemicals and then analyzed for TPC and TFC using gallic acid and rutin as reference standard. The highest TFC was reported in ethyl acetate peel extracts of both yellow and green lemon peels. TFC reported in ethyl acetate peel extracts of yellow and green lemon are 259.25 ± 0.95 mg RE/g extract and 256.25 ± 0.95 mg RE/g extract respectively. Higher value of phenolics and flavonoid indicates higher antioxidant activity. The present study revealed that ethyl acetate extract possess appreciable phenolic and flavonoid content. And therefore, lemon peel could be a potential bioactive agent with antioxidant property and further research could be carried out to determine its efficiency against diseases.

Keywords:*Citrus limon,*peel extracts, antioxidant potential, total phenolic content, total flavonoid content.

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I. INTRODUCTION

Antioxidants play a significant role in plant and human life as they act as free radical scavengers which are formed during severe Oxidative Stress (OS). Insufficient levels of antioxidants causes OS and may damage or kill cells. Medicinal and herbal plants are the potential source of antioxidant phytochemicals and are used in the treatment of many chronic diseases [1]. Polyphenols and carotenoids are mainly contributes to the antioxidant phytochemicals as they possess most of the antioxidant properties [2].

Flavonoids are basically phenolic substances which are present in large number of vascular plants. Flavonoids are potential antioxidants [3] as they have the ability to scavenge free radicals and thus there has been an increasing interest in establishing the structure and antioxidant activity relationship of flavonoids [4]. Most of the Citrus flavonoids have been classified into three groups: flavonones, flavones and flavonols. The most abundant Citrus flavonoids are flavanones which is found almost 90% in lemons [5].

Lemon is known for its ethanomedical, ancient and medieval significance. The lemon fruit helps in the digestion process whereas its peel is used as cardiac tonic, stomachic and pulmonary sedative, appetizer and antitoxic agents. Lemon pulp is also used as antihelmentic agent, antipyretic, appetizer and as vascular stimulant and in curing throat tonsils etc [6].

Citrus limon fruit is found to contain bioactive components such as phenolic compounds, flavonoids, vitamins, carotenoids etc. Flavonoids rich lemon fruit is particularly important for a balanced diet and is significantly used for the prevention against various diseases such as diabetes, cardiovascular diseases and other neurogenerative disorders. The lemon peel is found to contain good phenolic and flavonoid content [7]. The most common citrus flavonoids are hesperitin, hesperidin, diosmin, apigenin, limocitrin, rutin, limocitrol, isolimocitrol, quercetin, etc [8].

Preliminary investigations on the *Citrus limon* peel extract revealed that the lemon peels are highly rich in phenolic compounds, alkaloids, tannins and flavonoids etc [9]. The present study focus on the quantitative estimation of the total phenolic content (TPC) and total flavonoid content (TFC) of different peel extracts of *Citrus limon* which may provide a base for the further study of their antioxidant activity.

II. MATERIALS AND METHODS

2.1 Plant material

The *Citrus limon* peels were shade dried for 12-15 days. Dried lemon peels were first coarsely powdered and then grinded in a mechanical grinder and kept in different solvents(plant material:solvent ratio-1:5) with occasional shaking [10].

2.2 Preparation of extract

The plant material is extracted by maceration process, sequentially from non-polar to polar solvents with petroleum ether, chloroform, ethyl acetate and methanol respectively.The solvents were evaporated to dryness and stored in refrigerator for further investigations.

2.3 Chemical reagents

The chemicals used for the estimation are petroleum ether, chloroform, ethyl acetate, methanol, Gallic acid, Folin-Ciocalteu Reagent (1:10 in deionized water), Sodium carbonate solution (7.5% w/v), Rutin/Quercetin, 5% $NaNO_2$, 10% $AlCl_3$, 4% NaOH, Concentrated H_2SO_4, HCl etc.

2.4 Preliminary phytochemical screening of the different plant extracts [11]

Qualitative phytochemical testing of different extracts was performed to study the presence of various phytochemical constituents using standard tests[11]. Phytochemical screening of different extracts showed the presence of carbohydrates, flavonoids, phenolics, tannins, alkaloids, fats and oils, saponins, etc.

2.5 Quantitative estimation of Total Phenolic Content(TPC)

TPC of all the extracts was determined by Folin-Ciocalteu method [12-13]. Gallic acid was used as referencestandard. Different concentrations of gallic acid (10-100 µg/ml) were prepared in methanol. Test sample of each extract was prepared in methanol (100 µg/ml) or solvent of near about same polarity. A volume of 0.5 ml of different concentrations of gallic acid/test sample was added to 2 ml of Folin-Ciocalteu reagent followed by 4 ml sodium carbonate solution. The reaction mixture was then incubated at room temperature and allowed to stand for 30 minutes with intermittent shaking. The absorbance was recorded by the Ultra-Violet (UV) Spectrophotometer at wavelength 765 nm using methanol as blank. Standard curve of different concentrations of gallic acid was prepared to find the line of regression. The TPC was obtained from the calibration curve of gallic acid and expressed as mg gallic acid equivalent (GAE)/g extract or µg GAE/mg extract.

2.6 Quantitative estimation of Total Flavonoid Content (TFC)

Estimation of TFC was determined using colorimetric assay [14]. The reference standard used for the estimation and plotting of calibration curve is Rutin. Different concentrations of rutin (10-100 µg/ml) were prepared in methanol. Test sample of each extract was prepared in methanol (100 µg/ml) or solvent of near about same polarity. A volume of 0.5 ml of the diluted sample solution was mixed with 2 ml of distilled water followed by 0.15 ml $NaNO_2$ solution. After 6 minutes, 0.15 ml $AlCl_3$ solution was added and allowed to stand for 6 minutes. After that, 2 ml NaOH solution was added to the reaction mixture and allowed to stand for 15 minutes. The absorbance was recorded at wavelength 510 nm using water as blank by the UV Spectrophotometer. Absorbance of the test samples was measured by line of regression of standard curve of rutin. TFC is expressed as Rutin equivalent (RE), mg RE/g extract or µg RE/mg extract.

2.7 Statistical analysis

All the experimental data were replicated four times (n=4), and the results were reported in terms of mean ± standard deviation (Mean ± SD) of the four replicates. The data were analysed with the help of one-way variance analysis (one way ANOVA).

III. RESULTS AND DISCUSSION

3.1 Phytochemical screening

Phytochemical screening of different peel extracts indicated the presence of bioactive phytoconstituents like carbohydrates, oils, flavonoids, alkaloids, glycosides, phenolics, tannins, saponins, etc. as shown in (Table 1). Presence of tannins and phenolic compounds and flavonoids in the peel extracts of ethyl acetate and methanol indicates the polar nature of these phytoconstituents. + sign indicates presence and - sign indicates absence of phytochemical constituents.

3.2 Total Phenolic Content

The TPC of different peel extracts of yellow and green *Citrus limon* were calculated using regression equation with the help of standard curve of gallic acid. The highest phenolic content was reported in methanol extract of yellow as well as green lemon peel. The lowest phenolic content was found in chloroform extract (Table 2).

TPC ranged from 90.35 ± 0.5 to 111.2 ± 1.18 mg GAE/g extract in yellow lemon peel whereas it ranged from 81.3 ± 0.11 to 140.9 ± 1.50 mg GAE/g extract in green lemon peel. The phenolic content was found negligible in chloroform peel extract whereas the highest TPC was reported in methanol peel extracts of both yellow and green lemon as well.

TPC in methanol peel extract of yellow lemon is found to be 111.2 ± 1.18 mg GAE/g extract whereas in that of green lemon peel extract is reported as 140.9 ± 1.50 mg GAE/g extract. The order of TPC found is: Chloroform < Ethyl acetate < Methanol. TPC and the antioxidant activity of the phytoextracts can be directly related. The plant extracts with higher amount of phenolic content possess higher antioxidant activity [1]. This relationship provides a strong base to the medicinal applications of the herbal plants in the prevention and treatment of various diseases.

3.3 Total Flavonoid Content

The TFC of different peel extracts of yellow and green *Citrus limon* were calculated using regression equation with the help of standard curve of rutin. The highest flavonoid content was reported in ethyl acetate extract while the chloroform extract was found to contain the lowest flavonoid content (Table 3).

TFC ranged from 20.5 ± 0.57 to 259.25 ± 0.95 mg RE/g extract in yellow lemon peel extracts whereas it ranged from 46 ± 0.81 to 256.25 ± 0.95 in green lemon peel extracts. The flavonoid content was found negligible in chloroform peel extract whereas the highest TFC was reported in ethyl acetate peel extract.

Ethyl acetate peel extract in yellow lemon is found with TFC value as 259.25 ± 0.95 mg RE/g extract whereas the ethyl acetate peel extract of green lemon contains 256.25 ± 0.95 mg RE/g extract. The TFC has found to be in the order Chloroform < Methanol < Ethyl acetate. The plants with higher content of flavonoids are considered to be good antioxidants. Flavonoids are the most important bioactive components [15], and they provides the potential to fight against certain diseases.

TPC values were calculated with the help of line of regression obtained from the calibration curve of gallic acid and expressed as mg gallic acid equivalent (GAE)/g extract or µg GAE/mg extract (Graph 1). TFC values were measured by line of regression of standard curve of rutin and expressed as Rutin equivalent (RE), mg RE/g extract or µg RE/mg extract (Graph 2). Correlation curves for the TPC and TFC of yellow lemon peel extracts are indicated in Graph 3 whereas that of green lemon peel extracts are shown in Graph 4.

Table 1: Phytochemical screening

Phytoconstituents investigated in different peel extracts of *Citrus limon*				
Phytochemical constituents	**Pet ether**	**Chloroform**	**Ethyl acetate**	**Methanol**
Carbohydrates	+	+	+	+
Protein, Amino acids	-	-	-	-
Glycosides	-	-	+	+
Alkaloids	+	+	+	+
Saponins	-	-	+	+
Flavonoids	+	+	+	+
Triterpenoids	-	+	+	+
Tannins, phenolics	-	+	+	+
Fats and oils	+	+	+	+

Table 2: TPC of Yellow lemon and Green lemon peel extracts

Name of the extract	**TPC (mg GAE/g extract)**	
	Yellow lemon	**Green lemon**
Chloroform	90.35 ± 0.5	81.3 ± 0.11
Ethyl acetate	107.05 ± 0.34	136.15 ± 0.19
Methanol	111.2 ± 1.18	140.9 ± 1.50

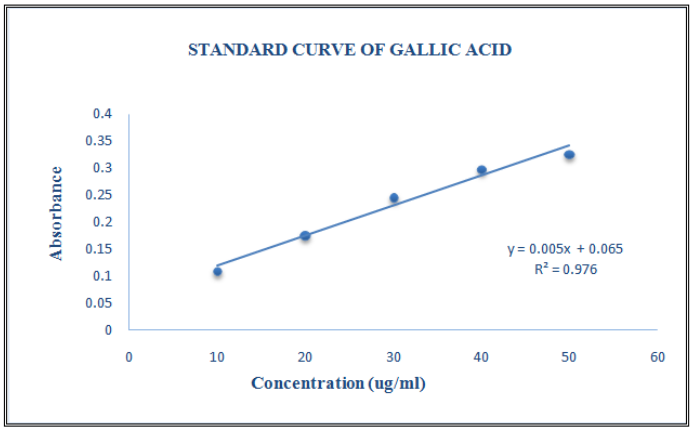
x represents quantity/concentration of phenols which is obtained from the equation: y=0.005x + 0.065, where y is absorbance of samples and R^2=0.9761. Results are expressed asMean±SD. SD: Standard deviation, GAE: Gallic Acid Equivalents.

Table 3: TFC of Yellow lemon and Green lemon peel extracts

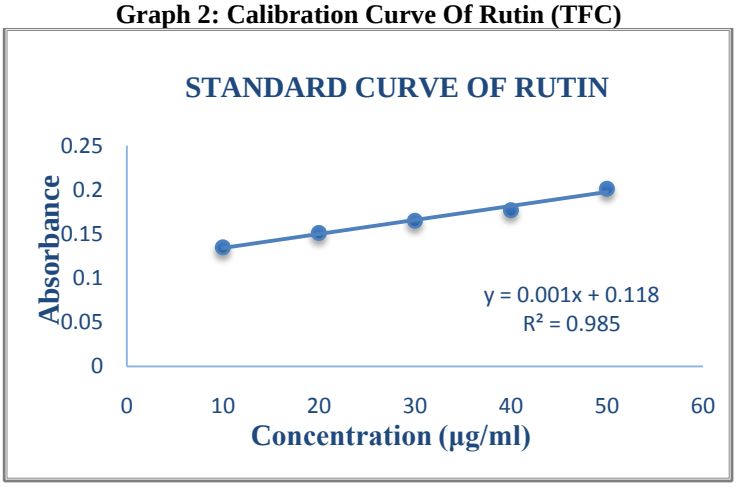
Name of the extract	TFC (mg RE/g extract)	
	Yellow lemon	Green lemon
Chloroform	20.5 ± 0.57	46 ± 0.81
Ethyl acetate	259.25 ± 0.95	256.25 ± 0.95
Methanol	212.25 ± 1.70	160.5 ± 0.57

x represents quantity/concentration of flavonoids which is obtained from the equation: y=0.001x + 0.118, where y is absorbance of samples and R^2=0.9856. Results are expressed in Mean±SD.SD: Standard deviation, RE: Rutin Equivalents.

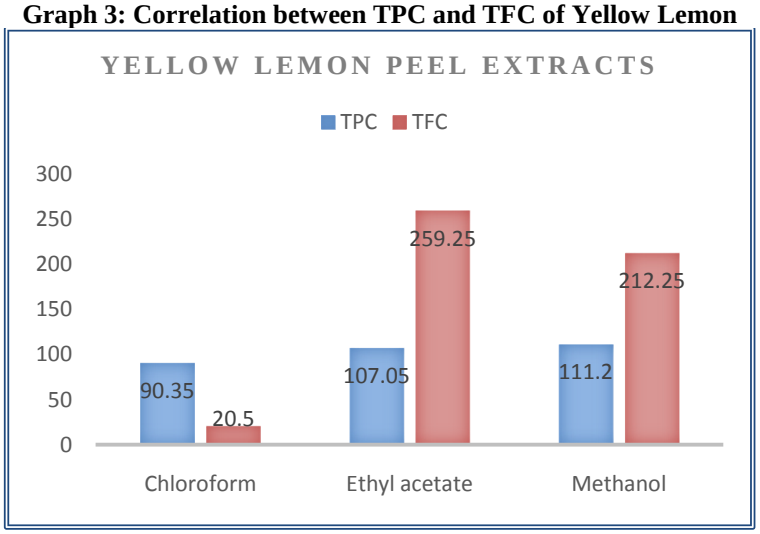
Graph 1: Calibration Curve Of Gallic Acid (TPC)



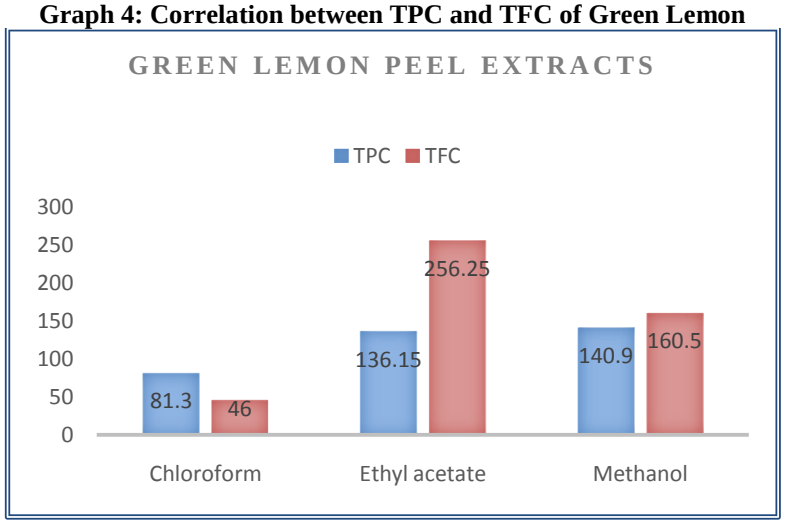
Graph 2: Calibration Curve Of Rutin (TFC)



Graph 3: Correlation between TPC and TFC of Yellow Lemon



Graph 4: Correlation between TPC and TFC of Green Lemon



IV. CONCLUSION

The present study was conducted on the different peel extracts of yellow and green lemon, which revealed that the ethyl acetate extracts of yellow as well as green lemon possess the maximum flavonoid content. The presence of high amounts of flavonoid and phenolic content in ethyl acetate and methanol extracts of *Citrus limon* suggests the presence of bioactive components which can be potential antioxidants for treating and preventing various neurodegenerative diseases. Lemon peel extracts possess appreciable amounts of phenol and flavonoid content and thus it may be considered a potential source of natural antioxidant. Thus, it may provide a base for further studies for the isolation and characterization of the bioactive component so as to widen the scope in neuromedical applications.

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