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Research Article

# HPLC Profiling of Phytoceutics in the Fruit Peel of Citrus Species of Tamilnadu

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## Abstract

Citrus fruits are the rich source of natural antioxidants to prevent lots of diseases and are widely consumed in the villages of Thanjavur region, Tamilnadu, India. Peel waste of citrus fruits are rich in bioactive constituents such as flavonoids that are power house of antioxidants. Flavonoids are used in health boosting ingredients in foods as free radical scavenger. Flavonoids are secondary metabolites in citrus fruits that may possess biological activity and have beneficial effects as antimicrobial, anti-inflammatory, anti-diabetic, anti-cholesterolemic, antioxidant and anti-cancer agents. This study aimed to analyze the chemical constituent of C*itrus medica and* C*itrus aurantium* fruits peel for the first time. The objective of this study is to assess the Flavonoid contents in peel of C.*medica and* C.*aurantium* fruits by HPLC technique. Results of this study indicated that gallic acid, qucertin, rutin, cathecin, chlorgenic acid are exist in *C.medica* and chlorgenic acid and other flavonoids in *C.aurantium* peel. This study high lights the citrus peel could serve as a alternative source to chemicals, may be used for larger scale production of flavonoids.

**Keywords:** *C.medica, flavonoids, C.aurantium peel, bioactive constituents, HPLC chromatogram.*

## Introduction

Plants are the power house of secondary metabolites that act as therapeutics to prevent diseases of human beings. Plants contain many bioactive molecules that act potentially as antioxidant and antimicrobial agents. Citrus peels are rich sources of phenolic compounds and flavonoids that have antioxidant potential (Ignat, I. *et al*., 2011). Citrus crops are one of the most important producers of high vitamin C and polyphenolic content, and the fruits are among the most valuable functional diets. 98% of citrus fruits are used in industries for juice extracts and other processed products (Mahugo, S.C. *et al*., 2006). Globally it is consumed highly in the form of fresh juices and shown to lower oxidative stress-related diseases and ageing (Sharma, B. *et al*., 2006). Citrus fruit juice constitutes larger amount of byproducts and peel wastes are used for animal feeding, fuel production and source of fiber due to the presence of pectin (Ormancey, X, 2001). The inherent phytoceutics of citrus fruits peel consist of phenolics, flavonoids and are mainly used for free radical scavengers (Giampieri, F. *et al*., 2014). However, recent studies have demonstrated that the biological activities of polyphenols is not only related to their role as antioxidants but also to other pathways involved in cellular metabolism and cellular survival (Mulero, J. *et al*., 2012). Flavonoids are widely distributed in plants and play important roles in many biological processes and citrus fruits are rich dietary sources of flavonoids. However, there have been very few reports about the comprehensive metabolic profile and natural diversity of flavonoids in different tissues of various *Citrus* cultivars.

It has been reported that flavonoids play important roles in some physiological processes (Lo Piero, A.R, 2015) and exhibit promising pharmaceutical activity such as anti-atherogenic, anti-inflammatory, antitumor and antioxidant activities and

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inhibitory activity of flavonoids against blood clots (Barreca, D. *et al*., 2011; Garcia-Salas, P. *et al*., 2013). Currently, flavonoids are the main source of human beings in dietary intake, and flavonoids are present in most edible fruits (Nogata, Y, *et al*., 2006). Citrus peel waste is also considered as a power source of antioxidants (Gattuso, G. *et al*., 2014). In recent years, the interest of phenolic compounds in fruits has been increasing in the scientific community. However, to the best of our knowledge, study on the flavonoids in *C.aurantium and C.medica* fruit peel has not been reported yet. Hence, the purpose of the current study is to investigate the flavonoids from *C medica* and *C. aurantium* fruit peel using HPLC techniques.

## Materials and Methods

### Sample Preparation

*C.aurantium and C.medica* fruit peel parts are used for the extraction and characterization of bioactive compounds. In the present work, fruit peel parts of wild *C. aurantium* and *C.medica* are collected in Thanjavur district, Tamilnadu and are shredded into small pieces and dried in air dryer at 60±5°C for 6-8 hours. Then dried peel is ground into fine powder and stored in polyethylene bags to avoid rehydration. The dried powder is then extracted with methanol for 24 h at room temperature (20–25 °C), with slow shaking. The methanolic extract is then filtered using Whatman No.4 filter paper and dried under vacuum at 40°C using a rotary evaporator. The extract is stored for a maximum of 2 weeks.

### HPLC Analysis

Separation and quantification of the various components in the ethanolic (70% v/v) extract is carried out using a reversed-phase high performance liquid Chromatographic system with the UV detector. The size of column 4.6 × 250 mm consisting of silica particles of diameter 5 µm and modified with alkyl chain having 18 Carbons ($C_{18}$)(Grace) as the stationary phase. In pair formation agent (0.1 % trifluoroacetic acid) enhances the retention of highly charged molecules due to charge compensation. The column was thermo stated at ambient temperature. Samples size of 20 µl is injected with flow rate 0.5 ml/min. The initial mobile phase consists of a mixture of water/acetic acid (98:2, v/v, solvent A). Aqueous acetonitrile (50:50, v/v) with 0.5 % (vol.) acetic acid additive is used as solvent B. Following gradient is used for elution: 10 % of B at 0 min, 55 % of B at 50 min, 100 % f B at 60 min and 10 % of B at 65 min. A wavelength of 280 nm is used for the detection of gallic acid, chlorgenic acid, catechin, rutin, quercetin etc. as standard compounds. The identification of each compound is carried out comparing the retention time and UV-Vis spectra of the peaks with those previously obtained by the injection of standards. Each compound is quantified as mg/100g of dry sample material by using the peak area because peak area is proportional to the quantity of that compound in the sample (Wilson, K. *et al*., 2005).

## Results and Discussion

Preliminary phytochemical analysis reveals the presence of steroids, alkaloids, phenols, tannins and flavonoids in the fruit peels of *C.medica* and *C.aurantium*. At the same time, cardiac glycosides, saponins and terpenoids are absent in citrus species. The chromatogram of citrus peel extracts through HPLC analysis has recorded the presence of flavonoid compounds by peaks. The resultant peaks obtained from HPLC are compared with the standard peak area, retention time and spectral exploration. HPLC assessment of citrus peel (Fig.1and 2) proved that the highest concentration of qucertin in *C.medica* as compared to *C.aurantium*. The current findings are in line with the findings of Kim and Kim (FAO, 2016; Shen, W. *et al*., 2012). Existence of quercetin, rutin and kaempferol are found in *C.medica* fruit peel. The results of the analysis showed the presence of compounds like gallic acid, catechein, rutin, chlorgenic acid, quercetine and some unknown components which need to be identified in *C.medica* fruit peel. The present study demonstrated that the 70% MeOH extract of *C.aurantium* fruit peel indicated the presence of chlorogenic acid that has retention time of 19.2.

Phenolics are compounds of bioactivity present in plant-derived foods and beverages and it is found in the formulations of well-marketed cosmetic and pharmaceutical products (Barreca, D. *et al*., 2011; McClay, W, 2000). Furthermore, polyphenols exhibit various biological activities such as anticancer, antioxidant, antimicrobial and anti- inflammatory activities. The flavonoids in citrus peel wastes have medical impact on human health (Liu, Y.Q. *et al*., 2012). The peel of *Citrus* fruits are rich source of flavanones and many polymethoxylated flavones which are very rare in other plants. Quercetin has many biological activities such as antioxidant, antimutagenic effect, analgesic, anti-inflammatory and chlorogenic acid has the potential to be used as a new natural product for the management of diabetes.

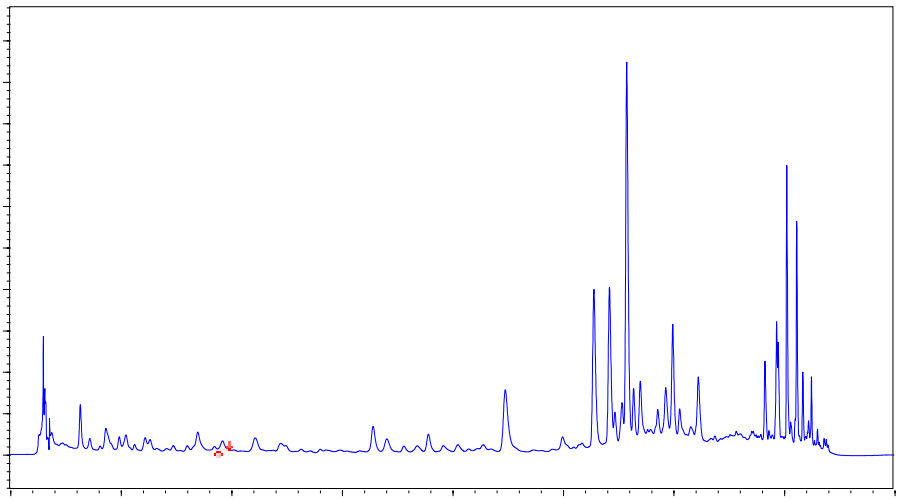

**Fig 1:** HPLC analysis of fruit peel of C.aurantium

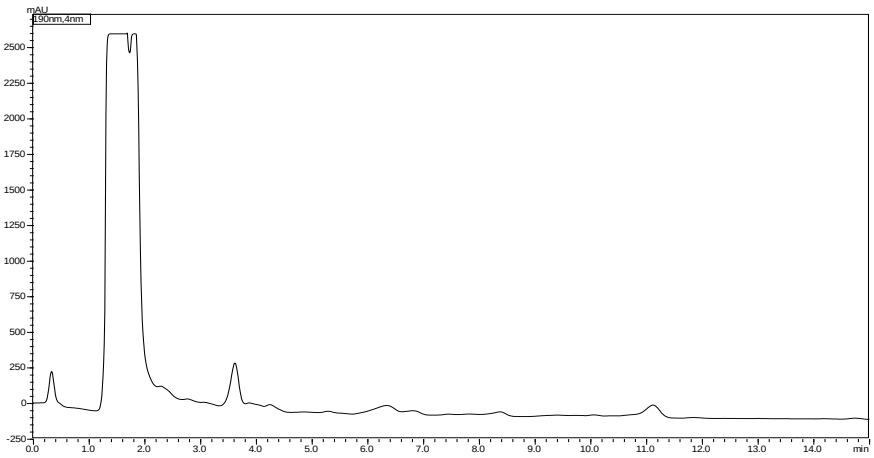


**Fig 2:** HPLC analysis of fruit peel of C. *medica*

Furthermore, citrus waste has abundant quantity of essential oils that are used in food flavors and perfumes. It is also used to prevent indigestion, cancer, constipation, nausea, sedative and cardiovascular disease in traditional medicines. Flavonoids are present in modified forms due to hydroxylation (Suryawanshi, J.A, 2011; Perumalsamy, H. *et al*., 2015), methylation (Park, H.J. *et al*., 2013) and, most importantly, glycosylation. The major flavonoids in citrus fruits are flavanone-*O*-glycosides, flavone-*O*/*C*-glycosides and their derivatives (Gattuso, G. *et al*., 2014; López, M.D. *et al*., 2010; Ghafoor, K. *et al*., 2009). Flavanones are very important citrus flavonoids, and some are responsible for citrus bitterness, such as naringin, neohesperidin, neoeriocitrin and poncirin. Various chemical compounds *viz*. alkaloids, steroids, phenols, glycosides from *Citrus medica* are known to be useful in the treatment of many diseases (Dong, X. *et al*.,

2014; Sukumar, D. *et al*., 2014). The peel extracts showed presence very important compounds especially phenolics and alkaloids which have their medicinal uses in recent years. The phenolic compounds in this plant may contribute to its antioxidant properties and thus the usefulness in herbal treatment (Kim, J.H. *et al*., 2011; Jadhav, D.R. *et al*., 2012). Hence during the preparation of some antimicrobial and antioxidant compounds phenols is found to use. The results of HPLC study suggested that the methanol extract contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. The data clearly indicated that methanol (70%), of *C.medica* showed good antioxidant activity.

In agriculture, these flavonoids are used to control insects and inhibitory effects of citrus flavonoids on starch digestion and antihyperglycemic effects in HepG2 cells (Sharma, R. *et al*., 2014; Islam, M.B, 2006). Bioefficacy of quercetin is studied against melon fruit fly. Inhibition of acetyl cholinesterase is studied by monoterpenoids and implications for pest control. Larvicidal activity and possible mode of action of four flavonoids and two fatty acids identified in *Millettia pinnata* seed toward three mosquito species. Rutin, in particular, considered to be interfered with physiological processes in the insects at the time of molting leading to delay in development (McClay, W, 2000; Harborne, B.J, 1984; Boué, S.M. *et al*., 2003). Quercetin adversely affected egg hatching and decreased significantly larval periods in the second and third instar of melon fruit fly. Flavonoids affecting insects, due to acetylcholinesterase (AChE) that is the main site of action of the flavonoids (Boué, S.M. *et al*., 2003; Hou, J. *et al*., 2011). Due to the great role of AChE in controlling impulses transmission between nerve cells, interference with this enzyme by itself will adversely affect the insects.

## Conclusion

In conclusion, based on HPLC study, methanol extract of *C.medica* fruit peel consist gallic acid, catechein, rutin, chlorgenic acid, quercetine. *C. aurantium* peel is a potential natural source of monoterpenes such as limonene, linalool, myrcene and β-pinene and has more of chlorogenic acid that are used for management of various diseases. In agriculture these compounds are used for pest and insect control and these compounds are to be isolated from *C.medica* and *C.aurantium* fruit peel. These compounds are used to produce plant-derived pharmaceutical products, medicinal foods and beverages that have well-marketed nutritional value.

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