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Research Article

PHYTOCHEMICAL SCREENING OF YELLOW & GREEN *CITRUS LIMON* PEEL EXTRACTS IN DIFFERENT SOLVENTS

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ABSTRACT

Lemon or *Citrus limon* is a small evergreen subtropical plant in the family Rutaceae. Lemon is grown for its edible fruit, which is used for preparing a variety of recipes, jams, jellies, pickles and drinks. It is also used as preservatives. It is known for immense medicinal values as it possess various pharmacological properties like antioxidant, antibacterial, antifungal, antimicrobial, and anticancer. To isolate its biologically active components for exploring its pharmacological uses, present study was carried out. For this purpose, phytochemical screening was carried out for the first time in peel extracts of Yellow and Green *Citrus limon* prepared in four different solvents. Results indicated the presence of secondary metabolites such as carbohydrates, reducing sugar, glycosides, alkaloids, flavonoids, phenolic compounds and tannins, triterpenoids and steroids in different peel extracts of Yellow and Green lemon.

KEYWORDS: *Citrus limon*, peel extracts, phytochemical screening, antioxidants, pharmacological importance.

INTRODUCTION

Citrus fruits are of great pharmacological importance[1] as they are rich in flavonoids, which possess antioxidant properties. They offer resistance and protection against pathogens[2]. Lemon is one of the most important commercial citrus fruit crop grown all over the world. It is a perennial evergreen subtropical plant of genus *citrus* of family rutaceae and sub family aurantioideae containing 130 genera in the seven sub families. It is a rich source of vitamin C and has uniquely aromatic peels. Its peel extracts are rich in bioactive components and acts as potential antioxidants[3]. Its peel extracts were found to possess a wide range of antioxidant, antibacterial[4], antifungal[5], antimicrobial[6], and anticancer[7] activities. Determination of biologically active compounds from plant material depends on the type of solvent used during the process of extraction. Thus, there exists a need to screen phytochemicals in different solvents. Present study illustrates the phytochemical screening in different solvents viz; methanol, ethyl acetate, chloroform and petroleum ether.

MATERIALS AND METHODS

Collection of samples

Lemon peels were collected from the local market and fruit juice shops of Bhopal, M.P., India. Peels were properly washed with normal water and then with distilled water and thereafter extra pulp was properly removed. Peels were then shade dried at an ambient temperature for 12-15 days. Dried lemon peels were first coarsely powdered using a mortar and pestle and then were further grinded using a mechanical blender.

Figure: Yellow Lemon Peels

Figure: Green Lemon Peels

Extract preparation

The plant extracts were prepared by maceration technique. The grinded yellow and green lemon peels were kept in four different solvents on the basis of increasing polarity of solvents. The four solvents used were petroleum ether, chloroform, ethyl acetate and methanol. Dried lemon peel powder was successively kept in different solvents for 48 hours and then filtered. The solvent was vapourized at 40°C in the Soxhlet apparatus to obtain the desired peel extracts.

Qualitative analysis

The different peel extracts of green and yellow lemon were qualitatively analyzed for the presence of different phytoconstituents like carbohydrates, proteins, flavonoids, glycosides, steroids, alkaloids, tannin and phenolic compounds. The qualitative tests performed for phytochemical screening[8] are as follows:

Test for carbohydrates:

1. **Molisch's test:** Treat 2 ml aqueous solution of extract with Molisch's reagent (alcoholic solution α-naphthol) and then add 1 ml of concentrated sulphuric acid carefully along the sides of the test tube, shake well and allow it to stand for few minutes. Formation of violet ring indicates the presence of carbohydrates.
2. **Fehling's test:** Treat 1 ml aqueous solution of extract with 2 ml Fehling's solution (equal mixture of Fehling's A and Fehling's B solution) and heat it on water bath for 10 minutes. Formation of red precipitate indicates the presence of reducing sugar.
3. **Benedict's test:** Equal volume of Benedict's reagent and extract were mixed and heated on water bath for 5-10 minutes. Appearance of green, yellow, orange or red colour indicates the presence of reducing sugar.

Test for Proteins and Amino acids:

1. **Biuret's test:** The extract was treated with 1 ml of 10% sodium hydroxide solution and heated. Now add a drop of 0.7% copper sulphate to this mixture. Formation of violet or pink colour indicates the presence of proteins.
2. **Ninhydrin test:** Solution of extract was heated with 5% ninhydrin solution on a water bath for 10 minutes. Formation of bluish colour indicates the presence of amino acids.

Test for Glycosides:

1. **Legal's test:** Dissolve 1 ml of extract in pyridine. Add 1 ml of sodium nitroprusside solution and make it alkaline using 10% sodium hydroxide solution. Formation of pink to blood red colour indicates the presence of cardiac glycosides.
2. **Keller- Killiani test:** To 2 ml of extract add 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride. Now carefully add 0.5 ml of concentrated sulphuric acid by the sides of the test tube. Formation of blue colour in the acetic acid layer indicates the presence of cardiac glycosides.

Test for Alkaloids: To the extract, dilute hydrochloric acid was added, shaken well and filtered. With the filtrate, the following tests were performed:

1. **Hager's test:** To 2 ml of filtrate add few drops of Hager's reagent. Formation of yellow coloured precipitate indicates the presence of alkaloids.
2. **Wagner's test:** To 2 ml of filtrate add few drops of Wagner's reagent. Formation of reddish-brown precipitate indicates the presence of alkaloids.

Test for Saponins:

1. **Froth test:** Diluted the extract with distilled water and shake it vigorously for 5 minutes. The formation of layer of persistent froth indicates the presence of saponins.
2. 0.5 gm of extract was boiled with distilled water and filtered. 10 ml of distilled water was added in it. Shake it well for few minutes and allow it to stand. Frothing along with the formation of honey comb indicates the presence of saponins.

Test for Flavonoids:

1. **Lead Acetate test:** The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.
2. **Alkaline reagent test:** The extract was treated with few drops of sodium hydroxide. Formation of intense yellow colour, which becomes colourless on addition of few drops of dilute acid indicates the presence of flavonoids.

Test for Triterpenoids and Steroids:

1. **Salkowski's test:** 0.5 ml of the extract was treated with 3 ml of chloroform and filtered. To the filtrate add few drops of concentrated sulphuric acid along the sides of the test tube, shake and allowed it to stand. If the lower layer turns red then it shows the presence of steroids. Appearance of golden yellow colour in the bottom layer indicates the presence of triterpenoids.
2. **Liebermann-Burchard's test:** The extract was treated with chloroform. To this solution few drops of acetic anhydride was added, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. Formation of brown ring at the junction of two layers, if upper layer turned green, it indicates the presence of steroids and if it turns deep red in colour then it indicates the presence of triterpenoids.

Test for Tannin and Phenolic compounds:

1. **Ferric Chloride test:** To the aqueous solution of the extract, add 2 ml of 5% ferric chloride solution. Formation of blue, green or violet colour indicates the presence of phenolic compounds.
2. **Lead Acetate test:** To the aqueous solution of the extract, add few drops of lead acetate solution. Formation of white precipitate indicates the presence of phenolic compounds.
3. **Gelatin test:** To the aqueous solution of the extract, add 2 ml of 1% gelatin solution containing 10% sodium chloride. Formation of white precipitate indicates the presence of phenolic compounds.
4. **Dilute Iodine Solution test:** To 2 ml of the extract, add few drops of iodine solution. Formation of transient red colour indicates the presence of phenolic compounds.

Test for Fats and Oils:

1. **Solubility test:** To 2 ml of the alcoholic solution of the extract, add few drops of chloroform and observe its solubility.
2. To 2 ml of the alcoholic solution of the extract, add few drops of 90% ethanol and observe its solubility.

RESULTS

The different peel extracts of Green and Yellow lemon were investigated for the various phytochemical constituents. The results of phytochemical screening revealed the presence of many bioactive components in different peel extracts. The results for the presence of these phytochemicals in Yellow lemon are tabulated in Table 1, whereas the phytochemicals reported in Green lemon are tabulated in Table 2. Positive sign (+) indicated the presence of phytochemicals whereas negative sign (-) indicated the absence of phytochemicals.

The methanol and ethyl acetate extracts of Yellow lemon peel and Green lemon peel showed presence of carbohydrates, reducing sugar, glycosides, alkaloids, flavonoids, saponins, phenolic compounds and tannins, triterpenoids and steroids. Presence of tannin, phenolic compounds and flavonoids in ethyl acetate and methanol extract indicates the polar nature of the phytoconstituents present. The chloroform and petroleum ether extract exhibited the absence of saponins, but the presence of other secondary metabolites like carbohydrates, alkaloids and flavonoids was reported. The petroleum ether extract showed the absence of glycosides, triterpenoids and steroids, phenolic compounds and tannins, but there exist possibilities that higher carbohydrates may be present in petroleum ether extracts[9] of natural plants, thus results indicated the presence of carbohydrates in petroleum ether extract. Various phytochemicals reported in the present study could be responsible for exhibiting versatile pharmacological activities.

Table 1: Phytochemical screening of Yellow lemon peel

S.No	Phyto-chemical constituent	Name of the test	Name of the extract			
			Petroleum ether	Chloroform	Ethyl Acetate	Methanol
1.	Carbohydrates	Molisch's Test	+	+	+	+
		Fehling's Test	-	-	+	+
		Benedict's Test	+	+	+	+
2.	Protein and Amino acids	Biuret's Test	-	-	-	-
		Ninhydrin Test	-	-	-	-
3.	Glycosides	Legal's Test	-	-	+	+
		Keller-Killiani Test	-	-	+	+
4.	Alkaloids	Hager's Test	+	+	+	+
		Wagner's Test	+	+	+	+
5.	Saponins	Froth Test	-	-	+	+
6.	Flavonoids	Lead Acetate Test	+	+	+	+
		Alkaline reagent Test	+	+	+	+
7.	Triterpenoids and Steroids	Salkowski's Test	-	+	+	+
		Liebermann-Burchard's Test	-	+	+	+
8.	Tannins and Phenolic compounds	Ferric Chloride Test	-	-	+	+
		Lead Acetate Test	-	+	+	+
		Gelatin Test	+	+	+	+
9.	Fats and Oils	Solubility Test	+	+	+	+

Table 2: Phytochemical screening of Green lemon peel

S.No	Phyto-chemical constituent	Name of the test	Name of the extract			
			Petroleum ether	Chlorofom	Ethyl Acetate	Methanol
1.	Carbohydrates	Molisch's Test	+	+	+	+
		Fehling's Test	-	-	+	+
		Benedict's Test	+	+	+	+
2.	Protein and Amino acids	Biuret's Test	-	-	-	-
		Ninhydrin Test	-	-	-	-
3.	Glycosides	Legal's Test	-	-	+	+
		Keller-Killiani Test	-	-	+	+
4.	Alkaloids	Hager's Test	+	+	+	+
		Wagner's Test	+	+	+	+
5.	Saponins	Froth Test	-	-	+	+
6.	Flavonoids	Lead Acetate Test	+	+	+	+
		Alkaline reagent Test	+	+	+	+
7.	Triterpenoids and Steroids	Salkowski's Test	-	+	+	+
		Liebermann-Burchard's Test	-	+	+	+
8.	Tannin and Phenolic compounds	Ferric Chloride Test	-	-	+	+
		Lead Acetate Test	-	+	+	+
		Gelatin Test	+	+	+	+
9.	Fats and Oils	Solubility Test	+	+	+	+

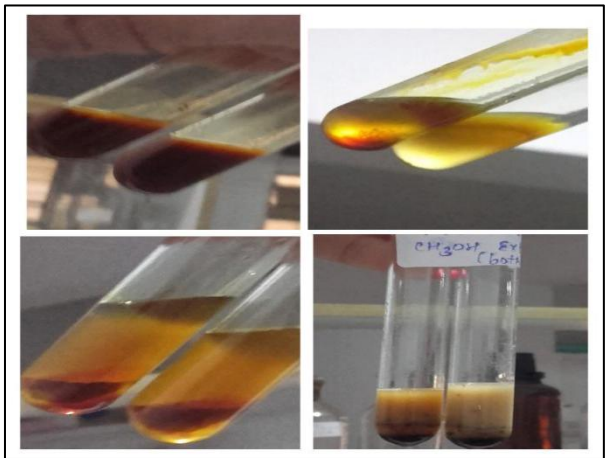

Figure: Colour change during phytochemical screening (Wagner's, Salkowski's, Legal's and Molisch's test) of methanol peel extract of both yellow and green lemon.

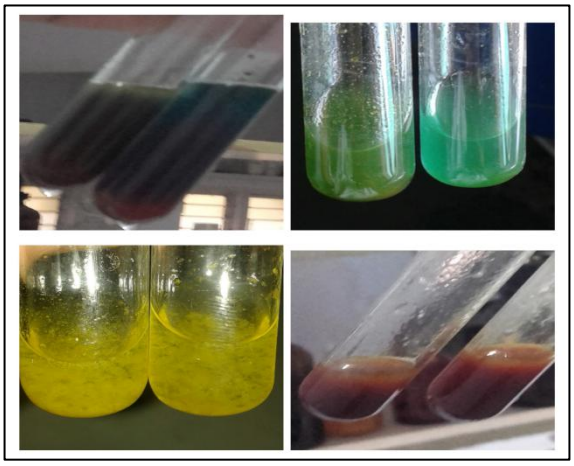

Figure: Colour change during phytochemical screening (Fehling's, Benedict's, Hager's and Wagner's test) of ethyl acetate peel extract of both yellow and green lemon.

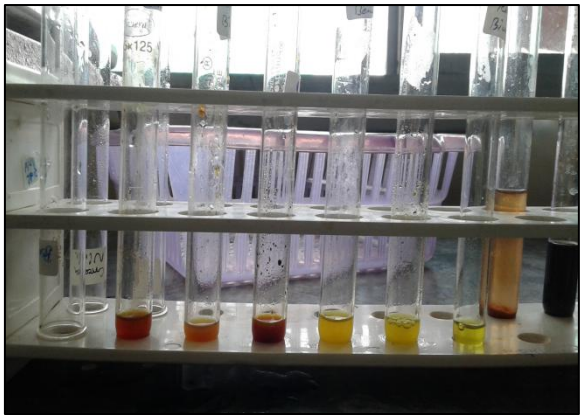

Figure: Colour change during phytochemical screening of chloroform peel extract.

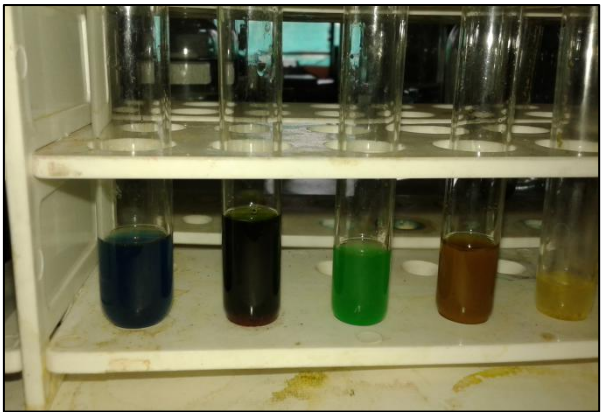

Figure: Colour change during phytochemical screening of petroleum ether peel extract

DISCUSSION

Medicinal plants are known for their bioactivities and medicated ailments. 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 designing of natural drugs as an alternative therapeutic approach for treating various diseases. The present activity describes for the first time the phytoconstituents reported in the yellow and green lemon peel extracts of methanol, ethyl acetate, chloroform and petroleum ether so as to search for the bioactivities of the same in further research which is under pipeline. Medicinal plants are usually screened for presence of phytochemicals that may lead to its further isolation, purification and characterization of the bioactive component. The bioactive component along with its bioactivity can be used as the basis of new pharmaceutical drug development. The ethnopharmacological application of plants is a good tool to explore their biological activities and therefore it can be the subject for extensive research area.

CONCLUSION

Medicinal plant species have been studied in the search of novel antioxidants. But the demand for potential bioactive antioxidants is still prevailing. From the present study it can be concluded that the different peel extracts of Yellow and Green lemon individually showed the presence of various phytochemicals which may be used in the treatment of various diseases. It could be a significant initiative to establish the relationship between the phytochemical components and their bioactivity. Presence of phytoconstituents in the peel extracts of *Citrus limon* can be explored for their bioactivity which may serve its efficacy as potential drug against various diseases.

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