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Article

Phytochemical analysis and secondary metabolite characterization of different extracts of Citrus fruits peel

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Abstract

Plants play a crucial role in human life, providing food, medicine, and industrial value. Citrus maxima, a member of the Rutaceae family, are traditionally used for its medicinal properties, especially its peel, which is rich in flavonoids such as Naringin and Naringenin. This study aimed to evaluate the phytochemical profile and antioxidant potential of C. maxima peel extracts compared to Carnation and C. sinensis. Peels were collected, dried, and subjected to Soxhlet extraction using methanol. Phytochemical screening, DPPH radical scavenging assay, TLC, HPLC, and LC-MS were performed to identify and quantify flavonoids. Methanolic extracts of C. maxima showed a high presence of flavonoids, particularly Naringin and Naringenin. DPPH assay confirmed significant antioxidant activity. TLC and chromatographic techniques confirmed the presence and concentration of bioactive flavonoids. C. maxima peel is a potent source of natural antioxidants, particularly flavonoids, and holds promise for pharmaceutical and nutraceutical applications. Utilizing the peels can also reduce environmental waste.

Keywords*: Citrus fruit, phytochemicals, secondary metabolite, liquid chromatography, fruit peel*

1. Introduction

Citrus fruits are well-known not just for their refreshing taste but also for their health benefits, thanks to the wide range of natural compounds they contain. While most of us enjoy the pulp, the peels are often overlooked and discarded—even though they are rich in valuable phytochemicals like flavonoids. These compounds have attracted attention for their antioxidant, anti-inflammatory, and potential disease-preventing properties.

Among citrus varieties, *Citrus maxima* (commonly known as pomelo) stands out due to its traditional use in herbal remedies and its high content of bioactive substances. Despite this, its peel remains underutilized compared to other citrus species such as *Citrus aurantium* and *Citrus sinensis*. This raised an important question: Could the peel of *C. maxima* actually be a richer source of beneficial compounds?

This study was undertaken to explore that possibility. The goal is to compare the antioxidant activity and flavonoid content of *C. maxima* with that of other citrus fruits. The underlying idea is that *C. maxima* peel may offer superior health-promoting properties, and recognizing this could encourage its use in nutraceutical or pharmaceutical products—turning what is often waste into something valuable.

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2. Materials and methods

Silica gel-G purchased from Himedia, Gypsum ($CaSO_4$), Methanol, Hexane, Chloroform. All reagents and chemicals were of analytical grade. Methanol : Chloroform : Hexane were used in the ratio 7:2:1 Naringin and Naringenin were taken from Sigma- Aldrich, USA. LC-MS grade and ultrapure Formic acid and Acetonitrile were used. Naringin and Naringenin were taken from Sigma- Aldrich,USA. HPLC- grade and ultrapure Orthophosphoric acid and Acetonitrile were employed.

2.1 Collection of plant material

Healthy *C. maxima* fruits were obtained from forests of Shimoga and Chikkamagalur districts of Karnataka. The fruits were cleaned using tap water followed by rinsing with distilled water. Further, then peel were separated from the pulp of the fruits. The peels were cut and dried in sunlight for 3-4days. The dried samples were powdered using mortar and pestle and electric blender. The powder was sieved through sieve plate and the resultant powder was stored in air tight boxes. This was used for further analysis.

2.2 Preparation of sample

Soxhlet extract of *Citrus maxima* peel was obtained by using Methanol as solvent. For the preparation of this methanolic extract, 15 g of dried citrus maxima peel was taken. 80% methanol was prepared by mixing 160ml methanol and 40ml distilled water to make up to 200ml. Soxhlet was carried for 2 hours at 70°C. The resultant extract was cooled and stored in a glass vial at 4°C for further analysis (Lin etal. 1999).

2.3 DPPH radical scavenging assay

The antioxidants found in the natural compounds and plant extracts can be analyzed by using DPPH radical scavenging assay. DPPH is a stable free radical at RT and purple in color. The reduction capacity of DPPH to accept an electron or a hydrogen from antioxidants is estimated by quantifying decrease in its absorbance at 517 nm.

2.4 Estimation of Total Flavonoid Content (TFC)

The total flavonoid content of peels of *C. aurantium, C. maxima* and *C. sinensis* using methanol, Ethyl acetate, Hexane and aqueous extracts, were examined by Aluminum chloride colorimetric method. Samples were analysed in triplicates. The results were expressed as equivalents of Quercetin as reference using standard curve (Pallab *et al.*, 2011).

2.5 Thin layer chromatography

A thin-layer chromatography apparatus was used for the preparation of silica gel thin layers on 20 x 20cm glass plates and TLC performed in 24 x 8cm glass jars. The slurry for TLC plate was prepared by mixing the silica gel G with a classical binder gypsum. 10% of gypsum was used for preparing slurry. 30g of Silica gel was mixed with 60ml distilled water and 10% gypsum. Constant shaking for 5-10 min is necessary to avoid clumps. The resultant slurry

was immediately to coated to clean glass plates of 20X20 using an applicator to make a layer of 0.25mm thickness. Then the plates were dried at RT and then at 100^0 C for 2hr. Further, plates were stored in oven at RT for analysis (Mokbel and Hashinaga, 2006; Abeysinghe, Wijerathne and Dharmadasa, 2014).

For qualitative analysis, few drops of the methanolic extract of *Citrus maxima* sample were spotted on the plates above 2cm from the bottom, using thin glass capillaries. The coated plates were activated at 100°C prior to their use. The plates were developed at room temperature in a vertical separating chamber, inthe selected solvent system of Methanol : Chloroform : Hexane, in the ratio 7:2:1 by ascending technique. The plates were dried after development and the spots were perceived by holding it against a bright light. Qualitative identification is based on characteristic colors produced combined with Rf values. In TLC, the separated compounds are identified based on the mobility in a solvent; which is referred to as Retention factor (Rf) of each compound. Rf values of sample should be ideally compared to standards in TLC systems. Hence the spots obtained from TLC of *C.maxima* were compared with the Rf values of standards.

2.6 LC-MS system

Sample of 10µl was injected to the manual injector using a micro syringe. The mobile phase used was 0.1% Formic acid in Water and Acetonitrile in an isocratic mode. The column used was Acquity UPLC-Beh C18 column of 1.7µm 1.0 x 50mm. The separated compounds were then ionized. The flow rate was maintained to 0.3ml/min with a temperature of 25 ± 2°C. Concentrations of the flavonoids in were estimated based on standard curve (Gattuso *et al.*, 2007; Jose, Sunilkumar and Antony, 2014).

2.7 HPLC system

The amount of flavonoids, Naringin, and its metabolite, Naringenin was determined using isocratic reversed-phase HPLC. A solid-phase extraction method was used with an Inertsil ODS-3V column (250x4.6 mm I.D., Particle size-5 µm). 0.1% Orthophosphoric acid and acetonitrile (70:30) was the mobile phase with a flow-rate of 1 ml min^{-1}. Absorbance was analyzed at 289 nm for Naringin and Naringenin. Concentrations of the flavonoids were determined using the obtained standard curve based on peak area and concentration of the standard compounds (Aturki, Brandi and Sinibaldi, 2004; Thorat *et al.*, 2017).

3. Results

3.1. Qualitative phytochemical analysis of different extracts of Citrus Fruits (*Citrus maxima* compared with *Citrus aurantium* and *Citrus sinensis*)

The phytochemicals of *C. maxima* extracted from different solvents are shown in Table 1. The tests revealed the highest yield of flavonoids in all solvents, followed by tannins and triterpenoids. Carbohydrates are also found in considerable amounts in all the four solvents, followed by saponins that were found to be absent in hexane solvent. Carotenoids are found

in lesser quantity in methanol and ethyl acetate extracts, and absent in aqueous and hexane extracts. Coumarins are absent in methanolic extract and found lesser in other three extracts. The least amount of phytonutrient found was alkaloids, as it was found absent in all the solvents except aqueous, in which it was found in lesser amount. Key:- ++ = High amount: dark color, + =Less amount: light color, - = Not detected.

Table 1: Screening for phytochemicals in the peel of *C. maxima*

Sl. No.	Phytochemicals	Tests performed	Solvents			
			Aqueous	Methanol	Ethyl acetate	Hexane
1	Tannins	Ferric chloride	++	++	++	+
		Sodium hydroxide	++	++	+	++
2	Flavonoids	Lead acetate	++	++	+	+
3	Triterpenoids	Chloroform	++	++	-	-
4	Coumarins	Ferric chloride	+	-	+	+
5	Alkaloids	Hager's	+	-	-	-
6	Carotenoids	Chloroform	-	+	++	-
		Ninhydrin	+	-	-	-
7	Proteins and amino acids	Lead sulphide	-	-	-	-
		Biuret	-	-	-	+
8	Carbohydrates	Benedict's	++	++	++	+
9	Saponins	Foam	+	+	++	-

Phytoconstituents of *Citrus sinensis* peel extracts obtained from different solvents are exposed in Table 2. It also showed the highest yield of flavonoids in all solvents, followed by tannins and Carbohydrates. Saponins are found in all the three solvents in lesser amounts and absent in hexane. Carotenoids, Triterpenoids and proteins are found in lesser quantity in Methanol and aqueous extracts, and absent in Ethyl acetate and Hexane extracts. Coumarins are absent in all the three extracts and found lesser in Hexane extract. The least amount of phytonutrient found was alkaloid, as it was found absent in all the solvents.

Table 2: Screening for phytochemicals in the peel of *C. sinensis*

Sl. No.	Phytochemicals	Tests	Solvents			
			Aqueous	Methanol	Ethyl acetate	Hexane
1	Tannins	Ferric chloride	++	+	+	+
2	Flavonoids	Sodium hydroxide	++	++	+	++
		Lead acetate	++	-	-	+
3	Triterpenoids	Chloroform	+	+	-	-
4	Coumarins	Ferric chloride	-	-	-	+
5	Alkaloids	Hager's	-	-	-	-
6	Carotenoids	Chloroform	++	+	-	-
	Proteins and amino acids	Ninhydrin	+	+	-	-
7		Lead sulphide	-	+	-	-
		Biuret	-	-	-	+
8	Carbohydrates	Benedict's	++	++	+	+
9	Saponins	Foam	+	+	+	-

The phytochemicals in *Citrus aurantium* peel extracts obtained from different solvents are shown in Table 3. Here also the yield of flavonoids was observed in considerable amounts in all solvents, followed by tannins and Carbohydrates. Saponins are found in all the three solvents in lesser amounts and absent in Hexane. Carotenoids, and Triterpenoids are found in lesser quantity in all the three solvents, except Hexane. Coumarins are absent in all the three extracts and found lesser in hexane extract. Alkaloids were found to be absent in all the solvents.

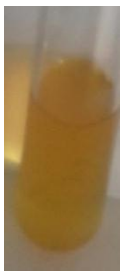
Table 3: Screening for phytochemicals in the peel of *C. aurantium*

Sl. No.	Phytochemicals	Tests	Solvents			
			Aqueous	Methanol	Ethyl acetate	Hexane
1	Tannins	Ferric chloride	++	++	+	+
2	Flavonoids	Sodium hydroxide	++	+	+	+

		Lead acetate	++	+	-	+
3	Triterpenoids	Chloroform	+	+	++	-
4	Coumarins	Ferric chloride	-	-	-	+
5	Alkaloids	Hager's	-	-	-	-
6	Carotenoids	Chloroform	-	++	+	-
	Proteins and amino acids	Ninhydrin	+	-	-	-
7		Lead sulphide	-	-	-	-
		Biuret	-	+	-	-
8	Carbohydrates	Benedict's	++	++	++	+
9	Saponins	Foam	+	+	+	-

Table: 4: Observation of tests performed in qualitative analysis of peels of three different citrus species.

Test	***C.sinensis***	***C.aurantium***	***C.maxima***	**Result**
Tannins				Dark green / dark blue colour indicates the presence of tannins.

Flavonoids NaOH Test Lead acetate Test	 	 	 	Yellow colour indicates the presence of flavonoids. Yellow precipitate indicates the presence of flavonoids.
Triterpenoids				Red brown colour at the interface indicates the presence of triterpenoids.

Protiens NinhydrinTest Lead sulphide test BiuretTest	  	  	  	Blue/violet colour indicates the presence of proteins. Black precipitate indicates the presence of amino acid. Red/ violet colour indicates the presence of proteins.
Coumarins				Yellow colour indicates the presence of coumarins.

3.2. Quantitative phytochemical analysis of extracts *of Citrus maxima, Citrus aurantium and Citrus sinensis*

3.2.1. Estimation of Total Flavonoid Content (TFC)

Fig.1 represents the standard curve for Quercetin in methanol. Table. 5. contains data for amount of flavonoid in Methanol extract of *C. aurantium, C. maxima* and *C. sinensis*.

Table 5. Total flavonoids content of methanolic extracts

Plant sample	OD value	Total flavonoid (mg/g)
C. aurantium	0.16	1.92
C. maxima	0.36	4.32
C. sinensis	0.35	4.20

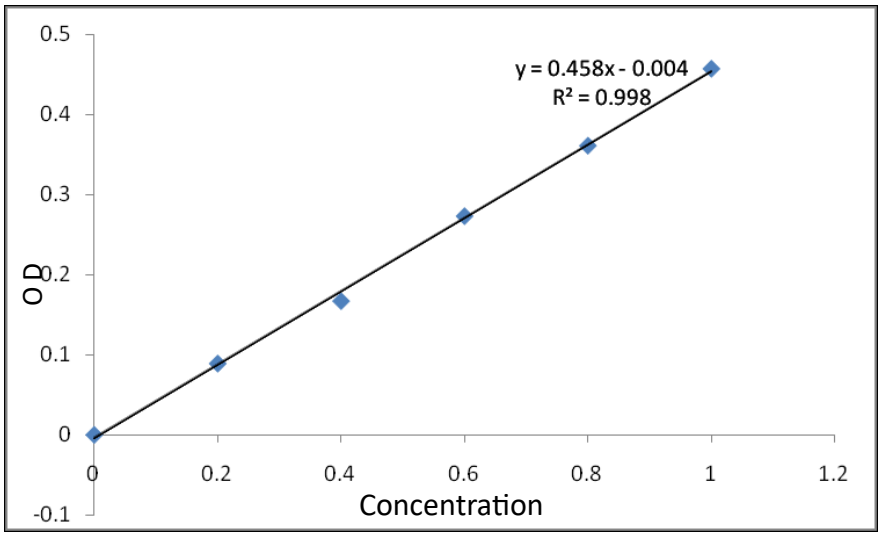


Fig.1: Calibration curve-Quercetin (Methanol extract)

The total flavonoid content of Methanolic extract of the above three different citrus fruit peel reveals that, Citrus maxima has highest amount of total flavonoids, followed by Citrus sinensis. Citrus aurantium shows the least TFC in methanolic extract of peels.

Fig.1. shows the standard curve for Quercetin in Ethyl acetate. Data for flavonoid content of the ethyl acetate extract of *C. aurantium, C. maxima* and *C. sinensis* is reported in Table.6.

Table 6: Total amount of flavonoids of Ethyl acetate extracts

Plant sample	OD value	Total flavonoid (mg/g)
C. aurantium	0.001	0.012
C. maxima	0.002	0.024
C. sinensis	0.006	0.07

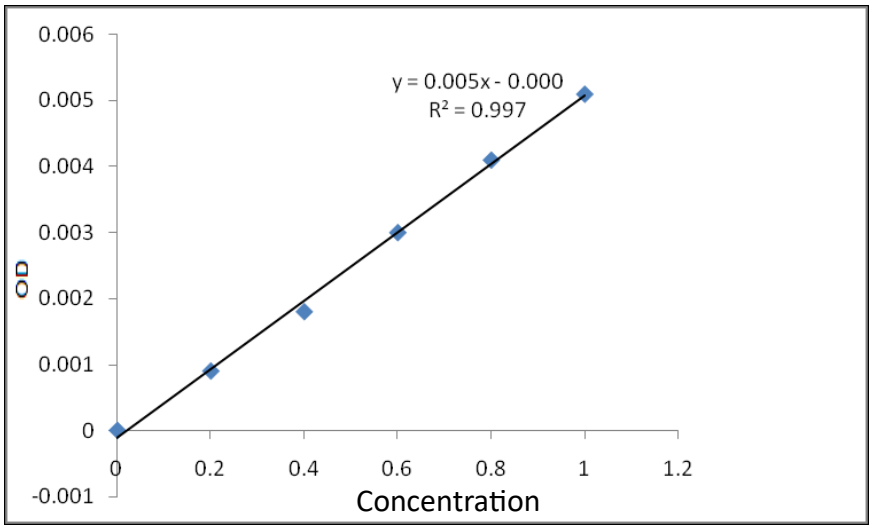


Fig 2: Calibration curve-Quercetin (Ethyl acetate)

The total flavonoid content of Ethyl acetate extract of the above three different citrus fruit peel shows that, Citrus maxima yielded highest number of total flavonoids, followed by Citrus sinensis. Citrus aurantium shows the least TFC in Ethyl acetate extract of peels.

Fig.3 and Fig.4 show the standard curve of Quercetin, for Hexane and Water respectively. Table.7 and Table.8 represents flavonoid content of the Hexane and Aqueous extracts of *C. aurantium*, *C. maxima* and *C. sinensis* respectively.

Table 7: Total amount of flavonoids in Hexane extracts of citrus fruits.

Plant sample	OD value	Total flavonoid (mg/g)
C. aurantium	0.009	0.11
C. maxima	0.02	0.24

C. sinensis	0.01	0.12

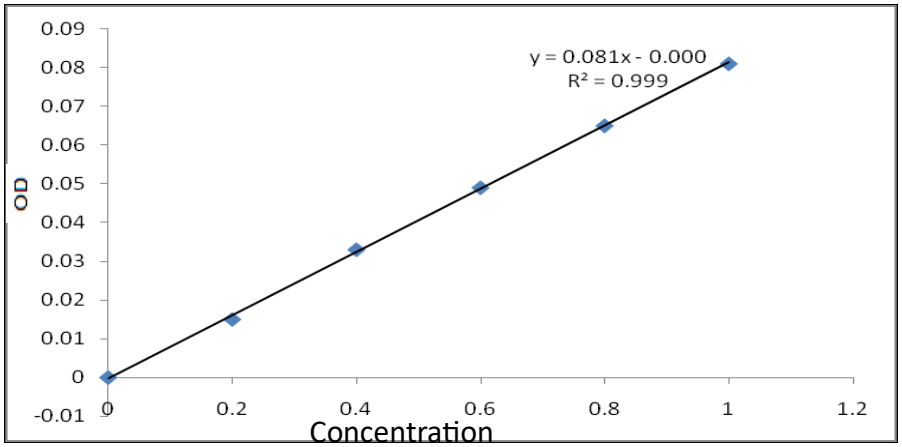


Fig 3: Calibration curve-Quercetin (Hexane extract).

Table 8: Total amount of flavonoids in aqueous extracts of *C. aurantium*, *C. maxima* and *C. sinensis*

Plant sample	**OD value**	**Total flavonoid (mg/g)**
C. aurantium	0.21	2.5
C. maxima	0.23	2.76
C. sinensis	0.20	2.4

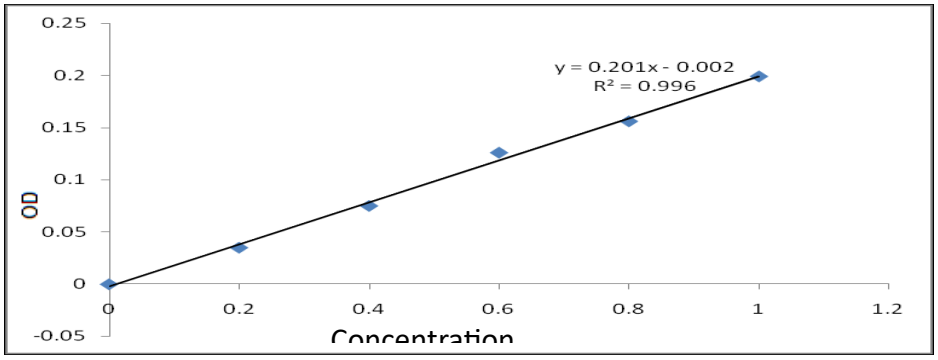


Fig 4: Calibration curve-Quercetin (Aqueous extract)

The total flavonoid content of Hexane extract of the above three different citrus fruit peel shows that, Citrus maxima yielded highest number of total flavonoids, followed by Citrus sinensis. Citrus aurantium shows the least TFC in Hexane extract of peels. Similarly, the aqueous extract of Citrus maxima fruit peel shows highest flavonoid content. But that of C. sinensis showed least flavonoid content when compared to C. aurantium and C. maxima in aqueous extract. Among all the four solvents, C. aurantium showed good amount of flavonoid content only in aqueous extract, whereas for C. sinensis and C. maxima methanol was found to be a better solvent to explore total flavonoid content in the peels.

In overall, the estimation of total amount of flavonoid in the peels of *C. aurantium, C. maxima* and *C. sinensis* using solvents like Methanol, Hexane, Ethyl acetate, and aqueous extracts, revealed the highest concentration of flavonoids in methanolic extract of peels of *C. maxima*. The above values indicates that the peels of *Citrus sinensis* showed less amounts of flavonoids followed *Citrus aurantium* with least amounts of flavonoids.

3.2.2. DPPH radical scavenging assay

The free radical scavenging effect of crude methanolic extracts peels of *Citrus aurantium, Citrus maxima* and *Citrus sinensis* were determined using the DPPH radical scavenging method (Mokbel and Hashinaga, 2006). The scavenging effect of different concentrations of peel extracts on DPPH free radicals was assessed in reference to gallic acid standard. Fig.5 shows the DPPH radical scavenging activity of the above three different citrus peel extracts in comparison with the standard Gallic acid curve. The percentage inhibition of DPPH free radical by peel extracts of *C. aurantium, C. maxima* and *C. sinensis* is shown in Table. 9.

Table 9: DPPH Radical Scavenging Activity of different citrus species and standard Gallic acid

Concentration in µM	**Percentage Inhibition**			
	Gallic acid	*C.maxima*	*C.aurantium*	*C.sinensis*
0.2	5.7	4.4	2.6	0.6
0.4	10	8.8	3.3	3
0.4	10	8.8	3.3	3
0.6	15.7	13.3	4.8	4.6

0.8	18.5	17.7	9	7.3
1	27.1	24.4	13.4	10.1

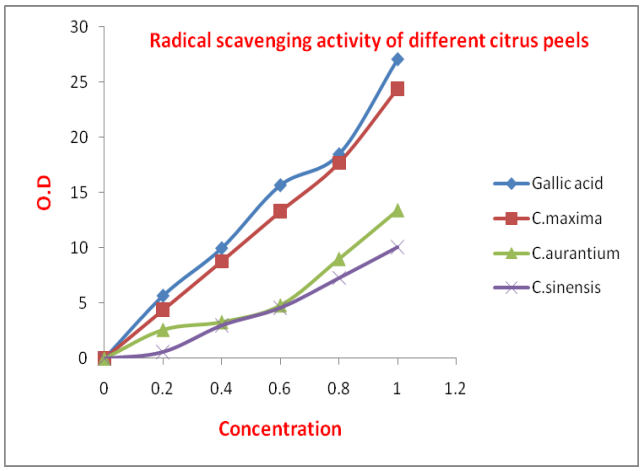


Fig. 5: DPPH radical scavenging activity of peels of Citrus aurantium, Citrus maxima and Citrus sinensis with respect to standard Gallic acid

3.3.Thin layer chromatography for flavonoids.

TLC of methanolic peel extracts of *Citrus maxima* showed the following results. TLC of methanolic extract of *Citrus maxima* peel is shown in Fig. 6. TLCplate showed the presence of two major spots. One with Orange coloured and anotherwith yellow coloured.

Fig: 6. TLC of *Citrus maxima* peel extract

These two colored spots were keenly observed. Their Rf values were determined by measur-

ing the distance traveled by solvent and distance traveled by the solute. The Rf values were found to be 0.31 and 0.61. It was compared with Rf values of standard flavonoids for the confirmation of flavonoid compound in the sample. TheRf values of standard flavonoids is given in Table. 10.

Table: 10. Rf values of standard flavonoids.

Compound	**Rf value**	**Colour**
Naringin	0.62	Orange
Prunin	0.58	Orange
Naringenin	0.3	Orange
Rutin	0.55	Yellow
Isoquercetin	0.53	Yellow
Quercetin	0.87	Yellow

(**Source**: Rodge, 2015)

3.4 .LC-MS analysis of *C.maxima* fruit peel

Liquid Chromatography Mass Spectrophotometric Analysis (LCMS) allowed the determination of the major flavonones in *Citrus maxima* peel extract in methanol.The results yielded by LC-MS analysis is given in the Fig.7.

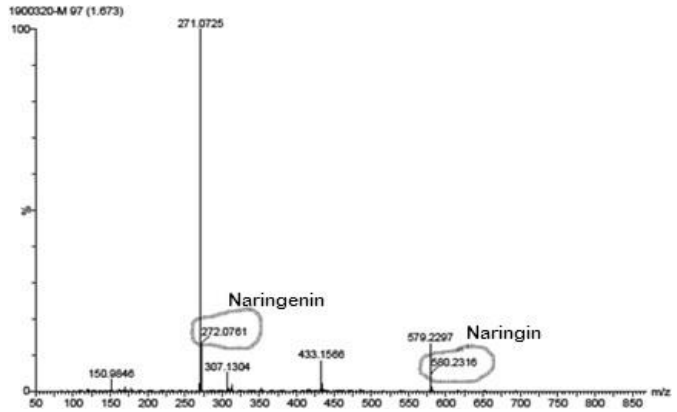


Fig 7. LCMS analysis of Methanolic extract of *C. maxima* peel extract.

The LC-MS analysis of Methanolic extract of *C. maxima* fruit peel indicated the presence of two flavonoids, Naringin and Naringenin. The molecular weight of Naringin was 580, and that of Naringenin was found to be 272 from literature. The peaks of these two molecular weights were found in the LCMS chromatogram of *C. maxima* peel.

3.5. HPLC analysis of flavonoids in *C. maxima* fruit peel

Since *C. maxima* is known to possess a range of flavonoids, their variety was examined using HPLC. HPLC analysis of methanolic extract of *Citrus maxima* revealed the following results.

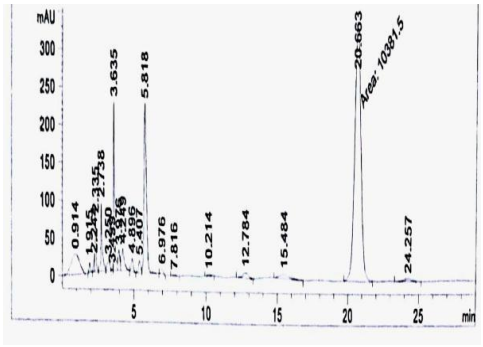


Fig.8. Chromatogram of Naringenin

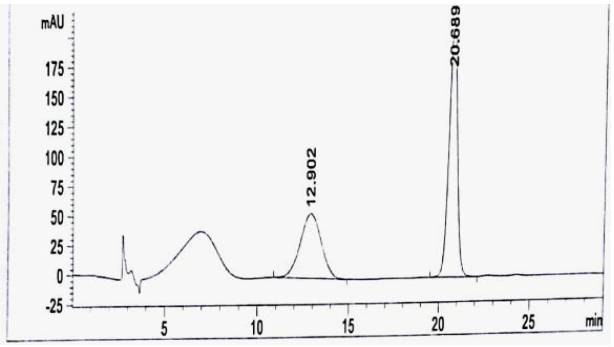


Fig.9. Chromatogram of standard Naringenin

The chromatogram of standard Naringenin is compared with the chromatogram of sample. It showed the presence of Naringenin in *C.maxima* peel with the same peak value. Similarly when chromatogram of standard Naringin is compared with the chromatogram of sample, it also exposed the presence of Naringin in the sample with the same peak value. The peak area of both satandards and that of Naringin and Naringenin is calculated to get the percentage yield of these two bioactive flavonones in Methanolic extract of *C.maxima* peel.

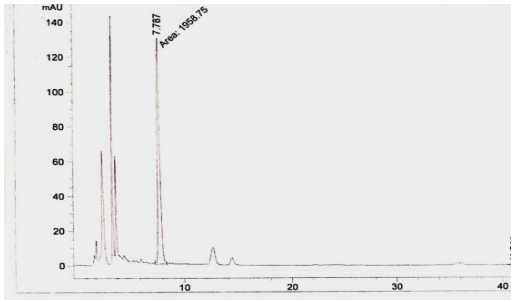


Fig. 10. Chromatogram of Naringin

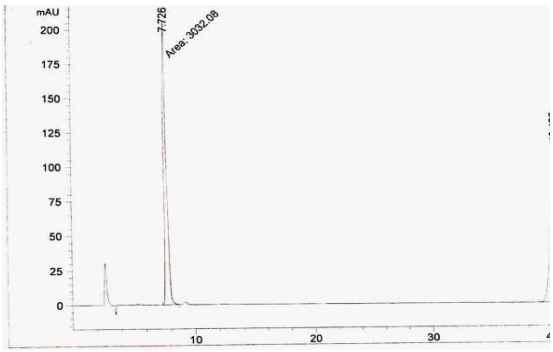


Fig. 11. Chromatogram of standard Naringin

4.Discussion

Phytochemical screening of extracts of the peels of *Citrus maxima*, *Citrus aurantium* and *Citrus sinensis* using distinct solvents yielded variable results. There were differences in intrinsic phytochemicals in the extracts obtained in various solvents. By considering the results from Table. 1, it is clear that the flavonoids showed highest yield of in all solvents, followed

by tannins and triterpenoids. Carbohydrates are also found in considerable amounts in all the four solvents, followed by saponins that were found to be absent in Hexane solvent. Carotenoids are found in lesser quantity in Methanol and ethyl acetate extracts, and absent in aqueous and hexane extracts. Coumarins are absent in Methanolic extract and found lesser in other three extracts. The least amount of phytonutrient found was alkaloids, as it was found absent in all the solvents except aqueous, in which it was found in lesser amount. Overall, it is evident that the phytochemical metabolites of peel extracts of *C. maxima* showed the maximum yield of flavonoids, in methanolic extract and followed by aqueous extract. Tannin was more in aqueous, Methanolic and Ethyl acetate extracts and temperately in hexane extract. According to Akiyama et al., (2001), tannins, are known to have antibacterial, antitumor and antiviral activities. Kumari and Jain, (2012), reported that, they work by precipitating microbial protein and thus making nutritional protein unavailable for them.

In overall the total flavonoid content could be deduced as:

Methanolic extracts: Citrus maxima > Citrus sinensis > Citrus aurantium

Ethyl acetate extracts: Citrus maxima > Citrus sinensis > Citrus aurantium

Aqueous extracts: Citrus maxima > Citrus aurantium > Citrus sinensis

Hexane extracts: Citrus maxima > Citrus sinensis > Citrus aurantium

The total flavonoid amount was efficient and preferable from methanol extract in *C. maxima*. This result is in accordance with the results of Sambandam et al., (2016), which says, that the Hexane and Ethyl acetate plant extracts revealed about one fold of TFC and the Methanolic extracts showed more than three folds of TFC with respect to absorbance with flavonoid Quercetin. By observing the above results, conclusion can be drawn that the Methanolic extract of Citrus maxima peel showed highest total flavonoid content when compared to the different extracts of Citrus aurantium and Citrus sinensis. In addition, the above results narrows the selection of solvents from broad spectrum to single one, i.e Methanol for further studies. Hence among the four solvents chosen for estimations, Methanol is selected for further quantitative estimation of Citrus maxima, Citrus aurantium and Citrus sinensis species.

DPPH radical scavenging activity of Methanolic extract of peels of Citrus aurantium, Citrus maxima and Citrus sinensis were compared with standard Gallic acid. The standard antioxidant had higher scavenging activity in all concentrations than the extracts. But the peels of *C. maxima* still showed good free radical scavenging activity with a maximum inhibition of about 24.4% at a concentration of 100µg/ml. The scavenging activity of Citrus aurantium peels is also satisfactory, that is about 13.4%. But peels of Citrus sinensis showed lesser scavenging activity of about 10.1%, when compared to the other two former extracts. The higher scavenging property of *C. maxima* may be one of the rationales why this fruit is effective as a traditional medicine. The consumption of Citrus maxima fruit peel can be beneficial in preventing oxidative stress related degenerative diseases (Fatma et al., 2001). Hence the

result can be drawn as the methanolic extract of Citrus maxima peel showed highest antioxidant activity when compared to the extracts of Citrus aurantium and Citrus sinensis.

Thin Layer Chromatography of *Citrus maxima* showed the rough presence of Naringin and Naringenin, their diversity was further examined by means of LCMS analysis. LCMS analysis enabled the determination of the flavonones in callus of *C.maxima* peel extract in methanol. The results yielded by LC-MS analysis shows the presence of two flavonones, Naringin and Naringenin. The molecular weight of Naringin is 580 and that of Naringenin is 272. The sample showed the presence of molecules with the above molecular weights, indicating their presence. Since the resultant peaks in LCMS analysis showed the molecular weights of Naringin and Naringenin, it could be proposed that the flavonoids found in *C. maxima* were the above two flavonones. Further this was confirmed by HPLC analysis of *C. maxima peel* extract by comparing with the chromatogram of Naringin and Naringenin standards to come to a proper conclusion.

The quantification of Naringin and Naringenin was done using HPLC (Isocratic reversed-phase). This is based on retention time of molecules and their comparison with standards. Fig.8 and 9 displays the chromatogram of Naringin and Naringenin in Fig.10 and 11 along with standard and respective retention time. The amount of Naringin in peels of *C. maxima* was 2.36% and that of Naringenin was 3.40%. Naringenin was found in highest level in *C. maxima* peel when compared to Naringin. This is in linewith the studies of Ramful et al., (2010), proving the presence of Naringin, and Naringenin in Mandarin fruit (Ramful *et al.*, 2010). But several reports highlight the absence of these flavoinoids in some species of citrus fruits (Clifford and Toma, 2000). Overall, here we conclude good levels of Naringin and Naringenin in Methanolic extracts of *C. maxima* peels.

Here, the flavonoids in the peels of three citrus fruits were studied and clear variety was observed. The quantitative analysis highlights the presence of good amounts of flavonoids in *Citrus maxima* peel extracts in comparison with the other two species. Thus, the present study reveals that, the citrus fruit peels possess useful biological products. The extracting methods used in this study showed that these bioactive molecules are more profuse in the fruit peels of Citrus maxima revealing their great industrial potential. The combination of techniques used in this study confirms that methanol is the most effective solvent for extracting flavonoids from the citrus peels.

Overall, this study provides information regarding the *Citrus maxima* fruit peel as a good source of flavonoids. They can be used as source of raw materials for conventional medicine, food supplements or production of drugs. Hence the above result narrows the selection of peels of Citrus maxima for further studies using methanol as solvent. Additional studies on effective mechanisms and evaluation with pure compounds are highly recommended for the definite conclusion.

5. Conclusions

Fruits are a rich source of polyphenolic molecules, especially flavonoids. Plant flavonoids are drawing interest as they have significant bioactivities. The work of Pandey and Rizvi (2009) and Vessal et al., (2003), reports the health benefits of flavonoids, including antioxidant, antithrombotic, antidiabetic, anticancer, and vasodilatory activities (Pandey and Rizvi, 2009). Recent research has shown that the consumption of plant flavonoids may provide protection against cardiovascular diseases (Knekt *et al.*, 2002). According to Xu et al., (2008), the most important flavanoids of pomelo are neohesperidin, hesperidin, naringenin, and naringin (Xu *et al.*, 2008). Therefore, it is important to investigate the flavonoid content and bioactivity of pomelo.

The partial purification of flavonoids by using Thin Layer Chromatography, roughly indicated the presence of the two flavonones, Naringin and Naringenin in the methanolic extract of *Citrus maxima* fruit peel. By taking this into consideration, the present study was concentrated on confirmation of the identified flavonones by LC- MS method. LC-MS analysis explored the presence of Naringin and Naringenin by showing the peaks of their respective molecular weights. This was followed by the quantification of these two flavonones using HPLC analysis, by comparing with the Naringin and Naringenin standards. Quantification of the above flavonones revealed the highest percentage of Naringenin in *C.maxima* peel. Naringin was also found to be in good amounts in methanolic peel extracts of *C.maxima.*

By considering the above results, it is clear that the *Citrus maxima* peel is a very good source of flavonoids, especially Naringin and Naringenin. Hene the present study was focused on *Citrus maxima* peel to be used as source material for further tissue culture techniques to enhance the production of the above two flavonones from the resultant callus obtained from *in vitro* culture of *Citrus maxima* fruit peels.

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