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Preliminary Phytochemical Evaluation of Methanol Extract Leaves of *Naringi crenulata* (WILD)

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ABSTRACT
Naringi crenulata is a useful medicinal plant belongs to the family Rutaceae. It is traditionally used to treat various diseases the plant root is used to curing vomiting, dysentery and colic disorders in India. The present study was aimed to investigate the phytochemical properties of methanolic extracts of leaves of Naringi crenulata. The Naringi crenulata plant materials were extracted using the solvent Methanol by Soxhlet method. The extracts were screened for preliminary phytochemical analysis for alkaloid, amino acid, carbohydrates, flavonoids, cardiac glycoside, saponins, mucilage, tannins, phenolic compound, and proteins. The quantitative phytochemical analysis was carried out for total flavonoid and total phenols using standard procedures. The preliminary phytochemical screening confirmed the presence of carbohydrates, glycosides, alkaloids, flavonoids, phenols, tannins, and saponins. The flavonoid and phenol contents of the plant extracts were found to be in the extract. The result showed the presence of phytochemical constituents and higher values of phenolic and flavonoid content make the plant useful for the formulation of the different drugs for human uses for treating various diseases.
Keywords: Naringi crenulata, Rutaceae, Carbohydrates, Flavonoid content, phytochemical.

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INTRODUCTION

Nature has given the enormous assortment of remedial plants and solid bioactive constituents for humanity as long various years; at any rate plants are the fortunes for the wellspring of solutions for the fundamental clinical benefits system [1]. Restorative plants play a critical capacity inside the human wellness care. Roughly 80 % of the world people is anticipated on utilizing ordinary medication that prevalently dependent on plant materials [2]. Plants inferred drugs have observed to be widely utilized in many nations since they are effectively available, more secure and less expensive. As of now, there is more number of lifesaving drugs got from plants [3].

Phytochemicals (Greek: phyton = plant) are substance intensifies normally present in the plants crediting to positive or negative wellbeing impacts. Therapeutic plants utilized in various infections and sicknesses are the most extravagant bio repositories of different phytochemicals. The therapeutic properties of the plants are dictated by the phytochemical constituents. A portion of the significant phytochemicals incorporate alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, and so forth which are appropriated in different pieces of the plants. Nature is an interesting wellspring of designs of high phytochemical variety addressing phenolics (45%), terpenoids and steroids (27%) and alkaloids (18%) as significant gatherings of phytochemicals [4].

Phyto constituents are the regular bioactive mixtures found in plants. This phyto constituents work with supplements and filaments to shape a coordinated piece of protection framework against different infections and stress conditions [5]. Phyto-synthetic compounds are fundamentally partitioned into two gatherings, for example essential and optional constituents; as indicated by their capacities in plant digestion. Essential constituents involves normal sugars, amino corrosive, proteins and chlorophyll while auxiliary constituents comprises of alkaloids, terpenoid, steroids and flavonoids, so on [6]. Phytochemical

constituents are the essential hotspot for the foundation of a few drug enterprises the constituents is assuming a critical part in the recognizable proof of unrefined medications. He therapeutic worth of these plants lies in some synthetic substances that produce a clear physiological activity on the human body. The main property of these bioactive constituents of plants is that they are more powerful with practically zero incidental effects when contrasted with the normally utilized engineered chemotherapeutic specialists [7].

Naringi crenulata (wild) is a therapeutic tree, having a place with Rutaceae family in found in India and other piece of the world. It is regularly called as Narinarakam, Kattunarakam and Tamil name it is called Megavilvam or Magavilvam [8]. Naringi class it's developing as understory trees in evergreen woods upto 1200m. Plant contains a few restorative properties specifically; the plant root is accustomed to relieving retching, diarrhea and colic issues in India [9]. Natural product decoction is utilized as a creepy crawly repellent specialist and furthermore disengages Pectic polysaccharides for a few organic exercises [10, 11]. Bark juice is applied remotely for getting fast help in sprain [12]. *N. crenulata* plant removes show great Anthelmintic action [13, 14] and because of these properties they are generally utilized for therapeutic reason. Therefore, qualitative phytochemical screening of some medicinal plants are necessary and the present study is designed to evaluate the bioactive chemical constituents of *Naringi crenulata* commonly used as medicine in India.

MATERIAL AND METHODS

Collection and authentication of plant

The plant *Naringi crenulata* was collected from Around the Trichy district. The plant was authenticated by Dr. T. Ramesh, HOD, Department of Botany, Srimad Andavan Arts and Science College, Trichy.

Plant processing and extraction

The Plant leaves were cut into small pieces dried under the shed for 4 weeks at room temperature. The entire plant was shaded and dried for grinding to get crude power. 100 g of crude powdered drug were taken and shied into filter paper thimble. 250 ml of methanol were poured into round bottom flask (1000 ml capacity) followed by fitting in on soxhlet apparatus. The powdered drug was extracted with methanol for 24 hours. A semisolid extract was obtained aier completed elimination of methanol under reduced pressure. The extract was stored in refrigerator until use.

Extraction

The leaves were shade dried and pulverized. The coarse powder of 500 gm packed in a soxhlet apparatus to continuous hot percolation, using 1.5 litres of methanol as a solvent. The extract was concentrated under vacuum and dried in a desiccator yield and 20.05 g.

Preliminary qualitative phytochemical analysis

The Methanol extracts were subjected to the preliminary phytochemical screening for the detection of the major phytoconstituents such as alkaloid, amino acid, carbohydrates, flavonoids, cardiac glycoside, saponins, mucilage, tannins, phenolic compound, and proteins [15-19].

Test for alkaloids

Required quantity (10 mg) of all three plant extracts was taken separately and dissolved it in 2% HCl, and filtered and the clear solution was used for the study.

Mayer's test

A small quantity of the extract was taken and treated with Mayer's reagent which was observed for the cream-colored precipitate.

Dragendorff's test

The test solution was treated with Dragendorff's reagent and observed for reddish-orange precipitate.

Wagner's test

A fraction of extract was treated with Wagner's reagent and the observed color was reddish-brown precipitate.

Test for flavonoids

Aqueous NaOH test

The test fraction of extract was treated with a drop of 1 N NaOH solution and observed for yellow-orange color.

Sulfuric acid test

The fraction of extract was taken and treated with concentrated sulfuric acid which was observed for the orange color.

Shinoda's test

The fraction of extract was treated with a piece of magnesium turnings and few drops of concentrated hydrochloric acid. This was slightly heated and observed for the formation of a dark pink color.

Test for tannins and phenolics

Ferric chloride test

The fraction of extract was treated with 5% ferric chloride solution and observed for deep blue color.

Lead acetate test

The fraction of extract was treated with lead acetate solution and observed for white precipitate.

Millon's test

To the test solution, 2 ml of Millon's reagent was added and observed for white precipitate.

Ninhydrin test

To the test solution, ninhydrin solution was added, boiled, and observed for the formation of violet color.

Test for carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used for check the existence of carbohydrates.

Molisch's test

Filtrates are tested in a test tube with two drops of alcoholic α-naphthol solution and analyzed at the junction of two liquids for the violet ring.

Benedict's test

Filtrates were treated and heated gently with Benedict's reagent. For the reduction of sugars, the orange-red precipitate was observed.

Fehling's test

Dil. HCl was used to hydrolyze the filtrates and neutralized with alkali and warmed with Fehling's A and B solutions. Red precipitate for the reduction of sugars was found.

Test for glycosides

Keller–Killiani test

About 0.5 g of extract was shaken with 5 ml of distilled water. To the sample solution, 2 ml of glacial acetic acid holding few drops of ferric chloride was added and then 1 ml of sulfuric acid along the side of the test tubes. The formation of a brown ring was observed at the liquid interface.

Legal test

The test solution was treated with pyridine and then added alkaline nitroprusside solution. The appearance of red blood color was observed.

Test for proteins

Heat test

Test solution was heated on a water bath and observed for the coagulation proteins.

Test with trichloroacetic acid

To the test solution, the trichloroacetic acid was added and precipitate formation was observed.

Biuret test

Two milliliters of the test solution was added to 2 ml of biuret reagent and the presence of protein is indicated for the violet color.

Test for saponins

Forth formations test placed 2 ml solution of extract in a test tube containing water. Shake well and observed for froth formation.

Quantitative estimation of phytoconstituents

Determination of total phenolic content

The total phenolic content of plant extracts was determined using the colorimetric methods. The plant extract (1 ml) was mixed with Folin–Ciocalteu's reagent (1 ml). Saturated sodium carbonate solution (1 ml) was added to the mixture after 3 min. The volume was made up to 10 ml using distilled water. The reaction kept 90 min in a dark place and after which absorbance was taken at 725 nm. Gallic acid was used as a standard solution for constructing the standard curve [20].

Determination of flavonoid content

Aluminum chloride colorimetric assay was used for the determination of total flavonoid content. The plant extract (1 ml) and distilled water (4 ml) were taken into the 10 ml volumetric flask. The 5% sodium nitrate (0.3 ml) was added to the flask and 5 min later 10% aluminum chloride (0.3) was mixed with it. After 5 min, the solutions treated with 1 M sodium hydroxide (2 ml) and make up the volume with water. Quercetin was used as a standard solution for constructing a standard curve in the same manner as described earlier. Absorbance was taken at 510 nm in the ultraviolet/visible spectrophotometer [21].

RESULTS

Preliminary phytochemical analysis

The phytochemical analysis methanolic extract of *Naringi crenulata* showed various compounds. Extract revealed the presence of secondary metabolites. This is shown in Table 1.

Table 1: Preliminary phytochemical screening

S.No.	Phytoconstituents	Indication
1	Alkaloids	++
2	Amino acid	++
3	Carbohydrates	++
4	Flavonoids	++
5	Cardiac glycoside	++
6	Saponins	++
7	Mucilage	++
8	Tannins	++
9	Phenolic compound	++
10	Proteins	++

++ = Presence, -- = Negative

Determination of total phenolic content

The total phenolic content was measured by Folin–Ciocalteu reagent, showed good phenolic content in methanolic extract The results are shown in Table 2.

Determination of total flavonoid content

The total flavonoid content was determined by the aluminum chloride spectrophotometric method. Methanolic extract of *Naringi crenulata* containing the highest flavonoid content. This is shown in Table 2.

Table 2: Determination of phenolic and flavonoid content

Extract	Phenolic content (µg of GAE/mg of extract)	Flavonoid content(µg RUE/mg of the extract)
Methanolic Extract	0.680	280.09

DISCUSSION

The phytochemicals present in the plant extricates profoundly liable for natural movement. The Phytochemical separating the current investigation has uncovered the presence of alkaloid, amino acid, starches, flavonoids, cardiovascular glycoside, saponins, adhesive, tannins, phenolic compound, and proteins. Flavonoids are a significant gathering of mixtures that go about as essential cancer prevention agents or free extreme foragers. Since these mixtures were found in the methanolic concentrates of Naringi crenulata, it very well may be answerable for the powerful cell reinforcement limit.

In, Quantitative analysis of *Naringi crenulata* presence of phenols and flavonoids. In the present study the phenols and flavonoids content seems to be more in methanolic extract of *Naringi crenulata*. Reported by Yanishlieva [22], flavonoids are found to be better antioxidants and have multiple biological activities including vasodilatory, anti-carcinogenic, antiinflammatory, antibacterial, immune-stimulating, anti-allergic, antiviral and radioprotective effects.

The phenolic compounds are one of the biggest and most pervasive gatherings of plant metabolites. They have natural properties, for example, antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular assurance and improvement of endothelial capacity, just as restraint of angiogenesis and cell expansion exercises [23]. A few investigations have depicted the cell reinforcement properties of restorative plants which are wealthy in phenolic compounds [24, 25]. Regular cancer prevention agent essentially comes from plants as phenolic mixtures like flavonoid, phenolic acids, tocopherols and so forth [26].

CONCLUSION

The phytochemical screening of Methanolic extracts of the Leaves of the *Naringi crenulata* has revealed the presence of a mixture of phytochemicals. The phytochemical screening showed that the all presence of phytoconstituents in both methanolic extract. It showed good values of phenolic content and flavonoid content. The presence of phytochemical constituents and higher values of phenolic and flavonoid content

make the plant useful for the formulation of the different drugs for human uses for treating various diseases. The obtained results explained the presence of possible phytoconstituents, potential usefulness and justified the traditional uses.

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