Original Research

PHYSICO-CHEMICAL EVALUATION, PRELIMINARY PHYTOCHEMICAL INVESTIGATION AND MICROSCOPIC EVALUATION OF *CITRUS MEDICA LINN.* SEEDS

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Submitted on: 25.09.2014 Revised On: 18.11.2014 Accepted on: 20.11.2014

ABSTRACT

Objective:

Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine. This article aims to provide for the physicochemical parameter, preliminary phytochemical screening and microscopic evaluation of *Citrus medica linn* seeds.

Methods: The fresh and powder *Citrus medica linn.* Seeds were studied by morphology, preliminary phytochemical screening, and microscopic evaluation of powdered drug. Other physicochemical parameters were also performed as per WHO guide lines.Results: The dried powder seed were investigated by morphology. The results of physic-chemical parameters such as loss on drying and ash values, extractive values, preliminary phyto-chemical screening, percentage of extractive values and microscopic evaluation are in the table no of 1, 2, 3, 4 and 5 respectively.

Conclusion: The present information on the pharmacognostic evaluation of the plant drug *Citrus medica linn.* seeds delivered the qualitative and quantitative parameters serve the important information to the identity and to determine the quality and purity of the plant material in the future. It also signify the important information of the closely related other species and varieties.

Keywords- *Citrus medica* linn, phytochemical screening, Phytoconstituents, physico-chemical evaluation.

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Indian Research Journal of Pharmacy and Science; 1(3);(2014) 55-65;
Journal home page: https://www.irjps.in

INTRODUCTION

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic mattes[1]. A number of medicinal plants, traditionally used for over 1000 years named rasa present in herbal preparations of Indian traditional health care systems[2]. In Indian systems of medicine most practitioners formulate and dispense their own recipies. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world [3].

Herbal remedies and alternative medicines are used throughout the world and in the past herbs often represented the original sources of most drugs[4-6]. The plant kingdom has provided an endless source of medicinal plants used as herbal teas, syrups, infusions, ointments, liniments and powders. Evidence of use of herbal remedies goes back some 60,000 years to a burial site in a cave in Northern Iraq, which was uncovered in 1960[7]. An analysis of the soil around the human bones revealed extraordinary quantities of plant pollen of eight species. Seven of these are medicinal plants and still used throughout the herbal world[8]. With the development of chemistry and Western medicine, the active substances of many species have been isolated and in some cases duplicated in the form of synthetic drugs[9]. Nevertheless, the synthetic preparation of some drugs is either unknown or economically impractical. For this reason, scientists continue to search for and test less known plants and conserve those properties have become crucial in the fight against diseases. Herbal-derived substances remain the basis for a large proportion of the commercial medications used today for the treatment of heart diseases, high blood pressure, pain, asthma and other illnesses. For example, ephedra is an herb used in traditional Chinese medicine for more than 2000 years to treat asthma and other respiratory problems. Ephedrine, the active ingredient in ephedra, is used in the commercial pharmaceutical preparations for the relief of asthma symptoms and other respiratory problems. It helps the patient to breathe more easily.

Today a great number of modern drugs are still derived from natural sources, and 25% of all prescriptions contain one or more active ingredients from plants. Herbal medicine can be broadly classified into four basic systems as follows: Traditional Chinese Herbalism, Ayurvedic Herbalism, Western Herbalism, which originally came from Greece and Rome to Europe and then spread to North and South America, and Arab traditional medicine, which forms the basis for alternative and herbal medicine in use today [10].

Citrus is a genus of flowering plants of the family Rutaceae and a common name for edible fruits of this family. Originating in tropical and subtropical Southeast Asia, these plants are among the oldest fruit crops to be domesticated. *Citrus* fruits are distinctive berry with the internal parts divided into segments and include oranges, lemons, limes, citrons, grapefruits, pomelos (pummelo, pommelo), and mandarins (tangerines). *Citrus* is likely the most

widely planted fruit for direct human consumption in the world. While fruit is a medium for plant multiplication, citrus fruits also provide human beings with a variety of aesthetic and sensual experiences as well as nutritional values. *Citrus* fruits are notable for their fragrance, partly due to flavonoids and limonoids contained in the rind, and most are juice-laden. The juice contains a high quantity of citric acid giving them their characteristic sharp flavour. They provide important health benefits, being good source of vitamin C, flavonoids, fiber, and folic acid [11].

Plant Profile

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Rosidae
Order	Sapindales
Family	Rutaceae – Rue family
Genus	*Citrus* L. – citrus
Species	*Citrus medica* L. – citron

Vernacular Names [12].

- **Sanskrit-** Matulunga
- **English-** Citron
- **Hindi-** Bijapura, Bara Nibu
- **Malayalam-** Ganapatinaraka
- **Gujurati-** Bijora
- **Punjabi-** Galgal
- **Urdu-** Turanj
- **Telgu-** Madi Phalam
- **Assam-** Jaradeda

Plant characteristics

Trees or shrubs, are about 3.6 m high usually spinous. Leaves foliate; petiole narrowly winged; leaflets entire or crenulate, coriaceous, persistent. Flowers white or pinkish in buds, sweet-scented, solitary, fascicled or in small cymes. Calyx copular or urceolate, 3-5-fid. Petals 4-8, linear-oblong, thick, imbricate. Stamens 20-60; filaments variously connate, compressed at the base; anthers oblong. Ovary many celled; ovules 4-8 in each cell, 2 seriate; style deciduous; stigma capitates. Berry globose or oblong, fleshy, many-celled; septa membranous; cells few seeded, filled with horizontal or fusiform with juice. Seeds horizontal or pendulous; testa coriaceous or membranous; embryos sometimes 2 or more in seed; cotyledons plano-convex, often unequal; radical small, superior[13].

MATERIALS AND METHODS

Collection of plant material and authentication:

The disease free fresh seeds were collected from the forest park of Nawabganj,Gonda (U.P.), India, in the month of January by hand picking method. The plant was identified and authenticated from National Bureau of Genetic Resources Regional Station, Niglat, Bhowali-263132, District-Nanital, (Uttarakhand), India, by Dr.KS Nagi, Principal Scientist & Officer In charge.

Preparation of the extract

The leaves of fresh samples were cleaned and washed under running tap water [14]. The samples were dried in the oven at 37°C for 6 days. After drying the samples were weighed and blended with warring

blender and soaked with methanol for 2 days and filtered using Whatman No. 1 paper. The methanol was completely removed by vacuum evaporator at 50°C till it gave a viscous mass. The crude extracts were weighed and stored at 4°C before analysis.

Macroscopic and microscopic studies

Macroscopic studies were carried out by simple determination, technique like the shape, size, colour, odour, margin and apex. Free hand sections of the fixed seed material were taken and boiled with Diluted HNO3 (1:3, 60% HNO3: Water) for 2-3 minutes to remove the coloring matter, washed with distilled water. Further it was kept in alkaline KOH solution for 2-3 minutes. Then almost seed was treated with 0.5 % safranin solution for staining purpose and mounted on a clean glass slide with glycerin and covered with cover slip. The sections were then viewed under low power (10 X) and subsequently under high power (40 X) microscope [15]. The microphotographs were taken using Nikon Phase Contrast microscope attached with Nikon Eclipse E600 camera**.** The powder seeds were also examined for its microscopic characters. The powders were passed through sieve no. 60 and studied for their organoleptic and microscopic characteristics [16].

Determination of physicochemical constants of plant materials [17]:

Various physicochemical constants like Total ash value, water and acid, soluble and insoluble ash value, and moisture content were determined as per Indian pharmacopoeia are described below.

Loss on drying: The powdered drug sample (10gm) without preliminary drying was placed on a tarred evaporating dish and dried at 105 ºC for 6 hours and weighed. The drying was continued until two successive reading matched each other or the difference between two successive weighing was not more than 0.25%. Constant weight was reached when two consecutive weighings after drying for 30 minutes in a desicator, showed not more than 0.01 gm difference.

Ash values: - Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form Total ash: The ground drug (1 g) was incinerated in a silica crucible at a temperature not exceeding 450oC until free from carbon. It was then cooled and weighed to get the total ash content.

Acid insoluble ash: Ash was boiled with 25 ml dilute HCl (2N) for five minutes. The insoluble matter collected on an ashless filter paper, washed with hot water and ignited at a temperature not exceeding 450oC to a constant weight.

Water soluble ash: Ash was dissolved in distilled water and the insoluble part collected on an ashless filter paper and ignited at 450oC to constant weight. By subtracting the weight of insoluble part from that of the ash, the weight of soluble part of ash was obtained [18]. **Sulphated ash:** 1g of plant powder was ignited in an electric furnace until the drug gets charred. The crucible was cooled and the residue was moistened with 1ml of H2SO4, heated gently until the white fumes were no longer evolved and ignited at 800o C ± 25o C until all black particles disappear. The crucible was allowed to cool; few drop of H2SO4 was added and again heated. The ignition was carried as before, allowed to cool and then weighed. This was repeated until the sample reaches a constant weight.

Extractive Values: - The air-dried coarse drug powder (1g) was macerated separately with solvents

(Petroleum ether, methanol and water) of volume 25 ml in a closed flask for 24 hours, shaken frequently during six hours and allowed to stand for 24 hours. It was filtered rapidly, taking precaution against loss of solvent, the filtrate evaporated to dryness in a tarred flat bottom dish and dried on water bath, to constant weight and weighed.

Foreign matter: Foreign matter in the crude drugs can be due to faulty collection or deliberate mixing. It was separated from the crude drug and percentage calculated.

Preliminary Phytochemical Analysis After collection and authentication, the plant material was shade dried and powdered. It was passed through sieve no. 40 and subjected to extraction. Weighed quantity of plant material was extracted separately with methanol and powderr by Cold extraction method, Qualitative screening of various extracts *Citrus medica* Linn. seed was performed for the identification of various classes of active chemical constituents like alkaloids, amino acids, carbohydrates, glycosides, proteins, steroids etc. using different methods [19]. The results of the phytochemical tests are shown in table 1.

RESULTS

Generally the herbal drugs are currently being used in the treatment of various diseases without standardization. The quantitative determination of some pharmacognostical parameters is useful for setting standards for crude drugs. The results of these investigations could serve as a basis for proper identification, collection and investigation of the plant.

Organoleptic study of plant material:-

In some cases, general appearance of the herb is similar to related species. Thus, detailed study of the morphological characters can be helpful in differentiating them. The organoleptic study of a drug includes its visual appearance to the naked eye along with its characteristics likes odour, taste, texture etc. For each particular organoleptic group, a particular systemic examination can be carried out.

Macroscopic evaluation

Mature seeds were whitish to cream in coloration, 5-6 mm in length, ovoid in shape shown in Fig 1& 2, with a woody seed coat. The seeds had a flattened ovoid shape, narrow at the tip and broader at the base. Seed possess acrid taste.

Microscopic characters of *Citrus medica* Linn. seed

Transverse section of the seed was somewhat triangular oval to irregular circular in outline and shows outer grooved testa often attached with ramnants of fleshy mesocarp tissue, followed by dark brown coloured tegmen and bulky endosperm embedding the cotyledons of the embryo.

The seed showed a layer of epidermis often attached externally with ramnants of fleshy mesocarp. The tissue of the testa was highly lignified and is composed of various type of compactly arranged stone cells and sclereids. A distinct zone of dark brown tegmen tissue lies underneath the testa, consisting of tangentially elongated cells embedded with few groups of stone cells, fibres and vascular strands; followed by the broad zone of endosperm filled with aluerone grains and oil globules. The usual

cellular structure of the cotyledon of the seed exhibiting, epidermis, palisade like cell underneath it. Different structures are clear in the photographs of transverse section of seeds shown in Fig 3& 4.

Phytochemical Study

The seed powder was extracted with methanol and the yield is reported in Table .2. The phytochemical screening of powder seed and methanolic extract is depicted in Table 3.

FIG-1,FRUIT AND SEEDS OF *CITRUS MEDICA* LINN.

Figure 2 – Fruit of *Citrus medica* Linn.

Figure 3 -TRANSVERSE SECTION OF *CITRUS MEDICA* LINN. SEED

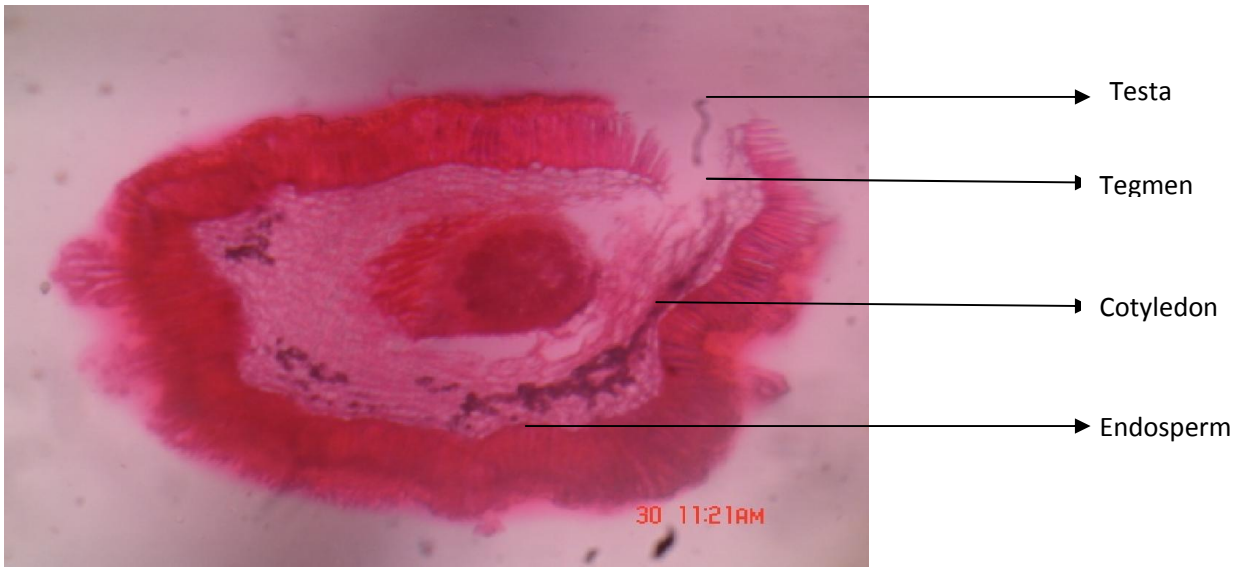

Figure 4 - Transverse section portion of seed of *Citrus medica* Linn.

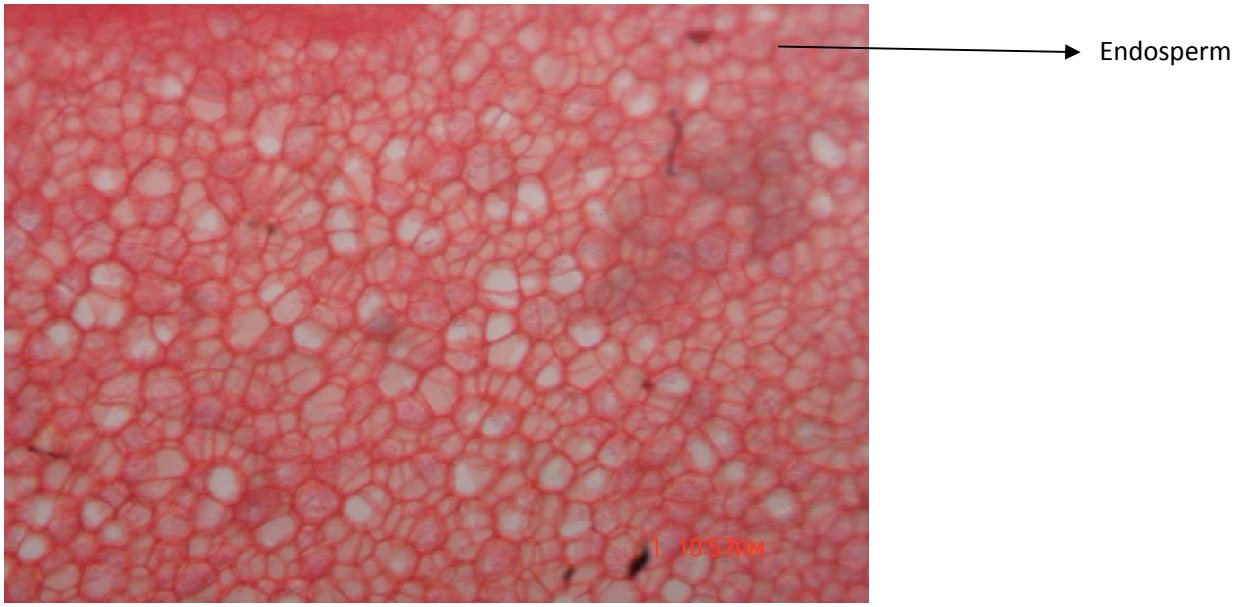

Table 1: Physicochemical Evaluation Parameters (*Citrus medica seed*)

Sl. No.	Parameter	Value (%w/w)
1.	Loss on Drying	6.3%
2.	Ash Values	
	Total Ash	4.14%
	Acid insoluble ash	2.25%
	Water soluble ash	2.12%
	Sulphated ash	4.67%
3.	Extractive Values	
	Water soluble extractive	3.4%
	Alcohol soluble extractive	4.2%
	Petroleum ether soluble Extractive	6.08%
4	Foreign Organic Matter	2.6%
5	Volatile Oil	-----

Table .2 Yield, colour and consistency of Extracts

Extracts	**Percentage Yield (w/w)**	**Colour**	**Consistency**
Methanol	3.68	Pale yellow	Slightly sticky

Table 3: Preliminary Phytochemical investigation of methanolic extract and Powder of *Citrus medica* seed.

Test	PD	ME
Test for Carbohydrates	-	-
Test for Gums and Mucilages	-	-
Test for Proteins and Amino Acid	+	+
Test for Fixed Oils and Fats	+	+
Test for Phytosterols	+	+
Test for Glycosides	-	-
Test for Saponins	-	-
Test for Flavonoids	+	+
Test for Alkaloids	-	-
Test for Tannins & Phenolic Compounds	-	-

+ Present, - Absent

DISCUSSION

Citrus fruits have been collected and used by man for centuries for medicinal, herbal and agricultural purposes [20,21]. Citrus fruits, belong to the family of Rutaceae, are one of the main fruit tree crops grown throughout the world [22]. All citrus fruits share in common their sweet and sour flavor. They possess refreshing juice and are available almost all round the year [23]. Citrus species are small to medium-size shrubs or trees that are cultivated throughout the tropics and subtropics. They are native to parts of India, China, and northern Australia.

According to UN 2007 data, Brazil, China, the United States, Mexico, India, and Spain are the world's largest citrus-producing countries. Of these, Brazil is the world's largest producers of oranges, China produces most of the world's mandarins, and India is the world's largest producer of lemons and limes, and the United States produce the most grape fruit[24]. It is reveals from the literature that the citrus fruit possess anti-cancer, antimicrobial, antioxidant, antiulcer, anti-inflammatory, and hypolipidemic antityphoid and hepatoprotective properties.

The traditional medicine requires intensive and urgent investigation in the next few years from botanical, chemical, and biological perspective, particularly for the rapidly increasing diseases in the developing world. Plants are important source of potentially bioactive constituents for the development of new chemotherapeutic agents. The first step towards this goal, the plant *Citrus medica* Linn *seed* was subjected to systematic organoleptic evaluation, physicochemical and phytochemical screening by different extracts to determine the amount of soluble constituents in a given amount of medicinal plant material and are helpful in determining the quality and purity of a crude drug, especially in the powdered form.

CONCLUSION

Before the introduction of modern medicines, disease treatment was entirely managed by herbal remedies. It is estimated that about 80% of the world population residing in the vast rural areas of the developing and under developed countries still rely mainly on medicinal plants. The study of Pharmacognostical features *Citrus medica* Linn *seed* had shown the standards which will be useful the detection of its identity and authenticity. The other study viz. physical evaluation, preliminary phytochemical test and Microscopic evaluation add to its quality control and quality assurance for proper identification.

ACKNOWLEDGEMENTS

The authors wish to thanks to all the staff of Sir Madanlal Institute of Pharmacy, Alum Pur hauz,Agra Road Etawah -206001,U.P., India and Department Of pharmaceutical Sciences, Gurukul Kangri University, Haridwar, Uttarakhand for their unresrved support and cooperation in collecting different articles for the preparation of this research article.

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Conflict of Interest Reported: Nil; Source of Funding: None Reported