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PHYTOCHEMICAL ANALYSIS OF *CITRUS KARNA* FRUIT

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ABSTRACT

Citrus fruits have long been valued as part of a nutritious and tasty diet. The flavors provided by citrus are among the most preferred in the world, and it is increasingly evident that *citrus* not only tastes good, but is also good for Health. The present study focuses on the phytochemical analysis of fruit of *Citrus karna.* The phytochemical screening of both ethanolic and aqueous fruit extracts revealed the presence of various secondary metabolites such as carbohydrates, alkaloids, Phytosterols, phenolic compounds, tannins, flavonoids, Cumarine, amino acids, terpenoids and saponins. Also in present work, total chlorophyll content of fruit peel is calculates which shows strong chlorophyll content in Ethanolic extract of peel. The FTIR analysis of the crude extract of peel gives information about the distribution of functional groups and provides a basis for comparison of compositional differences between isolates and among samples.

KEYWORDS: - ***Citrus karna,*** **Phytochemical, Chlorophyll content, FT-IR**

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INTRODUCTION

Citrus karna Raf (Family - Rutaceae) wild species found in India. Indigenous Citrus Germplasm in India Locally named as id-nimbu, jamuri, khatta nimbu. The leaves and flowers of *Citrus Karna* (karna-khatta) are similar to rough lemon but larger in size. *Citrus karna* is a native of India. It grows all over India upto an elevation of 1000 mt. *Citrus* fruits act as a fabulous source of vitamin C and a wide range of essential nutrients required by the body. Plants produce several secondary metabolite compounds including alkaloids, Cardiac glycosides, glucosinolates, flavonoids, saponins, steroids and terpenoids to protect themselves from the continuous attack of naturally occurring pathogens, insect, pests and environmental stresses (Ebel, 1986) [1]. The ethyl acetate extract of *Citrus karna* peel have been evaluated for antiulcerogenic property against water immersion and hypothermic restraint stress model at different doses (200, 300 and 400 mg/kg, b.w) in Wistar rats [2]. This paper presents a detailed physiochemical analysis of the *Citrus Karna* peel.

MATERIALS AND METHODS

Collection of sample

Fresh Fruits of *Citrus karna* were collected from Chikhaldara, Dist- Amravati, Maharashtra State (Central region of India) in the month of November – 2012, and authenticated by a taxonomist from Department of Botany ACS College Amravati. Fresh Fruits of *Citrus Karna* were washed well using tap water and twice using distilled water and it was dried in shade for a period of 20-25 days, at an ambient temperature of 25˚C. After drying the fruits of *Citrus Karna* the peel (Green color) were separated by cutting them into small pieces. The dried samples were grinded properly using a mortar and pestle and later using a grinder, to obtain the powdered.

Preparation of extracts

Aqueous extract

20 gm of sample of *Citrus Karna* the peel was suspended in 200 ml of distilled water and well stir by using a magnetic stirrer for several hours. Extraction was done at 70˚C for 30 minutes, followed by filtering of the extracts using Whatman filter paper No.1. Extracts were then evaporated at 45˚C for 72 hours to form a paste, and further transferred into sterile bottles and refrigerated until use [3].

Ethanolic extract

95% ethanol was added to 20 gm of sample of *Citrus Karna* the peel and well stir by using a magnetic stirrer for several hours. Extraction was allowed to stand for 72 hours at 27˚C, after which they were filtered using Whatman filter paper No.1. Extracts were then evaporated at 45˚C for 72 hours to form a paste, and further transferred into sterile bottles and refrigerated until use [4,5].

Phytochemical analysis (Qualitative analysis)

Test for carbohydrates

Molisch's reagent was added to 2 ml of both extracts. A little amount of concentrated sulphuric acid was added to it and allowed to form a layer. The mixture was shaken well, and allowed to stand for a few more minutes, which was then diluted by adding 5 ml of distilled water. Purple precipitate ring showed the presence of carbohydrates [5].

Test for reducing sugars

Fehling's test

1 ml of filtrate is boiled on water bath with 1ml each of fehling solutions A and B. A red precipitate indicates the presence of sugar.

Test for proteins

Biuret test (Gahan, 1984)

An aliquot of 2 ml filtrate is treated with one drop of 2 % copper sulphate solution. To this, 1 ml of ethanol (95%) is added, followed by excess of potassium hydroxide pellets. Pink colour in the

Ethanolic layer indicates the presence of proteins .

Test for amino acids

Ninhydrin Test :- To the sample extract, few drops of Ninhydrin reagent was added. After mixing it well, the solution was boiled in water for 2-3 minutes. A bluish-blackish color indicates the presence of amino acids [6].

Test for tannins

Gelatin test :- To the extract 1% gelatin solution containing sodium chloride was added. Formation of white precipitation indicates the presence of tannins .

Lead acetate test :- The extract (50 mg) is dissolved in distilled water and to this, 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of tannins .

Test for Phenolic compound

Ferric chloride test :-

The extract (50 mg) is dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution is added. A dark green color indicates the presence of phenolic compounds.

Test for Phytosterols

Libermann-Burchard Test :- A little quantity of the extract was dissolved in 5 ml of chloroform separately. This chloroform solution was treated with a few drops of concentrated sulphuric acid. To this a few drops of dilute acetic acid and 3ml of acetyl chloride was added. A bluish green color at bace & Brown ring at junction indicated the presence of Phytosterols. [7,8]

Test for steroids

0.5 ml of the each extract was dissolved in 3 ml of chloroform and was filtered. To the filtrate, concentrated sulphuric acid was added by the sides of the test tube, which formed a lower layer. A reddish brown colour ring with a slight greenish fluorescence was taken as the indication for the presence of steroids [8].

Determination of flavonoids

Two methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993; Harbrone,1973).

1. *Ethyl acetate test :-*

50 mg portion of each plant sample was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration was observed indicating the presence of flavonoids [5,8].

2. Alkaline Reagent test :-

Extract were treated with few drop of sodium hydroxide solution. Formation of intence yellow colour ,which becomes colourless on addition of dilute acid, indicating the presence of flavonoids [9].

Test for terpenoids (Salkowski test)

Five ml (1 mg/ml) of each extract was mixed in 2 ml of chloroform, and then 3 ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface was formed which showed positive results for the presence of terpenoids [10].

Test for diterpenes

Copper acetate test :- Extract were dissolved in water & treated with 3-4 drop of Copper acetate solution. Formation of emerald green colour, which becomes colourless on addition of dilute acid, indicating the presence of diterpenes [8].

Test for Alkaloids

0.4 g extract of each plant was mixed with 8 ml of 1% HCl, warmed and filtered. 2 ml of each filtrate was titrated separately with (a) Mayer's reagent and (b) Dragendroff's reagent Yellow precipitation for Mayer's reagent & Red precipitation for Dragendroff's reagent was observed to indicate the presence of alkaloids [10].

Cardiac glycosides

Legal's test :- Extract were treated with sodium nitropruside in pyridine solution and sodium hydroxide. Formation of pink to blood red colour indicating the presence of Cardiac glycosides [8].

Cumarine identification

Fluorescence test :-

0.3 g of each plant extract was taken in a small test tube and covered it with filter paper moistened with 1 N NaOH. The test tube was placed, for few minutes, in boiling water bath. Then the filter paper was removed and examined in UV light for yellow florescence to indicate the presence of Cumarine [11].

Identification of phlobatinins

For the identification of phlobatinins 80 mg of each plant extract was boiled in 1% aqueous hydrochloric acid, the deposition of a red precipitate indicates the presence of phlobatinins.

Test for Chalcones

2 ml of Ammonium hydroxide was added to 0.5 g each extract of each sample. Appearance of reddish colour showed the presence of chalcones [8].

Test for Saponins

Foam Test:- 0.5 gm of extract was shaken with 2 ml dist water if foam produce persist for ten minute it indicated the presence of saponins [12].

Froth test :-

1g of the each sample was weighed into a conical flask in which 10 ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5ml of the filtrate was added to 10 ml of sterile distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins [12].

Test for fixed oils and lipids

Small quantity of each extracts were separately pressed between two filter papers, and allowed to dry. Appearance of an oil stain or a grease spot on the filter paper when observed under direct sunlight, indicated the presence of fixed oils [13,14].

Table 1
Phytochemical analysis of Citrus karna peel

SN	Phytochemical	Tests performed	Aqueous Extract of Peel	Ethanolic Extract of Peel
1	Carbohydrates	Molisch's Test	++	+
2	Sugar	Fehling's test	-	-
3	Protein	*Biuret test*	+	-
4	Amino acids	Ninhydrin Test	++	+
5	Tannins	Lead acetate / Gelatin Test	+	+
6	Phenolic comp	Ferric chloride test	+	+
7	Phytosterols	Libermann-Burchard Test	-	++
8	Steroids	Ring test	+	++
9	Flavonoids	Ethyl acetate/Alkaline Reagent test	+	+
10	Terpenoids	Salkowski test	+	+
11	Diterpenes	Copper acetate test	++	++
12	Alkaloids	Mayer's test	++	-
13	Cardiac glycosides	Legal's test	+	++
14	Cumarine	Fluorescence test	+	++
15	Phlobatinins	Spot test	-	-
16	Chalcones	Spot test	-	-
17	Saponins	Foam test	-	+
18	Fixed oils and lipids	Spot test	+	+

++ indicates: strong presence, + indicates: weak presence, - indicates: strong absence

Total Chlorophyll content of fruit

Chlorophyll content was determined by the method of Aron (1968)

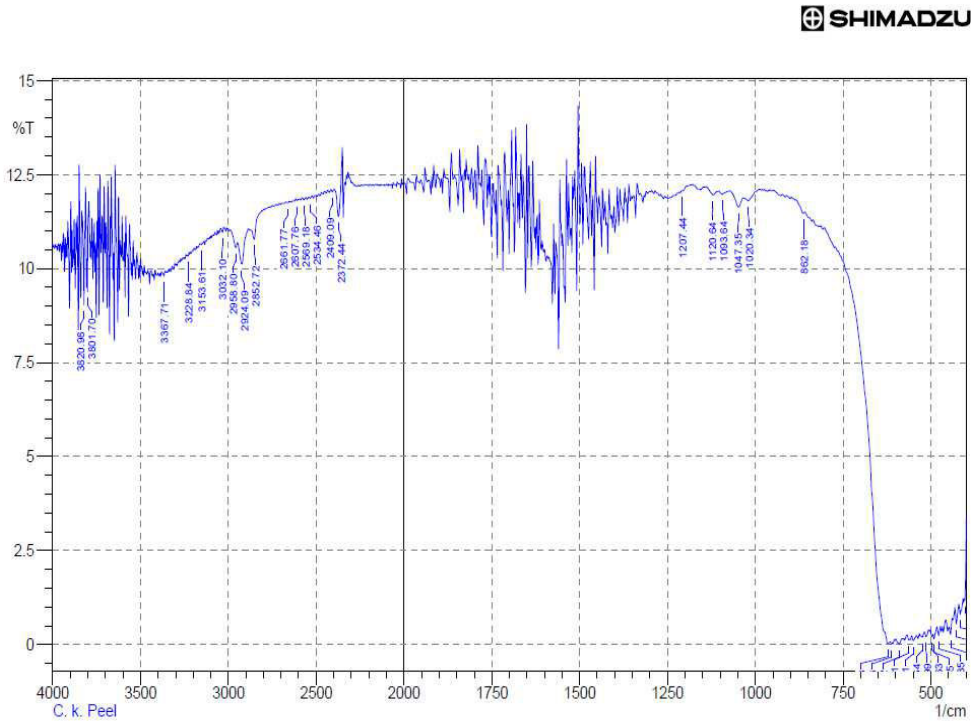
1 ml of crude preparation of each extract of *Citrus karna* peel was mixed with 4 ml of 80% (v/v) acetone and allowed to stand in dark at room temperature. It was centrifuged at 2000 rpm for 5 minutes to clear the suspension. Supernatant was used for chlorophyll determination. Absorbance of solution was read at 645 nm (chlorophyll a) and at 663nm (chlorophyll b) on spectrophotometer against 80% (v/v) acetone blank. Total chlorophyll of each extract was calculated by = (20.2 X A645) + (8.02 X B663) mg/l.

FT-IR Analysis of crude powder peel of Citrus karna

Fourier transforms infrared spectrophotometer (FT-IR)

FT-IR is perhaps the most powerful tool for identifying types of chemical bonds (functional groups). The wavelength of light absorbed is characteristic of the chemical bond as can be seen in this annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of fruit peel and pulp of *Citrus karna* plant materials was considered for instrumental analysis. The powdered sample plant specimens were treated for FTIR spectroscopy (Shimadzu, IR Affinity 1, Japan). Scan range: from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} [7].

Figure 1
FT-IR for Citrus karna fruit peel



RESULTS AND DISCUSSION

The *Citrus karna* fruit peel extracts was rich in phytochemical activity as shown in Table 1. Total chlorophyll content of *Citrus karna* fruit peel of both extract of aqueous and Ethanolic were found to be 8.2224 mg/l and 17.1516 mg/l respectively. In *Citrus karna* fruit peel, FTIR-Spectrum shows. strong absorption peaks at 3367.71 cm^{-1} which shows strong absorbency for N-H Stretch for aliphatic secondary amine, peaks at 3228.84,3153.61and 3032.10-2924.09 cm^{-1} represents identical absorbency of stretching frequency for N-H and Ar C-H it indicates aromatic amines, The peaks at 2852.72-2607.76 cm^{-1}stretching frequency and 862.18 cm^{-1} blended frequency shows for aliphatic hydrocarbon C-H group , The peaks at 1093.64 – 1020.34 cm^{-1} represents primary amine C-N stretch and peak at 1207.44 and 1120.64 cm^{-1} represents Tertiary amine C-N stretch [15].

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