Research Paper

E-ISSN NO : 2455-295X | IMPACT FACTOR VALUE : 5.983
VOLUME : 11 | ISSUE : 2 | SPECIAL ISSUE FEBRUARY-2025

SCREENING OF BIOACTIVE CONSTITUENTS IN KINNOW MANDARIN *(CITRUS RETICULATA L.)* FROM HANUMANGARH, RAJASTHAN

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ABSTRACT:

Kinnow mandarin (*Citrus reticulata*) is a medicinally important plant species known for its therapeutic potential in treating various diseases. The present study aimed to estimate the phyto-constituents present in different parts of the plant. Crude extracts were obtained using 50% ethanol, and the phytochemical analysis was conducted to determine the presence of carbohydrates, starch, proteins, lipids, indole-3-acetic acid (IAA), phenols, aliphatic amino acids, and essential vitamins such as thiamine, riboflavin, niacin, and vitamin C. The results revealed that ascorbic acid (vitamin C) was the most abundant phyto-constituent in all plant parts, followed by niacin, whereas riboflavin was found in minimal quantities. The significance of these bioactive compounds is discussed concerning the medicinal value of Kinnow mandarin in treating various ailments.

KEYWORDS:

KINNOW MANDARIN, PHYTOCONSTITUENTS, CRUDE EXTRACT, BIOACTIVE COMPOUNDS, VITAMIN C, MEDICINAL PLANT.

INTRODUCTION

In the struggle for survival, living things defend themselves in a variety of ways. Because of this, man has been familiar with plants since the beginning of time and has used them in a variety of ways to find food and effectively manage human suffering. Primitive men have started to differentiate between plants that are suitable for nutrition and those that have definite pharmacological action. As a result, certain plants were used as food, while others demonstrated positive effects against a range of human ailments, including illnesses and injuries. As a result, the plant-based medication has been a cornerstone in the treatment of human ailments and the maintenance of health throughout history [1]. According to historical references, people in China and Egypt were fully aware of a wide variety of medications and poisons as early as 5000 years ago. Some of these substances, such as opium, aconite, and croton, are still used in both traditional and modern medical systems. It is estimated that only 95 plant species are used to make the 120 or so plant-based medications that are given worldwide [2]. There are over 30,000 species in this group, spread across 5,000 genera and over 1000 families and sub-families. They include aquatic plants and lower plants like lichens and fungus, and they are found at a wide range of elevations, from the Great Tibetan Plateau to the seacoast [3]. In rural regions of many developing nations, the use of medicinal plants as traditional medicine is widely recognized [4]. More research should be done on medicinal plants, particularly those used as folk medicines, in order to encourage the use of herbal remedies and determine their potential [5,6]. Primary and secondary metabolites are two categories of chemicals that are found in all plants. Proteins, amino acids, sugars, nucleic acid purines and pyrimidines, chlorophylls, and other substances are examples of primary metabolites; alkaloids, terpenoids, flavonoids, tannin, and other substances are examples of secondary metabolites. Different plants and parts have different distributions of these metabolites, both in terms of quality and quantity. The relationship between a plant's pharmacological action and its phytochemical components is gaining attention [7].

The genus Citrus includes several important fruits such as orange, mandarins, lime, lemons and grapefruits. In mandarin, Kinnow mandarin (*Citrus nobilis* × *C. deliciosa*)[8,9]is one of the most economic fruit crop of India. It was first developed by H.B. frost at California, Regional fruit station USA[10,11] and first introduced in India during early 1940's at the fruit experiment station of Punjab Agriculture College and Research Institute Lyallpur by S. BhadurLal Singh[12]. Kinnow is rich in fiber, which is imported for production and maintenance of collagen[13]. Kinnow plant parts are used in pharmaceutical industries for the preparation of drugs and in the cosmetic industries for the preparation of soaps and perfumes for the home cleaning products. Peels, seeds, and pulps—which make up half of the raw processed fruit—are among the significant amounts of waste or by-products produced by the food

and agro-food processing industries. These by-products are thought to be a good source of dietary fiber, flavonoids, and essential oils, which are useful components.

The peel of *Citrus* fruits is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants[14]. Citrus peels are used for candied, feed of livestock, perfumes production and soap making products. Citrus sp. accumulates large quantities of flavonone glycosides in their leaves and fruit[15].Preparation from peel, flowers and leaves of bitter orange (*Citrus aurantium* L.) are popularly used in order to minimize central nervous system disorders[16].They supply humans with many other constituents such as simple sugars, Vitamin C, carotenoids, limonoids, fiber, folic acid and potassium which have important effect on health. They also help in lowering blood pressure levels and substantially reduce the risk of stroke. The peel of *Citrus* fruits is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants[14]. These compounds, not only play an important physiological and ecological role, but are also of commercial interest because of their multitude of applications in the food and pharmaceutical industries.

This study investigates the fundamental scientific basis for the use of Kinnow mandarin in herbal medicine. The contents of phytoconstituents and vitamins present in the species were determined with scientific methods.

MATERIAL AND METHODS

STUDY SITE

The study was conducted in Department of Botany, Pulkit College Hanumangarh District, Rajasthan. The Hanumangarh district, which is located between 29° 5' and 30° 6' North and 74° 3' and 75º 3' East, is bordered to the east by Haryana state, to the west by Sriganganagar district, to the north by Punjab state, and to the south by Churu district.

SAMPLE PREPARATION

The stems were cleaned with tap water and let to dry in the shade for ten days. A clean, sharp knife was used to cut the fruit and peel into smaller pieces. This promotes drying by decreasing surface area. This material was stored at 40–50°C in a hot air oven [17]. Using a grinding machine, the dry material was ground into a powder and kept in airtight vials until it was time for examination.

About 30g of powdered material was extracted using a Soxhlet device with 200ml of 50% ethanol at 20–25˚C in order to quantify the metabolites. The resulting extract was then concentrated using a Perfit-PMTC-3040 vacuum evaporator. For later usage, the concentrated extracts of different plant sections were stored in sealed vials in a refrigerator at 4˚C. The following techniques were used to further analyze the various components:

ESTIMATION OF PRIMARY AND SECONDARY METABOLITES

CARBOHYDRATE ESTIMATION

Standard solutions of dextrose were prepared at 0, 0.2, 0.4, 0.6, 0.8, and 1 mg/g respectively with the same treatment, and calibration curves of the absorbance values versus concentration of the standard were constructed [18]. 0.5g of sample (Stem, Leaf, Fruit, and Peel) were homogenized with 10mL of 80% ethanol and centrifuged at 2000rpm for 20 minutes. 1mL of the supernatant was added with 5% phenol, and then 5mL H2SO4 was added. The mixture was then agitated and left in a water bath at 26-30°C for 20 minutes to develop color.

STARCH ESTIMATION

After homogenizing 0.5g of fresh plant tissue with 10mL of 80% ethanol, the mixture was centrifuged for 20 minutes at 2000 rpm. Pellet was suspended in 5 mL of distilled water after the supernatant was disposed of, and then 6.5 mL of 52% perchloric acid was added to the residue. The mixture was centrifuged at 2000 rpm for 20 minutes. This process was repeated three times to collect and decant the supernatant. The supernatant was poured into a 100 mL volumetric flask and filled with distilled water to the 100 mL mark. The same method used for carbohydrate estimation was applied to the analysis of 1 milliliter of this filtrate. The amount of starch was determined by converting the value of dextrose for starch estimation using glucose equivalent [19].

PROTEIN ESTIMATION

0.5 g of fresh plant tissue was extracted using 5 mL of 5% TCA. The homogenized substance was centrifuged at 2000 rpm for 20 minutes. The pellet was dissolved in 10 mL of 0.1 N NaOH following the removal of the supernatant. 0.1 mL of this solution is diluted to 1 mL by the addition of distilled water. Alkaline copper reagent was added to dissolve the residue, which was then allowed to stand for 10 minutes before the addition of 0.5 mL of 50% Folin-Ciocalteu reagent. The optical density was assessed at 750 nm using a spectrophotometer. The standard curve was generated using a 0-1 mg/ml solution of BSA in 0.1N NaOH, and the protein concentrations in the sample were determined[20].

LIPID DETERMINATION

1g of the fresh tissue was taken and homogenized with 10 mL CHCl3 and methanol in 2:1 ratio. After being crushed, the material was placed in screw-capped tubes and left overnight. A glass funnel that had been sintered was used to filter the contents. Methanol and CHCl3 were used twice to wash the mixture. To eliminate water-soluble contaminants, a quarter of the volume of 1% NaCl was added to the crude extract. After a low-speed centrifugation step, a Pasteur pipette was used to remove the lower CHCl3 layer that contained the lipids [21].

ALIPHATIC AMINO ACID DETERMINATION

In 80% ethanol, 0.5g of the material was crushed. This mixture was centrifuged for 20 minutes at 2000 rpm after being made up to 10 mL. After collecting 1 mL of the supernatant, 2 mL of ninhydrin is added. For 15 minutes,

the tubes were maintained at 100°C. Absorbance was measured at a wavelength of 575 nm after the tubes were cooled to room temperature[22].

PHENOL ESTIMATION

5g of the sample was homogenized with 80% ethanol, suspend for half an hour in it, then centrifuge at 2000rpm for 10 minutes. 1 ML of the supernatant is liquated, followed by the addition of 1 ML of Folin-Ciocalteu reagent and 2 mL of Na2CO3 solution. The resultant mixture was boiled at 100°C for one minute, after which it was placed under running tap water and subsequently centrifuged for ten minutes at 2000 rpm. The supernatant is adjusted to a final volume of 10 mL with distilled water. The absorbance of the solution was measured at 650 nm. A blank sample was produced with phenol to achieve serial dilutions of 0, 20, 40, 60, 80, and 100 µg/ml. This was utilized to construct the calibration curve[23].

IAA ESTIMATION

5 mg of plant material was homogenized with 10 ml of 80% ethanol and centrifuged at 2000 rpm for a period of 20 minutes. Add 4 mL of Salkowsky reagent to the alcoholic supernatant once it has been collected, and then incubate for 30 minutes at room temperature in the dark. Once the solution turns pink, use a spectrophotometer to measure the absorbance at 530 nm. 50 mg/ml IAA at various concentrations was used to create the reference curve[22].

Determination of Vitamins The B complex vitamins comprising thiamin, riboflavin and niacin were determined[17]

DETERMINATION OF THIAMIN

50 mL of ethanolic sodium hydroxide was used to homogenize 5g of the sample extract. After filtering, it was placed in a 100 mL flask. 10 mL of the filtrate was pipetted, and 10 mL of potassium dichromate was added to develop the color. The reading was taken at 360 nm. Thiamin acid was used to create a blank sample at 100 ppm, and serial dilutions of 0, 0.2, 0.4, 0.6, and 0.8 ppm were created. This was used to plot the calibration curve.

DETERMINATION OF RIBOFLAVIN

100 mL of a 50% ethanol solution was used to extract 5g of the material, and it was agitated for an hour. A 100 mL flask was filtered out of this. A 50 mL volumetric flask was pipetted with 10 mL of the extract. After adding 10 milliliters of 5% potassium permanganate and 10 milliliters of 30% H2O2, the mixture was let to stand over a hot water bath for approximately half an hour. 2mL of 40% sodium sulphate solution was added and a yellowish pale color was formed. This was developed up to the 50 mL mark, and a spectrophotometer was used to measure the absorbance at 510 nm.

DETERMINATION OF NIACIN

5g of the sample were treated with 1N sulphuric acid and shaken for 30 minutes. The material was filtered after 3 drops of ammonia solution were added. After pipetting 10 mL of the filtrate into a 50 mL volumetric flask, 5 mL of potassium cyanide was added. 5 mL of 0.02N H2SO4 was used to acidify this, and the absorbance was measured at 470 nm in a spectrophotometer. The calibration curve was plotted using this.

DETERMINATION OF ASCORBIC ACID (VITAMIN C)

5g of the sample were weighed into an extraction tube and 100 mL of EDTA/TCA (2:1) extracting solution were mixed and mixture shaken for 30 min. After being moved into a centrifuge tube, this was centrifuged for roughly 20 minutes at 3000 rpm. The extracting solution was added to a 100 mL volumetric flask until the 100 mL mark was reached. To obtain a dark end point, 20 milliliters of the extract were pipetted into a volumetric flask, followed by the addition of 1% starch indicator and titration with 20% CuSO4 solution.

STATISTICAL ANALYSIS

All samples were tested and analyzed in triplicates. Results were calculated as the Mean ± SE (Standard Error) for each sample.

RESULTS AND DISCUSSION

The Kinnow Mandarin fruit is high in carbohydrates, protein, fat, IAA, phenol, and vitamins, according to the evaluation of phytoconstituents (Table 1&2). The plants' therapeutic value is shown by the presence of phytochemical substances. The results were displayed graphically as well.

Fruit contains the least amount of carbohydrates (.047 µg/g) and leaves the most (.067 µg/g). Carbohydrates are the main byproduct of photosynthesis and an essential metabolic intermediary for green plants. They are the direct or indirect source of almost all biological substances. In addition, they serve as food storage, a mechanical framework, and occasionally they combine with other groups of molecules to change many of their physical and chemical characteristics [24].

This plant has 0.25µg/g to 0.53µg/g of proteins. Fruit had the highest amount (0.53 µg/g), while the lowest amount (0.25 µg/g) was discovered in the leaf. The structure and functionality of living cells are influenced by proteins, which can exist alone or in conjunction with lipids, carbohydrates, nucleic acids, and many other substances. Drug discovery and development may result from the use of preliminary qualitative testing in the identification of bioactive principles [25].

One of the biggest and most common classes of plant metabolites are phenolic chemicals [26]. The plant's peel (.062µg/g) and stem (0.041µg/g) have the highest and lowest amounts of phenol, respectively. Citrus species may have anti-inflammatory, anti-coagulant, antioxidant, immune-boosting, and hormone-modulating properties because to the presence of phenol [27]. The capacity of phenols to inhibit particular inflammatory enzymes has been attributed to their role. Additionally, they alter the

prostaglandin pathway, which prevents platelets from clumping [28].

The vitamin components of Kinnow Mandarin are displayed in Tables 1 and 2. Ascorbic acid, riboflavin, thiamin, and niacin were all present in the plant. In fruit, thiamin contents range from.050µg/g to.108µg/g. Fruits contain niacin at levels ranging from.45 to.70 in the peel. Ascorbic acid content varied significantly, ranging from 14.3µg/g in leaves to 18.8µg/g in peels, whereas riboflavin content ranged from.02µg/g in leaves to.04µg/g in fruit. The body needs natural ascorbic acid to function properly. It has anti-scorbutic properties. The primary source of vitamin C for primate devices is citrus. The body's ascorbic acid helps the intestines absorb iron. It is necessary for the metabolism of connective tissue, particularly that of bones, teeth, and scar tissue [29]. It is essential as a stress reliever and a defense against dampness, cold, and chill. Ascorbic acid's function also explains why it's necessary for healthy wound healing[30]. Vitamin C is also necessary for collagen synthesis. It aids in improving fine wrinkles and promoting and restoring skin tone[31].

Plant portions contain trace levels of other vitamins, such as riboflavin, thiamin, and niacin. Despite being present in tiny amounts, they play crucial metabolic roles in the body. Pellagra, a condition that causes diarrhea, dermatitis (skin inflammation), and dementia, can be avoided with niacin[32]. A riboflavin deficit does not cause any particular, recognizable condition, but thiamin prevents beriberi. Riboflavin insufficiency manifests as tongue irritation, eye and lip lesions, conjunctival blood vessel congestion, and skin desquamation[30]. As dietary supplements, the herbal vitamins have nutritional benefits and can be taken safely as a preventative health strategy. Parts of particular plants, such as seeds, roots, stems, fruit, peels, leaves, or flowers, are used to make herbal vitamins.

To advance Indian herbal pharmaceuticals, it is imperative to assess the therapeutic potentials of these substances in accordance with WHO guidelines [33]. Patwardham et al. (2004) noted that 30% of global drug sales derive from natural products. Traditional indigenous medicine, confined to specific tribal and geographical regions known as "little traditions," serves as a valuable repository of knowledge regarding the medicinal attributes of botanical sources. Bioactive extracts must be standardized based on their phytochemical constituents[34]. Conducting phyto-chemical screenings of medicinal parts is crucial for identifying novel sources of therapeutically and industrially significant compounds. This communication aims to evaluate the phyto-chemical properties of Kinnow mandarin to enhance public health and facilitate its application in commercially relevant pharmaceutical and nutraceutical products.

CONCLUSION

The findings showed that the plants under study included components that were significant from a medical standpoint. Numerous findings from past research supported the found phytochemical's bioactivity. Numerous investigations have verified that the existence of these phytochemicals gives the plants under study both physiological and therapeutic qualities that aid in the treatment of various illnesses. As a result, these plants may be a valuable source of beneficial medications. In addition to the strong recommendation that these plants be used in traditional medicine, further research should be done to identify, isolate, and purify the key ingredients that give this plant its effectiveness.

TABLE 1 PRESENCE OF METABOLITES IN PLANTS

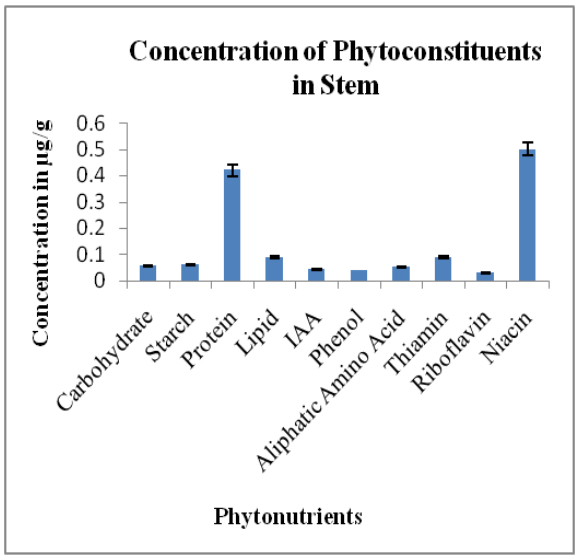
Phytonutrient	Peel	Leaf	Stem	Fruit
Carbohydrate	++	+	+++	++++
Starch	++	+	++++	+++
Protein	+	++	++++	+++
Lipid	++++	+++	++	+
IAA	++++	+	++	+++
Phenol	++++	++	+	+++
Aliphatic Amino Acid	+++	+	++	++++
Thiamin	+	++	+++	+++++
Riboflavin	+++	++	+++	++++
Niacin	++++	++	+++	+
Vitamin C	++++	+	++	+++

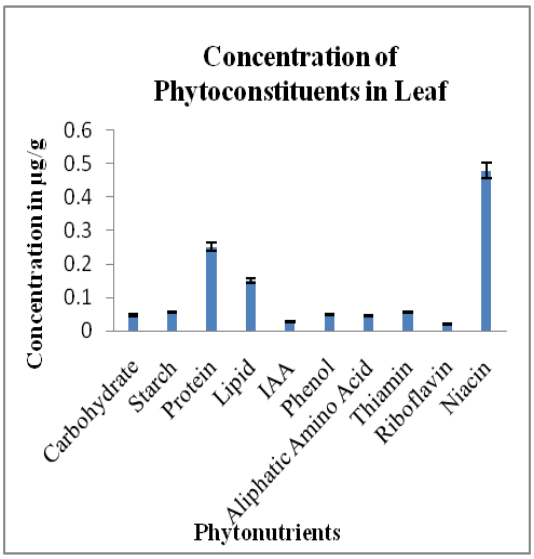
+ Lowest ++ Moderate +++ High ++++ Highest

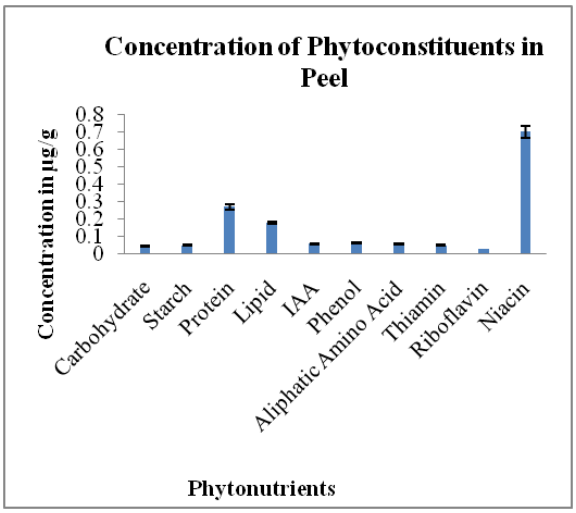
TABLE 2PHYTOCONSTITUENTS ESTIMATION IN VARIOUS PLANT PARTS (IN MICROGRAM/ML)

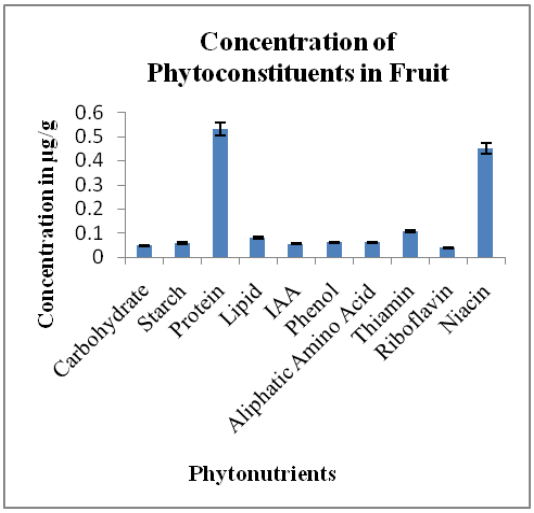
Phytonutrient	Peel	Leaf	Stem	Fruit
Carbohydrate	.047±.33a	.067±.56d	.057±.66b	.060±1.15c
Starch	.052±.89a	.055±1.10b	.062±1.76d	.058±1.20c
Protein	.27±.90b	.25±1.45a	.42±.85c	.53±.72d
Lipid	.18±.95c	.15±1.22b	.09±1.10a	.08±.66a
IAA	.060±1.15c	.026±.89a	.044±1.45b	.055±.76c
Phenol	.062±.52c	.049±.78b	.041±.38a	.060±.56c
Aliphatic Amino Acid	.057±.53bc	.044±1.33a	.052±1.20b	.061±.89c
Thiamin	.050±.56a	.056±.72b	.09±.76c	.108±1.45d
Riboflavin	.03±1.60bc	.02±.74a	.03±.88b	.04±1.20c
Niacin	.70±.88a	.48±.95c	.50±.38d	.45±1.15b
Vitamin C	18.8±1.45c	14.3±1.22a	16.5±.53b	18.7±.72c

Results as per shown in Mean±Standared Error









ACKNOWLEDGEMENT

The authors express gratitude to the Director of Pulkit College for Higher Education for providing all laboratory facilities necessary for the effective completion of this work.

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