International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 4 Issue 5, July-August 2020 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Qualitative and Quantitative Phytochemical Screening of Citrus Peel

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ABSTRACT

The objective of this study was to find out the presence of phytochemicals in the aqueous extracts of citrus peel of both qualitative and quantitative screening methods. In qualitative analysis, the phytochemical compounds such as alkaloids, saponin, tannin, phenol, a, flavonoids, glycosides, steroids, terpenes, flavonoid and were determined in the sample aqueous extracts by using standard methods. The aqueous extract of the citrus peel showed positive results for nine phytochemical tests. Also, quantitative analysis of the important secondary metabolites such as alkaloids, phenolic compounds, flavonoids, saponins and tannins were tested in the sample extracts. Results concluded that the presence of these active compounds may be responsible for the medicinal purposes of the plant.

KEYWORDS: *Phytochemicals, citrus peel qualitative, quantitative screening*

How to cite this paper: Arti Chandanshive | Ashpak Tamboli | Naziya Khan | Priyanka Karande "Qualitative and Quantitative Phytochemical Screening of Citrus Peel" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-4 | Issue-5, August 2020, pp.964-967, URL: www.ijtsrd.com/papers/ijtsrd33036.pdf

IJTSRD33036

INTRODUCTION

Plants are endowed with various phytochemical molecules such as vitamins, phenolic, stilbenes, tannins, flavonoids, quinones, coumarins, and other metabolites which are rich source of free radical scavengers [1-3]. They are also antioxidant compounds which possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities hepaptoprotective antiarthritic antimicrobial antianginal anticancer antidiabetic. Furthermore, ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing. Even though pharmacological industries have produced a number of new antibiotic in the last three decade, resistance to these drug by microorganism has increased. In journal, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agent (Gislene et al., 2000). For a long period of time, Plant have been valuable of natural product for maintain human health. The use of plant extract and phytochemical, These product are known by their active substance e.g. the phenolic compound which are part of the essential oils, as well as tannin Essential oil are more effective in controlling biofilm culture due to their better diffusibility and mode of contact (Al-Shuneigat et al., 2005).Hence the essential oil and other extracts of plant evoked interest as source of natural product. They have been screened for their potential use uses as alternative remedies for the treatment of many infectious disease. (Tepe et al., 2004

METHODOLOGY

A. Collection of plant material:

The peel of citrus Linn were collected from market in Sangola Village, Maharashtra, India. The Peel were dried under shade for 15 days, grounded into fine powder and sieved using a laboratory test sieves of 212mm. aperture.

B. Authentication of Plant material:

The plant was authenticated by Department of Botany, Sangola College of Arts, Science and Commerce Sangola.

C. Extraction:

The fine powder of Citrus Peel Linn was defined with Chloroform, Ethyl acetate, Diethyl Ether, Ethanol, and Water as solvent using Soxhlet extraction. The extraction were carried out for about 4 days at temperature between 65°C and 70°C.

- **Qualitative phytochemical test:**

The extract were subject to various chemical test to detect the chemical constituent present in them. Test methods are described below.

➢ **Detection of Alkaloids:-**

Extracts were dissolved individually in dilute HCL and filtered. The filtrates was used to test for the presence of alkaloids.

A. Mayer's Test:

Filtrate was treated with Mayer's reagent (potassium mercuric iodide). Formation of cream colored precipitate indicated the presence of alkaloid.

B. Wagner's Test:-

Filtrate was treated with Wagner's reagent (Iodine in potassium iodide) Formation of brown\ reddish brown precipitate indicated the presence of alkaloid.

C. Dragendroff'sTest:-

Filtrate was treated with Dragendroff's reagent (solution of potassium bismuth iodide). Formation of red precipitate indicate the presence of alkaloid.

D. Hager's Test:

Filtrate was treated with Hager's reagent (Saturated picric acid solution) Formation of yellow colored precipitate indicate the presence of alkaloid.

➢ **Detection of Carbohydrate:-**

Extract were dissolved individually in 5 ml distilled water and filtered. The filtrate was used to test for the presence of carbohydrate.

A. Molisch's Test:-

filtrate was treated with 2 drops of alcoholic naphthol solution in a test tube and 2ml of conc sulphuric acid was added carefully along the side of the test tube. Formation of violet ring at the junction indicated the presence of carbohydrate.

B. Benedict Test:-

Filtrate was treated with benedict's reagent and heated on water bath. Formation of orange red precipitate indicated the presence of reducing sugars.

C. Fehling's Test:-

Filtrate was hydrolyzed with diluted HCL, neutralized with alkali and heated with Fehling's A and B solution. Formation of red precipitate indicated the presence of reducing sugar.

D. Barfoed'sTest:-

Extract heated with Barfoed'sreagent.(Copper acetate and glacial acetic acid). Formation of red color indicate presence of reducing sugar.

Detection of Glycoside:-

A. Legal Test:-

Hydrolysed extract was treated with sodium nitroprusside in pyridine and methanolic alkali. Formation of pink to blood red color indicated the presence of cardiac glycosides.

B. Liebermann Burchard's Test:-

hydrolized extract was treated with chloroform and a few drops of acetic anhydried, boiled and cooled. Conc. Sulphuric acid was added carefully along the side of the test tube. Formation of brown ring at the junction indicated the presence of steroidal glycoside.

➢ **Detection of Saponins:-**

A. Foam test:-

Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15min. Formation of 1cm layer of foam indicated the presence of saponins.

Detection of Tannin:

A. Gelatine test:

To the extract 1% gelatine solution containing sodium chloride was once added. Formation of white precipitate shows the presence of tannin.

Detection of flavonoids:-

A. Alkaline reagent Test:-

Extract were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which become colorless on addition of dilute acid, indicated the presence of flavonoids.

B. Lead acetate Test:-

Extract were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicated the presence of flavonoids.

C. Shinoda Test:-

To the alcoholic solution of extracts, a few fragment of magnesium ribbon and Conc. HCL were added. Appearance of maganeta color after few minute indicated the presence of flavonoids.

➢ **Quantitative evaluation of phytochemical constituents:**

- **Determination of alkaloid:**

Quantitative determination of alkaloid was according to the methodology by Harborne (2) Exactly 200cm3 of 10% acetic acid in ethanol was added to each wood powder sample (2.50g) in a 250 cm3 beaker and added allowed to stand for 4 hours. The extract was concentrated on water bath to one-quarter of the original volume followed by addition of 15 drops of concentrated ammonium hydroxide dropwise to the extract until the precipitation was complete immediately after filtration. After 3 hours of mixture sedimentation, the supernatant was discarded and the precipitate were washed with 20 cm3 of 0,1M of ammonium hydroxide and then filtered using Gem filter paper (12.5). using electronic weighing balance Model B-218, the residue was dried in an oven and the percentage alkaloid is expressed mathematically

% Alkaloid = Weight of alkaloid/ Weight of sample ×100

- **Determination of flavonoid:**

Flavonoid determination was by the method reported by Ejiken et al and Boham and Kocipai. Exactly 50 cm3 of 80% aqueous methanol added to 2.50 g of sample in a 250 cm3 beaker, covered, and allowed to stand for 24hours at room temperature. After discarding the supernatant, the residue was reextracted (three time) with the same volume of ethanol. Whatman filter paper number 42 (125mm) was used to filter hole solution of each wood sample. Each wood sample filtrate was later transfer into a crucible and evaporate to dryness over a water bath. The content in the crucible was cooled in a desiccator and weight until constant

weight was obtained. The percentage of flavonoid was calculated as.

% Flavonoid = weight of flavonoid/ Weight of sample×100

Determination of saponin:

Saponin quantitative determination was carried out using the method reported by Ejikeme et al. Exactly the 100cm3 of 20% aqueous ethanol was added to 5grams of each peel powder sample in a 250cm3 conical flask. The mixture was heated over a hot water bath for 4 hours with continuous stirring at a temperature of 55°C. The residue of the mixture was reextracted with another 100cm3 of 20% aqueous ethanol after filtration and heated for 4hours at a constant stirring. The combined extract was evapourated to 40 cm3 over water bath at 90°C. 20cm3 of diethyl ether was added to the concentrate in a 250 cm3 separator funnel and vigourously agitated from which the aqueous layer was recoverd while the ether was discarded. This purification process was repetated twice. 60cm3 of n-butanol was added and extracted twice with 10 cm3 of 5% sodium chloride. After discarding the sodium chloride layer the reaming solution was hetated in water bath for 30minutes, after which the solution was transferred into a crusible and was dried in a oven to a constant weight. The saponin content was calculated as per percentage.

% saponin = weight of saponin/ weight of sample ×100.

The saponin content, flavonoid content, and alkaloid content, in different extract of Arial part of Citrus Peel Linn were determined. Result are tabulated in

Result:

Qualitative phytochemical analysis of citrus peel:

Inqualitative analysis of extract of citrus peel exhibited positive and negative test phytochemical tests (table no 1). Phytochemical compound such as alkaloid, saponin, tannin, phenol, flavonoids, glycoside, terpenes, flavonoid and were screened in the extract. Although, among these compound result for compounds, alkaloid, phenolic compound, flavonoids, saponins are importantly secondary metabolites and are responsible principle for medicinal value of the respective plant furthermore, the extract was subjected to further analytical testes for the quantification of phytochemical compound.

Table no:1 Qualitative Chemical Test of The Different extract of Aerial Part Of Citrus Peel.

Chemical Constituent	Tests	Petroleum ether(60-80% extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
Alkaloid	Meyers test	+	+	++	++	_
	Dragendroff's reagent	+	+++	+++	++	+
	Wagner's reagent	+	_	++	_	+
	Hager's reagent	_	+	+	++	++
Glycoside	Legal Test	_	+	++	+	_
	Liberman buchard's test	+	_	+	+	_
Saponin	Foam Test	_	++	++	+	++
Flavonoid	Alkaline reagent	_	++	+++	+	_
	Lead acetate test	_	++	++	+	_
	Shinoda test	_	++	+++	_	++

Note: +ve Indicate presence of phytoconstituent; whereas -ve Indicate Absence of phytoconstituents.

- **Quantitative evaluation of phytochemical constituents:**

Table no:2 Quantitative phytochemical analysis:

SI no	Extract	% Saponin content	Flavonoid content as mg/100mg of rutin, hesperidine.	% Alkaloid content
1	Ethyl acetate Extract	0.5	0.15	0.055
2	Chloroform Extract	-	0.02	0.1
3	Ethanol Extract	0.6	0.725	0.8
4	Aqueous Extract	0.35	0.375	0.4

Quantitative analysis Citrus peel was found to posses flavonoid (0.725); Chloroform extract (0.02) Aqueous extract (0.375); etc and alkaloid contain in chloroform extract (0.1); ethanol extract(0.8); Aqueous extract(0.4); Ethyl acetate extract (0.055). other extract are represent in table.

Discussion:

Plant that have biological activities usually contain secondary metabolite which are chemical substance responsible for such activities.

Phytochemical screening of the peel powder extract of citrus peel shows the present Saponin of which are steroid or saponin.

Triterpenoid glycoside characterized by their bitter or astringent taste, forming properties and hemolytic effect on red blood cells. Saponin possess both of beneficial (cholesterol-lowring) and deleterious (cytotoxic permeabilization of the intestine) properties and also exibite structure dependent biological activities. Saponin cause reduction of blood cholesterol by preventing its reabsorption

which make it useful in cardiovascular disease. In addition, it has been documented that saponin have antitumor and antimutagenic activity and can lower risk of human cancer cell from growing saponin are belived to react with the cholesterol rich membranes of cancer cell, thereby limiting their growth and visibility plants produce saponin has potential to fight infection by parasites and in humans saponin serves as immune system booster and also protect against viruses and bacteria. The non sugar part of saponins has direct antioxidant activity which may result in reduced risk of cancer and heart disease. Flavonoids are also responsible for the colouring of fruits, vegetable and Herbs. Flavonoids have antioxidant activities as well as much health promoting effect such as anti-allergic, anti cancer, antioxidant, anti-inflammatory, anti-thrombotic, vasoprotective, tumor flavonoid containing plant are diuretics, antispasmodic and other have antimicrobial properties. Also epidemiological studies have shown that heart disease are inversely related flavonoid intake and that flavonoids prevent the oxidation of LDL therefore the reducing the risk for the development of atherosclerosis. The usefulness of flavonoids as hypoglycaemic and antidibetichave been documented. Moreover, the effect of flavonoid, quercetin and ferulic acid on pancreatic β-cells leading to their proliferation and secretion of more insulin have been proposed by as the mechanism of their hypoglycaemic activity in streptozocin induced diabetic rats. These may make the peel aqueous extract in the management of diabetes mellitus. Alkaloid are very important in medicine and constituent most of the valuable drugs. They have marketed physiological effect on animal Okwa and JOSIAH (2006) have documented and important of tannin in promoting wound healing. Iwu (1983) have also reported that tannin containing anti- diabetic properties. The presence of phenol in citrus peel serves as antiseptic and reduces inflammation when taken internally. These bioactive agent have an irritant effect when applied to the skin Cardiac glycoside have a strong and direct action on the heart, help insupporting its strength and rate of concentration when it is failing.

Conclusion:

This finding show that the peel extract of citrus contain active phytochemical compound with hypoglycemic, antidiabetic anti arthritic and other degenerative disease potencial

Acknowledgement:

Miss. Chandanshive ArtiHanumantacknowledged to Mr. Patil Manoj sir vice-principle, Sahyadri College of Pharmacy, for their kind support during the progress of these work.

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