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Research Article

Pharmacognostic and Phytochemical Evaluation of Citrus *Reticulata blanco* peel

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ABSTRACT

Citrus reticulata Blanco belongs to the family Rutaceae. It is commonly known as Narangi or Santra. Peel is the outer part of the pericarp of the ripe or nearly ripe fruits of Citrus reticulata Blanco. The present study deals with the pharmacognostic and phytochemical evaluation of Citrus reticulata Blanco peel. This study includes the macroscopic and microscopic analysis, physicochemical evaluation and preliminary phytochemical study. Thin layer chromatography of alcoholic extracts has also been performed and results were mentioned as R_f value. Macroscopy reveals the organoleptic properties of peel and microscopic study shows the presence of oil glands and vessels responsible for essential oil production in rind. Water soluble ash value is more than acid insoluble ash indicates that the amount of acid-insoluble siliceous matter present was less than that of water soluble ash. The preliminary phytochemical investigations indicate the presence of carbohydrates, amino acids, flavonoids, Tannins and Phenolic derivatives, Steroids etc. The entire study provides useful information in the botanical identification, standardization for purity and quality in authentication of the plant.

Key words: Rutaceae, Macroscopy, Microscopy, Physicochemical analysis, TLC fingerprinting.

INTRODUCTION

Citrus reticulata Blanco (Rutaceae) is commonly known as Narangi or Santra (Orange). It is
a small, spiny tree with a dense top of slender branches, believed to have been introduced sometimes in eight century A.D. from Indo-China, widely grown in India. Ayurvedic description
of fruit is as follows:
Sanskrit name: Naranga
Synonyms: Nagararіga, Tvaksugandha, Mukhapriya
Properties: Rasa: Madhura, amla; Guna: Guru, snigdha, sara, visada
Actions: Vatahara, rucya, dipana, pacana, trisnahara, hrdya, sramhara, balya
Therapeutic uses: Krimiroga, sula, agnimandya, jvara [1].
The fruit is laxative, aphrodisiac, astringent, tonic and relieves vomiting[2]. The fruit rind is traditionally used as tonic, stomachic, astringent, carminative and antiscorbutic. The fruit peel is also useful in skin care. It regulates the skin moisture, softens hard and rough skin and has a cleaning effect on oily skin[3]. Husain et al collected essential oil from rind by hydro-distillation method using Clavenger apparatus and shown the presence of l-limonene (92.4 %) followed by
r -terpinene (2.6 %) and β- phellandrene (1.8 %) mainly[4].
In the present work, various pharmacognostic, physicochemical and phytochemical parameters have been investigated which could serve as a measure of authentication and serve as a tool for the identification of Citrus reticulata Blanco. The present work describes various pharmacognostic, physicochemical and phytochemical characteristics of Citrus reticulata Blanco peel.

MATERIAL AND METHODS

Collection and identification of plant material: Fruits of Citrus reticulata were purchased from local market of Mumbai, India and authenticated at Agharkar Research Institute, Pune. Fruits were washed thoroughly under water. Peels were separated from fruits and dried under shade. Then the dried material subjected to the powder form and stored in an airtight container at room temperature.
Reagents and Chemicals: All reagents and chemicals used for testing were analytical grade obtained from Fisher Chemicals Ltd., Mumbai, SD Fine Chemicals Limited, Mumbai and Qualigens Chemicals, Mumbai.
Macroscopic analysis: Various macroscopic characters such as color, odor taste, shape, size, etc of fruit peels were studied and reported[5,6].
Microscopic analysis: Microscopic evaluation was carried out for transverse section of peel and dried powder material. The outer epidermal layer (in fragments) were cleared in chloral hydrate, stained with phloroglucinol and concentrated HCl and mounted with glycerin and observed under a compound microscope[7,8].
Physicochemical analysis: Physiochemical values such as percentage of ash values and extractive values were determined according to the official methods[9] and Water and alcohol soluble extractive values were estimated by

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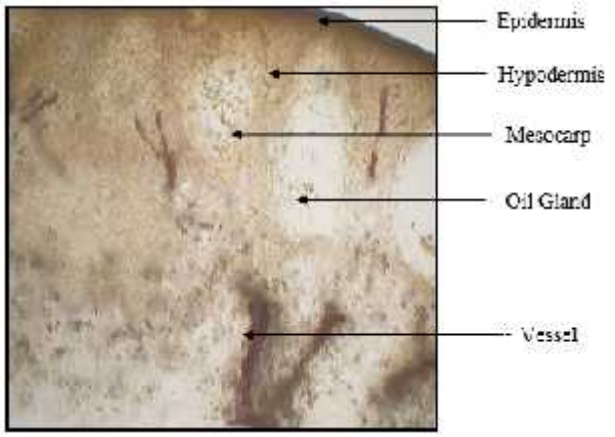

Fig. 1: Epidermis, Hypodermis, Oil Gland, Vessels in T.S. of dried Citrus reticulata Peel

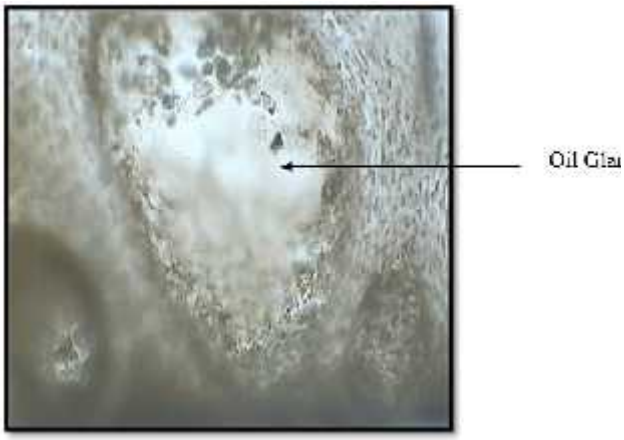

Fig. 2: Oil Gland in T.S. of dried Citrus reticulata Peel.

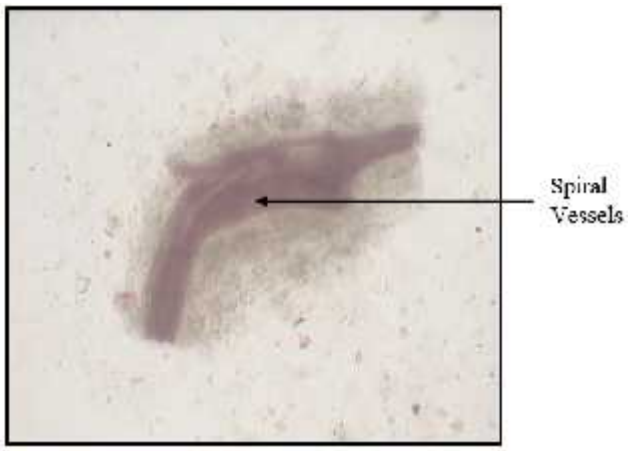

Fig. 3: Spiral Vessels in dried powder of Citrus reticulata Peel

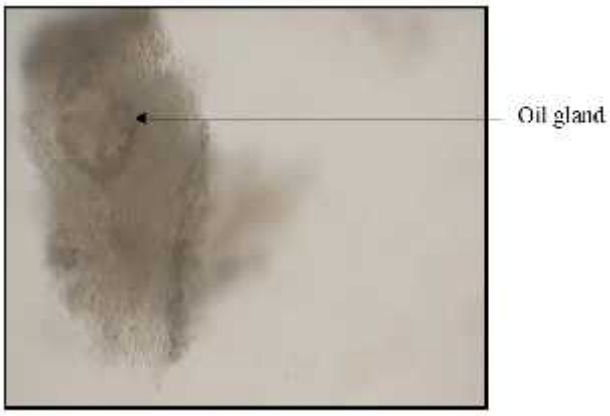

Fig. 4: Fragments of Oil glands in dried powder of Citrus reticulata Peel

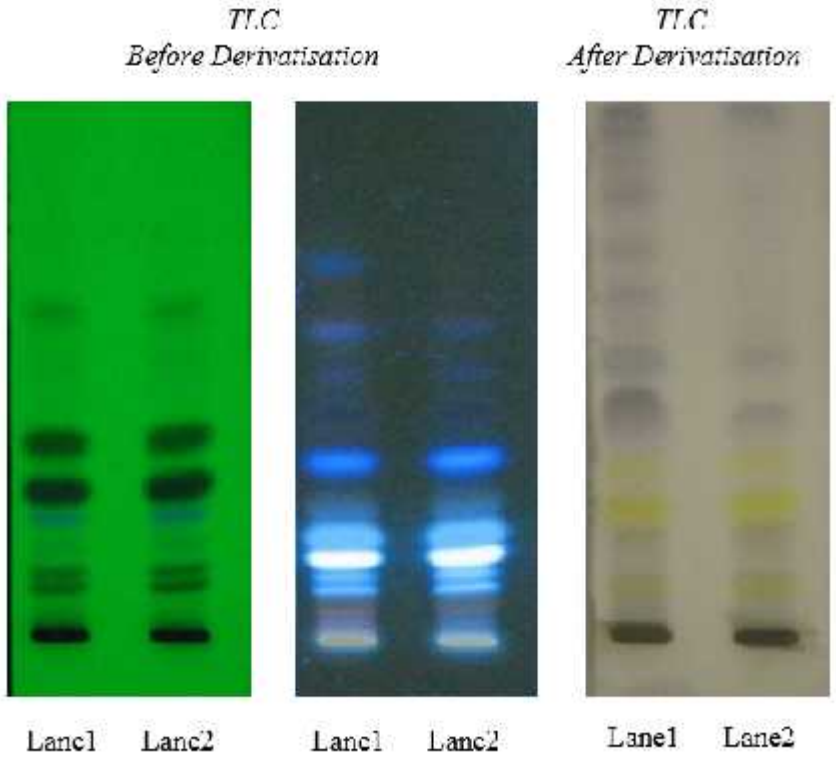

Fig. 5: TLC profile of Citrus reticulata Peel Extracts Lane 1: Hot Alcoholic Extract of Citrus reticulata Peel. Lane 2: Cold Alcoholic Extract of Citrus reticulata Peel. TLC Conditions: Silica gel 60F254 as stationary phase and Toluene: Ethyl acetate: methanol (8.5:1.5:0.5) as a mobile

Table1: Physicochemical analysis of dried peel powder of Citrus reticulata Blanco

Sr. No	Parameter	Average Value
1	Total ash content	3.56 %
2	Acid insoluble ash	0.35 %
3	Water soluble ash	0.46 %
4	Alcohol soluble extractive	9.68 %
5	Water soluble extractive	32.88 %

Table2: Percentage yield determination of alcoholic extracts of Citrus reticulata Blanco peel.

Sr. No	Type of Extract	Percentage yield
1	Hot Alcoholic extract (HAE)	25.00 %
2	Cold Alcoholic extract (CAE)	11.08 %

Table 3: Preliminary phytochemical analysis of alcoholic extracts of Citrus reticulata Blanco peel.

Test performed	Name of the test	HAE	CAE
Test for carbohydrates	Fehling's test	+ve	+ve
Test for Amino acids	Ninhydrin Test	+ve	+ve
Test for Flavonoids	Shinoda Test	+ve	+ve
Test for Alkaloids	Dragendroff	- ve	- ve
	Mayer's	- ve	- ve
	Wagner's reagent	- ve	- ve
Test for Tannins and Phenolic compound	5% FeCl3	+ve	+ve
Test for Steroids	Salkowski reaction	+ve	+ve

+ Denotes the presence of respective class of compound

cold maceration according to the method as described by WHO[10].

Preparation of plant extracts: Collected plant material was cleaned and shade-dried. Fruits of Citrus reticulata Blanco were washed thoroughly under water. Peels were separated from pulp. They were dried under shade. The dried peel was subjected to powder form. 50 gm of dried powder was extracted with 300 ml alcohol. Peel extracts were prepared by Soxhlet method and cold maceration. Soxhlet extraction was carried out at 65° C for 10-12 hrs. Cold maceration was carried out by constant shaking; where the mixture of peel powder and alcohol on a rotary shaker was set at 100 rpm at RT for 72 hrs. The extracts were filtered using Whatman's filter paper no. 1, and the filtrates were then evaporated under reduced pressure and dried using a rotary evaporator set at 50° C and the percent yield of both the extracts were determined. Dried extracts were stored at 4° C, in labeled, sterile, capped bottles until further use.

Preliminary phytochemical analysis of peel extracts: The extracts as mentioned above, were subjected to various qualitative phytochemical tests for the identification of chemical constituents present in the plant material according to the method described by Harborne, 1998[11].

Thin layer chromatography (TLC) of peel extracts: TLC of the Citrus reticulata Hot Alcoholic Extract (CR HAE) and Citrus reticulata Cold Alcoholic Extract Cold Extract (CR CAE) was performed. TLC fingerprinting was achieved by using Silica Gel 60F 254 Merk plates. Toluene: Ethyl Acetate: Methanol (8.5:1.5:0.5) was used as a solvent system. Separated spots were visualized under UV light at 254 nm and 366 nm. Anisaldehyde Sulphuric acid reagent was used as a derivatising reagent for detection of Terpenoids, Essential oils, Propylpropanoids, Pungent and bitter principles, Saponins etc and R_f values were calculated[12].

RESULTS AND DISCUSSION

Macroscopic analysis: The color of the fresh Orange peel is dark orange and it turned to yellowish brown after drying. The dried peel is brittle and hard. They vary in size and shape. Peels are approximately 3- 4 mm in thickness. Outer surface of the peel is rough which represents oil glands. Inner surface is yellowish white and turned brown after drying. It has an aromatic odor and bitter mucilaginous taste.

Microscopic analysis: T.S. of peel shows a layer of small-celled epidermis, below this lies the 2-3 rows of hypodermis which is composed of small parenchymatous cells. These cells are loosely packed thin-walled cells. Scattered in parenchyma are large oil glands in two irregular rows and vessels with spiral or annular thickening. Adjacent to oil glands, parenchymatous cells are arranged in a circle of 3-4 layers **(**Fig. 1, 2). The powder characters of a drug are mainly used in the identification of the drug in the powder form. Fragments of parenchyma containing oil gland, and vessels with spiral or annular thickening are found in powder form (Fig. 3, 4).

Determination of physicochemical parameters: The result of the physicochemical parameters of raw material lie within the limit which is mentioned in (Table 1) signifies that the quality and purity of raw material was good enough. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign matter such as metallic salts or silica. Water soluble ash value is more than acid insoluble ash indicates that the amount of acid-insoluble siliceous matter present was less than that of water soluble ash. Extractive values give an idea about the chemical constituents present in the drug and indicate the presence of sugar, acids and inorganic compounds; Water soluble extractive value found to be more than that of alcohol soluble extractive value suggests that the powdered drug have high water soluble extractive value.

Determination of percentage yield of extracts: Percentage yield of both the alcoholic extracts such as Hot Alcoholic Extract (HAE) and Cold Alcoholic Extract are calculated and indicated in (Table2)

Table 4: Thin layer Chromatography-R_f Values of alcoholic extracts after derivatisation with Anisaldehyde Sulphuric acid reagent

R_f Value	Color of the Band
0.02	Brown
0.04	Purple
0.08	Yellow
0.12	Brown
0.15	Blue
0.18	Brown
0.22	Yellow
0.30	Yellow
0.35	Navy blue
0.38	Black
0.41	Black
0.48	Blue
0.55	Purple

Preliminary phytochemical analysis: The preliminary phytochemical investigations of alcoholic extracts of powdered peel indicate the presence of carbohydrates, amino acids, flavonoids, Tannins and Phenolic derivatives, Steroids etc. The results of the Preliminary phytochemical screening are listed in (Table3).This screening obtained the useful information of chemical constituents mainly present in powdered drug and on that basis further ; quantification of principal phytoconstituents can be obtained.

Thin Layer Chromatography study: Fingerprint construction has become an important quality control tool of herbal samples in the light of constantly growing interest in natural origin medicines. Fingerprint analysis has been accepted by WHO as a methodology for the quality control of herbal samples[13,14]. In the present study, we have performed TLC analysis of two extracts i.e. CR HAE and CR CAE. R_f values were calculated for both the extracts and summarized in table (Table 4) (Fig. 5).

CONCLUSION

Standardization is an important tool for herbal drugs in order to establish their identity, purity, safety and quality. In order to standardize a drug, various macroscopic, microscopic is done. Microscopic method is one of the cheapest and simplest methods to start with establishing the correct identification of the source material. The above studies provide information with respect to the identification, chemical constituents & physicochemical characters of Citrus reticulata Blanco peel. Morphological and microscopical studies of the peel will enable to identify the crude drug. The information obtained from preliminary phytochemical screening will be useful in finding out the quality of the drug. These studies can also help the manufacturers for identification and selection of the raw material for drug production. In conclusion, the parameters which are reported here can be considered as distinctive enough to identify and decide the authenticity of this drug in herbal industry and this can be included as microscopic standards in Herbal Pharmacopeia.

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