Research Paper
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Phytochemical screening and evaluation of leaves of *Citrus limon* (L.)Osbeck.

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Abstract

Plants have some specific bioactive compounds which are called Phytochemicals. They usually don't have any nutritional property but are used as defense by the plants and also to prevent many diseases. Human body can uptake only minimal amount but they are very useful to minimize the free radical damage caused to the cells, as a result of oxidative stress. Lemon is a citrus fruit considered to be rich in Phytochemicals. This study gives a brief perception of the *Citrus limonum*leaf, the bioactive compounds or Phytoconstituents present in them. Phytochemical screening was carried out in four extracts i.e. petroleum ether, acetone , methanol and chloroform, to identify the bioactive compounds such as alkaloids, carbohydrates, glycosides, steroids, Anthocyanins, Saponins, phenols, triterpenoids.

Keywords:*Citrus limon*, Phytochemicals, extracts, medicinal properties.

Introduction

Phytochemicals are those compounds which are produced naturally in plants. Secondary metabolites are derived from primary metabolites and are used as drugs or to obtain medicines (Akerle Heywood and Synge, 1991). They play an important role in preventing number of diseases such as asthma, arthritis, cancer etc. These Phytochemicals do not have any side effects and are also called as 'human friendly' constituents.Some, particularly those belonging to the Rutaceae family have been found to be very effective to natural antioxidants and prevents many diseases. Our current study mainly deals with collection, extraction, qualitative and quantitative analysis of Phytochemicals or bioactive compounds which are present in the leaves of plant *Citrus limon*(L.)Osbeck. Nearly, all the Citrus fruits are considered to be rich in phytoconstituents.

Lemon is very famous in many parts of the world because of its unique and different flavor, taste, and aroma as well as multiple health benefits associated with it. Consumption of lemon according to recommended dietary allowances, they are very beneficial to maintain and improve health and alsoprovide prevention against many different kinds of diseases because of the presence of different kinds of Phytochemicals (Pellegrini*et al.*, 2003; Peterson *et al.*, 2006). The presence of phytochemicals is responsible for their therapeutic effects. The qualitative analysis of bioactive compounds of leaves of *Citrus limon* (L.) Osbeck for the four extracts i.e. Petroleum ether, acetone, chloroform and methanol have been analyzed in this study and there is wide range of phytochemical compounds present in the four extracts which are shown below in brief.

Materials And Methods

Materials

The selected plant species for the experiment are *Citrus limon* of Rutaceae family. Plant part used are leaves. The selected solvents for extract preparation are Petroleum ether, Acetone, Methanol and chloroform.

Plant collection:

The leaves of plants species *Citrus limon* were collected from region of Ahmedabad city, Gujarat, India in the month of December-2016.

Sample preparation:

The leaves of *Citrus limon* were carefully separated, cleaned, washed well to remove all the dirt and are shade dried separately until all the water molecules were evaporated, mechanically grinded and coarsely powdered. The powder was subjected to solvent extraction with petroleum ether, acetone, methanol and chloroform.

Extraction Technique:

10gm of each leaf powder was added to 100 ml of solvent (petroleum ether, acetone, methanol and chloroform) in a conical flask. They were kept in shaker for 24 hours at room temperature. All the extracts were filtered by using Whattman No.1 filter paper and the concentrated extracts were collected in the petridishes and allowed to evaporate properly. Petridishes were weighed before and after the evaporation of filtrate to know their weight of extracts and then stored in a cool and dry place.

Phytochemical Screening

Test for alkaloids:

Extracts were mixed individually with a small amount of dilute HCland it wasfiltered. Thefiltrate was evaluated carefully with different alkaloidal reagents.

Mayer's test:2ml of Mayer's reagent was added in 1 ml of filtrate. Formation of cream precipitate indicates the presence of alkaloids.

Dragendroff's test: 2ml of Dragendroff's reagent was added in 1 ml of filtrate. Formation oforange brown color indicates the presence of alkaloids.

Hager's test: 2 ml of Hager's reagent is added in 1 ml of filtrate. Formation of yellow precipitate indicates the presence of alkaloids.

Wagner's test:2ml of Wagner's regent is added to 1 ml of filtrate. Formation of reddish brown indicates the presence of alkaloids.

Test for Carbohydrate:

The small amount of extract was dissolved in 5 ml of distilled water and it is filtered. The filtrate was subjected to analysis for the presence of carbohydrates.

Molisch test: The filtrate was mixed with 2 to 3 drops of 1% alcoholic alpha napthol and along the sides of test tube; 2 ml of concentrated sulphuric acid was added and appearance of purple color ring at the junction of two liquids.

Fehling's test: Fehling A and Fehling B was mixed and few drops of extract are added and boiled. A brick red colored precipitate of cuprous oxide forms if reducing sugars are present.

Benedicts test: 2 to 3 drops of benedicts regent was added to 1 ml extract and heat almost to boiling. Appearance of red, brown, orange or yellow color indicates the presence of carbohydrates.

Test for glycosides:

Keller-Killiani test: To 2 ml extract, add glacial; acetic acid. 1 drop 5 % $FeCl_3$ and conc. H_2SO_4. Brown ring appears indicates the presence of glycosides

Test for proteins and free amino acids:

Small quantity of extracts were dissolved separately in a few ml of water and treated with following reagents:

Millon's test: Few mg of extract was added with millon's reagent. Formation ofred color shows the presence of proteins and amino acids.

Ninhydrin test: 0.1% ninhydrin solution was added to few mg of extract. Appearance of purple color shows the presence of protein and free amino acids.

Biuret's test: Equal volume of 5 % solution of sodium hydroxide and 1% solution of copper sulphate were added. Appearance of pink color shows the presence amino acids and proteins.

Test for flavonoids:

Few ml of extract was mixed with sulphuric acid. The formation of yellow orange indicates the presence of flavones, formation of yellowish orange color indicates the presence of anthocyanins and the formation of orange to crimson color indicates the presence of flavonones.

Test for steroids:

Salkowski test: To 2 ml of extract add 2 ml chloroform and 2 ml conc. H_2SO_4 and was shaken for few minutes. Chloroform layer appeared red (upper layer) and acid layer showed greenish yellow fluorescence (lower layer) indicates the presence of steroids.

Liebermann-Burchard test: Mix 2 ml extract with chloroform. Add 1 to 2 ml acetic anhydride and 2 drops conc. H_2SO_4 from the side of test tube. First red, then blue and finally green color indicates the presence of steroids.

Test for Triterpenoids:

Liebermann-Burchard test: Mix 2 ml extract with chloroform. Add 1 to 2 ml acetic anhydride and 2 drops conc.H_2SO_4 from the side of test tube. First red, then blue and finally green color indicates the presence of steroids

Test for Tannins:

Gelatin test: to the extract, gelatin (dissolved in warm water immediately) solution was added. Formation of white precipitate indicates the presence of tannins.

Lead acetate test: 10% of lead acetate solution was added in few ml of extract. Formation of white precipitate indicates the presence of tannins.

Tests for Phenol:

Ferric chloride test: Few mg of extract is dissolved in 5 ml of distilled water. 2 ml of 1 % solution of gelatin containing 10% NaCl is added to it. White precipitates indicate presence of phenolic compounds.

Sodium hydroxide test: Five mg of extract was dissolved in 0.5 ml of 20% sulphuric acid solution. Add few drops of aqueous sodium hydroxide solution. Formation of blue color indicates the presence of phenols

Ellagic Acid Test: Add few drops of 5% glacial acetic acid and 5% $NaNO_2$ solution was added in few ml of extracts . Muddy or Niger brown precipitate occurs.

Tests for Saponins:

Foam test: small amount of extract is shaken with little amount distilled water. Formation of foam indicates the presence of saponins.

Result and Discussion

The lemon leaf extractwas rich in Phytochemicals activity and constituents as shown in table 1. In present study, preliminary Phytochemical study was carried out to identify the bioactive compounds such as alkaloids, carbohydrates, glycosides, steroids, Anthocyanins, Saponins, phenols, triterpenoids were present.Phytochemical analysis of *Citrus limon* plant shows the presence of alkaloids, carbohydrates, glycosides and steroids in high amounts in all the four extracts. Anthocyanins are present in petroleum ether, acetone and chloroform extracts, while flavanones

are present in methanolic extracts in moderate amount. Saponins, phenols, triterpenoids and tannins are also present in considerably moderate amount. Proteins and free amino acids are present in very few amounts in all the extracts except the chloroform extract (Prasad M. P. *et al.*, 2014). Tannins, which were absent in methanolic and acetonic extracts (Saumendu Deb Roy *et al.*, 2012).

Phytochemical screening of *Citrus limon*(L.) Osbeck leaf extract

SRNO	CONSTITUENTS	TESTS	RESULTS			
			PE	AE	ME	CE
A	Alkaloids					
		Mayer's test	+	+	-	+
		Dragendroff's test	+++	++	+++	++
		Hager's test	+	+	+	++
		Wagner's test	-	-	-	-
B	Carbohydrates					
		Molisch test	++	+++	++	+++
		Fehling's test	-	-	-	-
		Benedicts test	++	+++	++	+
C	Glycosides					
		Keller-killiani test	++	++	+++	+
D	Proteins and free amino acids					
		Millon's test	+	++	+	-
		Ninhydrin test	+	+	-	-
		Biurets test	-	+	+	-
E	Flavonoids					
		H_2SO_4 test	+ (antho cyanin)	+ (antho cyanin)	++ (flavo nones)	+ (antho cyanin)
F	Steroids					
		Salkowski test	++	+++	+++	-
		Liebermann-Burchard test	++	++	++	+
G	Triterpenoids					
		Liebermann-Burchard test	++	++	++	+

(-) Absent, (+) Very few, (++) Moderate, (+++)High

Conclusion

Plants are mostly used for medicinal purposes and considered as important source to obtain drugs in many different regions of the world. India is considered as one of the largest source and producer of many important medicinal herbs and plants. Many people still uses traditional methods to obtain drugs from different medicinally important plants. Drugs from the plants can be made easily available and can be isolated through different methods. They are also less expensive, more safe, and efficient and have negligible side effects in comparison to the pharmacological chemicals. The presence of bioactive constituents in the leaves of *Citrus limon*can be used in a multitude of ways for obtaining medicines. Alkaloids are important compounds which have a metabolic role in the living systems. Flavonoids have been foundto prevent the cancer causing tumors. It also inhibits growth and progression of tumors.Phenols together with the flavonoids compounds in plants showmultiple activities like antioxidant, anti-carcinogenic, anti-inflammatory etc.Tannins acts against the pathogenic fungi and shows antimicrobial activity. Saponins are responsible for the leakage of proteins and degradation of cell wall .The plant based compounds which are naturally produced have the effective dosage response and minimal side effects when compared to the synthetic compounds with much more number of health benefits.It reflects a ray of hope for the development of many more new drugs which will prevent chemotherapeutic tumors.With the help from such plants in future they may serve for the production of drugs to cure the deadly diseases.

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