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LC-MS ANALYSIS OF RIND AND PULP OF *CITRUS LIMON* (L.) BURM. F. FRUITS

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Abstract: *Citrus limon* fruits are commonly used as soft drink. It possesses medicinal values also as a part of nutraceutical studies. Liquid Chromatography Mass Spectrophotometric Analysis (LCMS in *Citrus limon* fruits were done for the determination of the major components in it. The medicinal and health promoting properties are due to the presence of active principles on it.

Key Words: *Citrus limon,* Terpenoids, Phenolic compounds, Coumarins

INTRODUCTION

Several up to date research work and practical experience have shown that using medicinal plants is better than allopathic drugs by being safer besides having synergistic effect. The medicinal properties of plants could be based on the antioxidant, antimicrobial and antipyretic effects of the photochemical in them (Cowman, 1999; Adesokan *et al.,* 2008). Medicinal plants produce bioactive compounds used mainly for medicinal purposes. So the identification of bioactive compound in plants, their isolation, purification and characterization of active ingredients in crude extracts by various analytical methods are important.

The plant selected for the study is *Citrus limon.* Citrus fruits contain bioactive compounds including vitamin C, flavonoids, coumarins, carotenoids and limonoids with potential health promoting properties (Lam *et al.,* 1989, Miller *et al.,* 2000, Tian *et al.,* 2001, Guthrie *et al.,* 2000). The fruits of Citrus *limon is* ovoid in shape and has a pointed tip at one end. They have thick covering over it. The unripened fruits are hard, dark green and non-juicy but on ripening it turns to bright yellow, becomes soft and juicy. The objective of the study is to identify the different compounds in rind and pulp using LCMS method and to explore the medicinal and nutraceutical aspects of lemon fruit. By this study list out the primary and secondary metabolites using Liquid Chromatography Mass Spectrophotometric Analysis (LCMS) in *Citrus limon* fruit.

MATERIALS AND METHODS

Taxonomical description of Citrus limon (L.) Burm. f.

Citrus limon (L.) Burm. f. is a shrubs, spinous; branchlets glabrous; bark green. Leaves unifoliolate, reddish or purplish when young; petioles narrowly winged, leaflet blade elliptic-oblong, obtuse to rounded at base, obtuse at apex, glandular-serrulate along margins, 5.5-11.5 × 2-5cm. Inflorescence axillary condensed racemes, usually 5–7 flowered. Flowers shortly pedicellate, usually bisexual or staminate, 5–merous, purplish in buds. Calyx urceolate; sepals suborbicular, minute glabrous. Petals ovate-oblong, obtuse, 1.5-2 × 0.3-0.5cm, coriaceous, glabrous, purplish tinged abaxially, greenish white adaxially. Stamens 30-40; filaments connate at base, free above, glabrous; anthers oblong, apiculate, 0.4-0.6cm long, greenish-yellow. Disk annular, fleshy, 0.3cm broad, glandular, glabrous, greenish white. Ovary sub cylindric, 0.5-0.7 × 0.3-0.4cm, greenish; style thick, 0.4cm long, caducous; stigma globose. Fruit ovoid-oblong or oblate, small to medium-sized up to 6cm across, 8-12 locular; rind thick, glandular, strongly adherent, mamillate, yellowish when ripe, pulp-vesicles pale green to yellowish; juice scanty or abundant, sour, acidic; seeds ovoid, acute, small, 0.5–1cm long, 0.5-0.6cm, whitish inside when cut.

Collection and authentication

The fruits of *C. limon were* collected different localities and the collected plant samples are authenticated from the Regional herbarium Kerala (RHK) in S. B. College, Changanassery, Kerala. The fruits were rapidly processed on the same day. At least 10 fruits were carefully washed under running tap water and patted dry. The peel was carefully removed with a manual peeler and the pulp obtained was homogenized in a Warring Commercial blender (Dynamics Corporation, CT, USA). Samples were ground into a fine powder in a coffee grinder and stored in airtight containers at –4°C until analyzed.

Extraction

All the materials are dried under hot air oven. 3gm each of the fruit and peel (rind) were separately extracted using petroleum ether (60- 80) and methanol sequentially using soxhlet apparatus for three hours each using 50ml of the corresponding solvent. The extracts were then dried and dissolved in 10 ml petroleum/ methanol (HPLC grade, Merck). It was then filtered through 0.20mm membrane filter. The extract was used for the analysis.

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LCMS Analysis

Liquid Chromatography Mass Spectrophotometric Analysis (LCMS) allowed the determination of the major components in *Citrus limon*. 10µl of the filtered sample was injected to the manual injector using a Micro syringe (1-20µl, Shimadzu). The mobile phase used was water: methanol (50:50) in an isocratic mode. The column used was RP-C-18 (phenomenex). The separated compounds were then ionized using APCI method and using the split mode (50:50). The flow rate was maintained to 2ml/mn with a temperature of 25 ± 2°c. The class VP integration software was used for the data analysis. The Library used for the analysis was Metwin- LS. The version of the library was version 1.0- 52.09.

PASS

Computer program PASS (Prediction of Activity Spectra for Substance) (Filimonov *et al.,* 1995) provides the activity spectra of these compounds.

RESULT

Primary Metabolites

The primary metabolites present in the rind and pulp of *Citrus limon* are the following:

Table 1: Showing the presence of Fatty Acids & Lipids in Rind and Pulp of Citrus *limon* Fruit

S. No.	Fatty acids & lipids	Lemon Pulp	Rind
1.	Linoleic acid	✓	-
2.	Arachidic acid	✓	-
3.	Palmittic Acid	✓	-
4.	Linolenic acid	✓	-

There is no fatty acid and lipids are present in the rind of citrus limon. Linoleic acid, Arachidic acid, Palmittic acid, Linolenic acid are the fatty acids and lipids present in the pulp of Citrus *limon* fruits. The purines and pyramidines like Uracil is present in the pulp. Thiamine is the only aminoacid present in the pulp and Methionine and proline are present in the rind. The organic acid citric acid is present in both rind and pulp and pimelic acid is present only in rind.

Secondary Metabolites

Secondary metabolites present in the *Citrus limon* fruits are the following.

Terpenoids: The terpenoids like nomilin, Friedelin, limonin, Valencene are common in both rind and pulp of *Citrus limon* fruits. Paracymene and Forskolin are present only in pulp and Lemonol, Sabinene, Nivalenol are present only in rind.

Table 2: Showing the presence of terpenoids in Rind and Pulp of Citrus *limon* Fruit

S. No.	Terpenoids	Lemon Pulp	Rind
3.	Nomilin	×	×
4.	Friedelin	×	×
5.	Limonin	×	×
6.	Valencene	×	×
7.	Paracymene	×	-
8.	Forskolin	×	
9.	Lemonol	_	×
10.	Sabinene	_	×
11.	Nivalenol	-	×

Phenolic compounds: The phenolic compounds present in the rind and pulp of *Citrus limon* are the following.

Phenyl Propanoids: Phenyl propanoids present in the rind and pulp of *Citrus limon* fruits are the following.

Table 3: Showing the presence of Phenyl propanoids in Rind and Pulp of Citrus *limon* Fruit

S. No.	Phenyl Propanoids	Lemon Pulp	Rind
1.	Cinnamic acid	×	×
2.	Trans Para coumaric acid	×	-
3.	Caffeic acid glucoside	-	×
4.	Rosmarinic acid	-	×

Phenyl propanoids like Cinnamic acid is common in Pulp and rind of *Citrus limon* fruits. Trans para coumaric acid is present only in Pulp and Caffeic acid glucoside and Rosmarinic acid are present only in Rind.

Flavonoids: The flavonoids present in the rind and pulp of *Citrus limon* fruits are the following.

Table 4: Showing the presence of Flavonoids in pulp and rind of *Citrus limon fruit*.

S. No.	Flavonoids	Lemon pulp	Lemon Rind
1	Naringenin	×	×
2	Diosmetin	×	×
3	Eriodictyol	×	×
4	Tangeretin	×	×
5	Diosmin	×	×
6	Hesperidin	×	-
7	Hesperetin	×	-
8	Quercetin	×	-
9	Nobiletin	-	×
10	Iridin	-	×

The flavonoids like naringenin, eriodictyol, diosmetin, tangeretin and diosmin are common in both rind and pulp of *Citrus limon* fruits. Quercetin, hesperetin and hesperidin are present only in pulp and nobiletin and iridin are present only in rind. The literature shows that the availability of flavonoids in these citrus fruits, prevent platelet stickiness and hence platelet aggregation. Citrus flavonoids are a good digestive tonic, with an appetizer effect and it aids in digestion. Antioxidant flavonoids from citrus helps in the digestion and assimilation of food to the body system. The presence of flavonoids in citrus fruits

may be the reason for their use in herbal medicine for the treatment of capillary and vascular weakness (edema, varicose vein, blood clotting, and dysfunction) (Rogar, 2002, Oliver-Bever, 1986).

Coumarins: The coumarin Byakangelicol is common in both rind and pulp of *Citrus limon*. Byakangelicin and Epoxybergamottin are present only in Pulp. Umbelliferone is present only in rind.

Table 5: Showing the presence of Coumarins in Rind and Pulp of Citrus *limon* Fruit

S. No.	Coumarins	Lemon Pulp	Rind
1.	Byakangelicol	×	×
2.	Byakangelicin	×	-
3.	Epoxybergamottin	×	-
4.	Umbelliferone	-	×

Phenol and phenolic acid: Phenolic acids like Vanillic acid and Methyl ellagic acid are present only in pulp and cresol is present in the rind only. The stilbinoid Astringin is present in the pulp only. Cinnamaldehyde (Organic compound), Lipoic acid (Organo sulfur compound), Isothebine and caffeine (Alkaloid), Crocetin (Carotenoid Dicarboxylc acid) present only in pulp of *Citrus limon* fruit. Theophylline (alkaloid) present only in rind. The glucosinolates glucoconringin is present in both rind and pulp. Based on the PASS and literature studies the uses of compounds are the following.

The antioxidant property of the plant material is due to the presence of many active phytochemicals including vitamins, flavonoids, terpenoids, carotenoids, coumarins, curcumins, lignin, saponin, plant sterol. *etc.*, (Lucia *et al.,* 2008). Citrus fruits and juices are an important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids, phenolic compounds and pectins that are important to human nutrition (Fernandez *et al.,* 2005).

The presence of above said compounds in *Citrus limon* fruits is significant to note that soft drinks that are so called lemonade or that they are made of lemon not only lack medicinal properties but also are noxious for health due to their content of carbonic gas, artificial colorings and aromatizes and sugar or other flavours (Rogar, 2002).

CONCLUSION

Based on the present data the best way to take advantage of the many medicinal values of lemon juice is to consume them just after they have been squeezed from the fruits. Avoid canned juices and soft drinks. This will help the good health of the body. So protect and conserve *Citrus limon* plants.

Table 6: Shows the activity of compounds.

Compound Name	Activity
Linoleic acid	Antioxidant, anti- inflammatory (Maillard *et al.,* 2003)
Thiamin	Prevents beriberi
Limonin	Phosphatase inhibitor, Polarization stimulant, Inflammatory bowel disease treatment (PASS).
Valencene	Retinol dehydrogenase inhibitor, Carminative, Sigma receptor agonist, Opioid dependency treatment (PASS).
Friedelin	Muco-membranous protector, Membrane integrity antagonist, Oxidoreductase inhibitor, Antiviral (influenza), Cholesterol synthesis inhibitor, Phosphatase inhibitor, Hypercholesterolenic, Antipuritic, Hepatic disorders treatment, Cardiovascular analeptic, Dermatologic, Antinephritic, Tocolytic (PASS).
Diosmetin	Kinase inhibitor, Anti-neurotoxic, Cyto-protectant, Antioxidant, Chemo preventive, Antiseborrheic, Antineoplastic (lung cancer), Antithyroid, Cardio protectant, Chemo protective, Free radical scavenger, Retinal oxidase inhibitor, Haemostatic, Carbonyl reductase (NADPH) inhibitor, Choleretic, Aldehyde oxidase inhibitor, Fibrinolytic, Apoptosis agonist, Vasoprotector, Nitric oxide antagonist, CYP1 substrate, Aryl alcohol dehydrogenase (NADP+) inhibitor, Leukotriene, Chalcone isomerase inhibitor etc. (PASS)
Naringenin	Vascular disease treatment, Membrane integrity against, Capillary fragility treatment, NAD(P)H dehydrogenase (quinine) inhibitor, CYP1A1 substrate, Antineurotoxic, Muco-membranous protector, Pectate lyase inhibitor, Transcription factor inhibitor, Free radical scavenger-benzoquinone reductase (NADPH) inhibitor, Aryl alcohol dehydrogenase (NADP+) inhibitor, Cytoprotectant, antioxidant etc., (PASS)
Hesperidin	Reduce cholesterol and blood pressure, anti-inflammatory effects and also a potential sedative (Emim JA, 1994).
Hesperetin	Free-radical scavenging activity, Anti-elastolytic activity anti-inflammatory, antimicrobial, antioxidant, anti-carcinogenic properties and prevent bone loss (PASS)
Eriodictyol	Bitter masking flavonone.
Quercetin	Prevent the oxidation of low density protein, chelating transition metal ions, prevention of cancer, atherosclerosis, chronic inflammation and antioxidant (Hollman and Katan, 1997).
Diosmin	Potential in the treatment of Alzheimer's disease, anti-inflammatory, anti-apoptotic (Sanjay, 2005)
Tangeretin	Cholesterol lowering, anticancer agent (Datla KP).

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