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# Pharmacognostic and Phytochemical Evaluation of *Citrus limon* (Lemon) Fruit and Leaf

Rathod Zalak R., Makwana Sonal K., Christian Venisha V., Saraf Meenu S.\*

Department of Microbiology and Biotechnology, University School of Sciences, Gujarat University, Ahmedabad, Gujarat, India.

**ABSTRACT:** *Citrus limon* (Lemon) belongs to Family Rutaceae, is commonly known as Kagdi Limbu or Lime. The peel is a leathery exocarp, or skin of the fruit containing numerous oil glands. Lime peel tea was traditionally used as an appetite stimulant, for cold and cough, for sore throat and as a digestive stimulant. It can also be used as an astringent for skin. Lime peel essential oil is use as an aroma and flavour enhancer. The present study deals with the pharmacognostic and preliminary phytochemical evaluation of *Citrus limon* (Lemon) peel extract including macroscopic, microscopic characters, physiochemical evaluation and preliminary phytochemical study. Thin layer chromatography has also been performed. Microbial load of dried powder material was determined to avoid the contamination and for safety of crude drug. The present work will provide referential information for the correct identification and standardisation of the crude drug and will ensure the use of only genuine and uniform material in preparation of herbal formulation.

**KEYWORDS:** *Citrus limon* (Lemon), pharmacognosy studies, physicochemical analysis, TLC.

## I. INTRODUCTION

Herbal medicines are currently in demand and their popularity is increasing day by day. In the healthcare sector, WHO recommends and encourages the use of traditional herbs or remedies because huge amount of raw material is easily available. Plants are very complex in nature. Their therapeutic activity varies according to species, geographical location and harvesting processes. Improper authentication of herbs, adulterations by microorganism, pesticide residue; has made standardization of herbal drug of primary importance. According to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken.

### Lime fruit

Scientific classification (Liogier, 1988)

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Sapindales

Family : Rutaceae

Genus : Citrus

Species : C. limon

Binomial name : *Citrus limon* (L. Burm. F.)

Common name : Acid Lime, Key lime (Mexican), Kagdi Limbu (Gujarati)

## II. MATERIALS AND METHODS

The whole *Citrus limon* plant was collected from Department of Horticulture, AAU Anand, Gujarat, India. *Citrus limon* fruits and leaves were washed thoroughly under sterile distilled water surface sterilization too. Peels were separated from fruits; both leaves and peels dried under shade. The dried material was then subjected to fine powder and passes the powder through sieve no. S25#. Then the dried material subjected to the powder form and stored in an airtight container at room temperature.

### 2.1. Reagents and Chemicals

All reagents and chemicals used for testing were analytical grade, SRL, Himedia and Sigma brand.

### 2.2. Pharmacognostical evaluation

Macroscopic Characters The organoleptic characters of fruits such as shape, size, odour, colour, surface, taste etc. were studied for morphological evaluation (Dutta, 2007 [5]; Handbook of Ayurvedic Pharmacopoeia, 2001).

### 2.3. Macroscopic and Microscopic analysis

The macroscopic characters of lime fruit peels were studied according to standard methods for its morphological characters such as colour, odour, taste, shape, size, etc and reported. Microscopic evaluation was carried out for transverse section of Lime peel and dried powder material. The outer epidermal layer (in fragments) was cleared in chloral hydrate, stained with phloroglucinol and concentrated HCl and mounted with glycerine and observed under a compound microscope.

### 2.4. Physicochemical analysis

Physicochemical properties such as Total ash, Acid insoluble ash, Water soluble ash, were determined according to the specifications as per Indian Pharmacopoeia. (4) Percentage of ash value (indicative of the purity of the drug) and extractive values (represent the presence of polar and non-polar compounds) were performed according the official methods. Water and alcohol soluble extractive values were estimated by cold maceration according to the method as prescribed by WHO guidelines on quality control methods for medicinal plants materials (WHO, 2011)

### 2.5. Determination of volatile oil

The determination of volatile oil in peels and leaf were carried out by steam distillation using Kjeldahl according to the method of Khandelwal. 10 gm of chopped peels and shadow dried powder of leaf were taken into round bottomed flask and mixed with 100 ml of Hexane: glycerine (1:1) then the Clevenger's apparatus was assembled. The round bottom flask was heated for 2 hrs and the oil was collected in the graduated tube. The oil was obtained from which percentage of oil in the peel of fruit and leaves of *Citrus limon* was calculated.

### 2.6. Preliminary phytochemical analysis

The powdered plants were subjected to preliminary Phytochemical screening for qualitative detection of phytoconstituents. The dried and coarsely powder (50g) were extracted with n-Hexane (65°C-80°C) and Methanol (64.5°C-65.5°C) by Soxhlet extraction and by cold maceration. The extracts obtained were evaporated to dryness by Rota evaporator and weighed to calculate percentages in terms of initial air-dried plant material. 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 of Harborne, 1998, Khandelwal 2002.

### 2.7. Thin Layer Chromatography (TLC)

Methanolic extract of *Citrus limon* extracts of fruit and leaf were prepared by using Soxhlet extraction method. The extract was filtered and concentrated on rotary evaporator. Thin layer chromatography fingerprinting was achieved by using Silica Gel G as a stationary phase. The concentrated extract was spotted using a capillary tube on a normal phase plate previously activated at 110 °C for 2 h. The plate was developed using mobile phase of Benzene: Acetone (9:1). Isolated spots were visualized under ultra violet light at 254 nm and 366 nm. Further, the plate was sprayed with 10% vanillin sulphuric acid. The retardation factor (Rf) was determined using following formula (Wagner et al. 1996).

$R_f = \text{Distance travelled by solute} / \text{Distance travelled by solvent}$

## III. RESULTS AND DISCUSSION

### 3.1. A Brief Taxonomic Description of the Plant

*Citrus limon L. Burm. f.* is shrub, about 3.6 m high with tiny, thick and spiny branches, cultivated sparingly throughout the warm-moist regions of the country (Handbook of Ayurvedic Pharmacopoeia, 2001). Its leaflets are 3-6 inch long, ovate lanceolate with short, wingless or nearly wingless petioles; flowers are small, 5-10 in a raceme; petals are generally more or less pink; fruit is globose ovoid or oblong often mamillated at the apex, with a leathery peel, green when unripe and turns to yellow when ripe. This plant is found apparently wild in Gujarat, Khasia Hills, Garo hills, Chittagong, Upper Yunzalin valley, Kumaon, Pachamarhi, Sikkim, Western Ghats and Saputara range in Central India (Kirtikar and Basu, 2007)

### 3.2. Macroscopic and Microscopic analysis

Macroscopic characters of fresh and dried lime peel vary. Colour of outer surface of fresh peel is Bright Green to Yellow and turns to Brownish Yellow when dried. Inner surface of fresh peel is white and turn to Whitish Brown after drying. Fresh Peel has strong and aromatic odour and dried peels are not very strong and less aromatic. Taste is sour and bitter. Shape of fruits is globose or ovoid. Dried peels are triangular to spiral shaped. These brittle strips are about 1 to 2 cm long, 2 to 2.5 cm broad and 0.1 to 0.2 cm in thickness, externally rugose, marked with numerous minute pits which represent oil glands.

The powder characters of a drug are mainly used in the identification of the drug in the powder form. Powder also showed the presence of spiral vessels and fragments of broken oil glands.

### 3.3. Determination of physicochemical parameters

The physical-chemical parameters are mainly used in judging the purity and quality of the drug. Ash values of a drug give an idea of the earthy matter or inorganic composition. Water soluble ash value is more than acid insoluble ash. Extractive values give an idea about the chemical constituents present in the drug as well as useful in the determination of exhausted or adulterated drugs. The results suggest that the powdered drug have high water-soluble extractive value (Table 1).

**Table 1: Physicochemical analysis of dried peel powder of *Citrus limon* fruit and leaf**

Parameter	Average Value of <i>Citrus limon</i> fruit	Average Value of <i>Citrus limon</i> leaf
Total ash content	4.0%	2.8%
Acid insoluble ash	0.062%	0.05%
Water soluble ash	0.70%	0.78%
Alcohol soluble extractive	11.12%	14.30%
Water soluble extractive	30.89%	42.69%

### 3.4. Determination of volatile oil

Volatile oils are characterized by their odour, oil-like appearance and ability to volatilize at room temperature. Aromatic compounds predominate in certain volatile oils. *Citrus limon* peel and leaf volatile oil has characteristic smell. The percentage of oil calculated in the peels was 3.08% and leaf was 4.62% of *Citrus limon*.

### 3.5. Preliminary phytochemical analysis

The information obtained from preliminary phytochemical screening will be useful in finding out the genuity of the drug. Preliminary phytochemical analysis indicated the presence of carbohydrates, amino acids and flavonoids in methanolic extracts (Table 2).

**Table 2: Preliminary phytochemical analysis of *Citruslimon* fruit and leaf extracts.**

Test performed	Name of the test	Fruit extract	Leaf extract
Test for carbohydrates	Fehling's test	+	-
Test for Amino acids	Ninhydrin Test	+	+
Test for Flavonoids	Shinoda Test	+	+
Test for Alkaloids	Dragendroff Mayer's Wagner's reagent	-	+
Test for Tannins and Phenolic compound	5% FeCl <sub>3</sub>	-	+
Test for Steroids	Salkowski reaction	+	+

(Key: + Test positive, - Test negative)

### 3.6. Thin Layer Chromatography study

TLC is a simple, low-cost, versatile and specific method for the identification of herbal medicines. The fingerprint has potential to determine authenticity and reliability of chemical constituents of herbal drug and formulations. Rf values were calculated for methanolic and n-hexane extracts (Table 3).

Table 3: Thin Layer Chromatography analysis of *Citrus limon* fruit and leaf extracts.

Colour of the Band	R <sub>f</sub> Value of Fruit extract	R <sub>f</sub> Value Leaf extract
Dark Purple	0.2	0.22
Dark Green	0.34	0.37
Dark Purple	0.51	0.49
Brown	0.54	0.52
Dark Blue	0.60	0.60
Green	0.66	0.65
Purple	0.74	0.74
Dark Blue	0.88	0.86

#### IV. CONCLUSION

Standardization is essential measure for quality, purity and sample identification. Microscopic method is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials. The majority of the information on the identity, purity and quality of the plant material can be obtained from its macroscopy, microscopy, physio – chemical parameters and TLC fingerprinting. The present work is undertaken to obtain some pharmacognostical standards. The above studies provide information with respect to the identification, chemical constituents and physicochemical characters of *Citrus limon*. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy.

#### V. CONFLICT OF INTEREST

The authors have no conflict of interest in preparing of this manuscript.

#### VI. ACKNOWLEDGEMENT

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