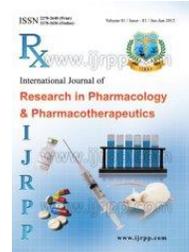




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In vitro studies on phytochemical analysis and antioxidant activity of *citrus maxima*

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ABSTRACT

Citrus fruits are rich source of vitamin-C (Ascorbic acid). In the present study the antioxidant activities of the pulp of some of the citrus fruits has been investigated. The results indicate that the aqueous extracts from citrus fruit pulp contain significant antioxidant activity. In vitro antioxidant activity was determined by various procedures and it has been determined that aqueous, methanol and petroleum ether fraction of pulps of citrus fruits possessed maximum antioxidant activity in reference to standard antioxidant. The study thus revealed that pulp of *Citrus maxima* fruits are useful for consumption and are beneficial for health. This study may thus lead to the phytochemical analysis and antioxidant activity of *citrus maxima*. They can be used as a potent natural antioxidant additive or food products and as a dietary supplement.

Keywords: Citrus fruits, phytochemical analysis, antioxidant activity, aqueous extract, methanol extract and petroleum ether extract.

INTRODUCTION

Citrus fruits are rich source of active compounds and beneficial for human health e.g. vitamin C, carotenoids, flavonoids, limonoids, essential oils, acridone alkaloids, minerals and vitamin B complex. Flavonoids especially polymethoxy flavones, flavanone glycosides and limonoids are natural secondary metabolite compounds of citrus [1].

Antioxidants are the substances that inhibit oxidation of the substrate even when present in low concentration as compared to oxidisable substrate.

Nutritional value and pharmacological properties of different parts of indigenous fruit of *C. maxima* have been greatly reported, but there has been very little information on antioxidant and medicinal properties Of juices [2]

In recent years, more attention had been paid on phenolic compounds from pomelo fruit, and some publications have suggested they might play an important role on the antioxidant capacity of pomelo fruit juice [3]. Hence, the present study attempts to

investigate the total phytochemicals and antioxidant activity of fresh juice from the fruit of *C. maxima*

MATERIALS AND METHODS

The material and methods pertaining to the study of “*In vitro* studies on phytochemical analysis and antioxidant activity of *Citrus maxima*” is presented under the following headings,

Collection of plant materials

The pomelo fruits were obtained from a local supermarket of Mayiladuthurai, Nagai District, Tamil Nadu, South India. The mature (140 - 160 days from fruit set and light green to yellow color) and medium size classification (651 - 900 g) of the pomelo was used.

Preparation of fruit juice

About 1kg of pomelo fruit was taken. The peel of the fruit and the pulps were collected. Those pulps were mashed by grinding method and the fruit juice was extracted.

Preparation of fruit extract

About 50ml of the fresh fruit juice of pomelo fruit exhaustively extracted in 50ml of 60% aqueous, methanol, Petroleum ether, the resultant extract were boiled in a water bath until a syrupy consistency was obtained.

Preliminary phytochemical screening

Qualitative phytochemical examinations were carried out for all the extract as per the standard methods (4).

RESULTS AND DISCUSSION

The present investigation was carried out to screen the phytochemical and antioxidant activity of *Citrus maxima*

Antioxidant screening

DPPH RADICAL SCAVENGING ACTIVITY

Briefly a 2 ml aliquot of DPPH (2, 2-diphenyl picrylhydrazyl), 1 ml methanol solution (25µg/mL) was added to 0.5ml sample solution at different concentration. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance was measured at 517 nm in a spectrophotometer. Lower absorbance of the reaction mixture indicate higher free radical scavenging activity [5].

TOTAL ANTIOXIDANT CAPACITY

The total antioxidant capacity was evaluated by the phosphor molybdenum method. To 1ml of extract of different concentrations was treated with 1 ml of reagent solution (0.6mm sulphuric acid, 28mm sodium phosphate and 4mm ammonium molybdate) in eppendeff tube. Capped tubes were incubated in thermal block at 95° C for 90mins. After cooling to room temperature the absorption was measured at 695nm against blank. The activity was compared with ascorbic acid as standard [6].

HYDROGEN PEROXIDE SCAVENGING ACTIVITY

Scavenging activity of extract was evaluated by hydrogen peroxide. 1ml of sample was mixed with 3ml of phosphate buffer 1 ml of H₂O₂ and incubated for 10mins at 37°C after incubation the absorbance value of the reaction mixture was recorded at 230 nm. ascorbic acid used as standard [7].

Table 1: Phytochemical screening of *Citrus maxima*

S.No	Name of the test	Aqueous	Methanol	Petroleum Ether
1	<i>Steroids</i>	+	+	+
2	<i>Phenols</i>	+	+	+
3	<i>Resins</i>	-	-	-
4	<i>Tannins</i>	+	+	+
5	<i>Flovanoids</i>	+	+	+
6	<i>Alkaloids</i>	+	+	+
7	<i>Glycosides</i>	+	+	+

8	Terpenoids	-	-	-
9	Saponins	+	+	+
10	Cardiac glycosides	+	-	-
11	Coumarins	-	-	-
12	Quinine	-	-	-
13	Xanthoprotein	-	-	-
14	Phlobatannins	-	-	-
15	Carbohydrate	+	+	+
16	Anthraquinones	-	-	-
17	Amino acid	+	+	-
18	Protein	-	-	-

(+)= Indicates Presence

(-)= Indicates Absence

Citrus fruits contain a wide variety of phytochemicals. The biological action of these flavonoids is possibly linked to their interactions with key regulatory enzymes involved in cell activation and receptor binding. Qualitative phytochemical analysis for alkaloids, carbohydrate, tannins, phenols, saponins, proteins are screened in methanolic, aqueous, petroleum ether extract of *Citrus maxima* by their respective chemical tests.

The screening of aqueous, methanolic and petroleum ether extract indicates the presence of steroids, phenols, alkaloids, glycosides, flavonoids, saponins, cardiac glycosides, carbohydrate, amino acid and absence of resins, terpenoids, coumarins, quinine, xanthoprotein, phlobatannins, anthraquinones, protein. Amino acid is absent in petroleum ether extract and cardiac glycoside is absent in methanol and petroleum ether extract of fruit juice of *Citrus maxima* is given in table 1.

From the above results, the phytochemical analysis of Methanolic and Aqueous extracts showed more

phytonutrients than Petroleum ether extract. The phytochemical analysis of *Citrus maxima* showed the presence of phyto constituents like alkaloids, saponins and carbohydrates. This indicates that this fruit can be useful for treating different diseases because the therapeutic activity of a plant is due to the presence of particular class of compounds [8].

Phytonutrients are mainly natural bioactive compounds from plants with general benefits to human health. Citrus plants synthesize and accumulate in their cells a great variety of phytochemicals including low molecular phenolic, acetophenones, terpenoids, flavanoids, stilbenes and condensed tannins [9].

The majority of these beneficial effects are at least in part due to the presence of phytochemicals in vegetables and fruits. In this context phytochemicals may be defined as "non nutrient" chemicals found in plants that have biological activity against chronic diseases [10].

Table 2 : Hydrogen peroxide scavenging activity of *Citrus maxima*

S. No	Extracts	H ₂ O ₂ (%)
1.	Aqueous	20.14±2.02
2.	Petroleum ether	52.23±1.99
3.	Methanol	47.58±1.08

Figure 1

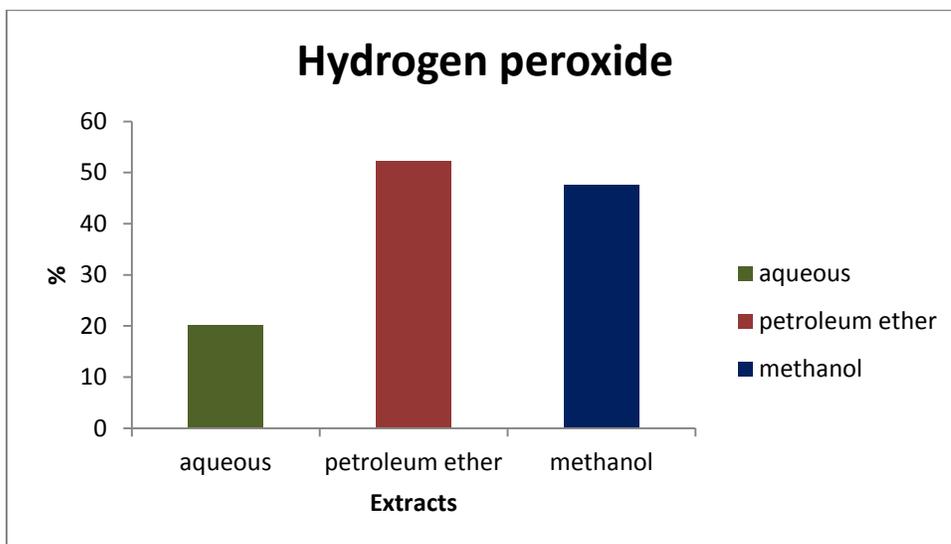


Figure.1 and **Table 2** shows the hydrogen peroxide scavenging activity of *Citrus maxima* in comparison with Ascorbic acid standard. It shows the Hydrogen peroxide scavenging activity of *Citrus maxima* which is increased in petroleum ether when compared to methanol and aqueous extract.

Hydrogen peroxide is weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can probably react with Fe^{2+} possibly Cu^{2+} ions to form hydroxyl radicals and this may be the origin of many of its oxide effects. From the results, it appeared that H_2O_2 scavenging activity of the plant extract is

significant compared to that of the standard ascorbic acid.

H_2O_2 is highly important because of its ability to penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells. The results showed that ethanol extract of *Citrus maxima* had an effective H_2O_2 scavenging activity. Hydrogen peroxide absorbs the ultraviolet radiation at 230 nm. The addition of scavenger containing extract to the H_2O_2 solution caused a fast decrease in the concentration of hydrogen peroxide, which is monitored at 230nm. Hydrogen peroxide scavenging ability was increased on increasing concentration of the extract [11].

Table 3: DPPH activity of *Citrus maxima*

S. No	Extracts	DPPH (%)
1.	Aqueous	15.25±1.08
2.	Petroleum ether	0.14±1.88
3.	Methanol	54.62±1.9

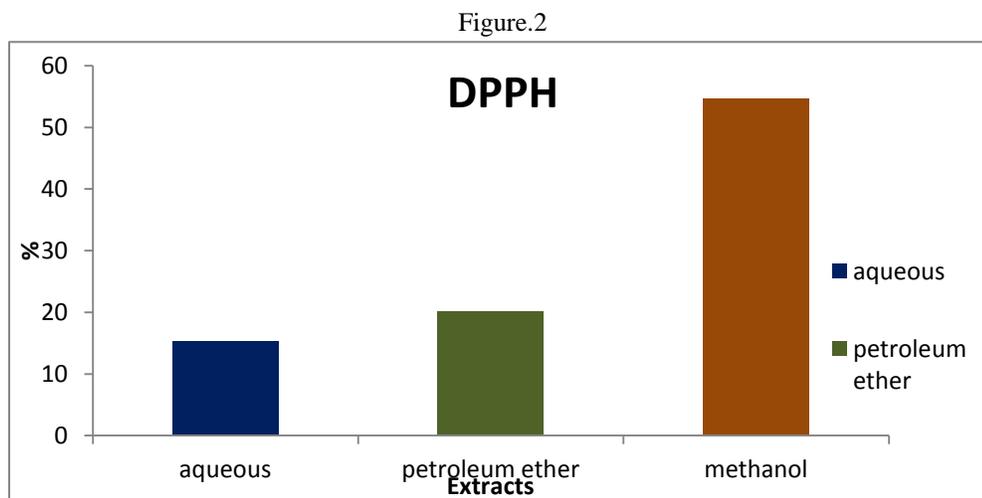


Figure 2 and **Table 3** show the DPPH activity of *citrus maxima* in comparison with Ascorbic acid standard. It indicates the level of DPPH activity of *Citrus maxima* which is increased in methanolic extract when compared to aqueous and petroleum ether extracts.

DPPH is a stable free radical compound with a characteristic absorption at a wavelength of 517 nm.

Antioxidants upon interaction with DPPH either transfer an electron or hydrogen atom to DPPH, thus neutralizing its free radical character. The colour of the reaction mixture changes from purple to yellow resulting in an absorbance decrease. The degree of discoloration indicates the scavenging potential of the antioxidants [12].

Table 4: Total antioxidant activity of *Citrus maxima*

S. No	Extracts	Total antioxidant (%)
1.	Aqueous	27.78±1.08
2.	Petroleum ether	38.62±0.96
3.	Methanol	35.24±0.89

Figure.3

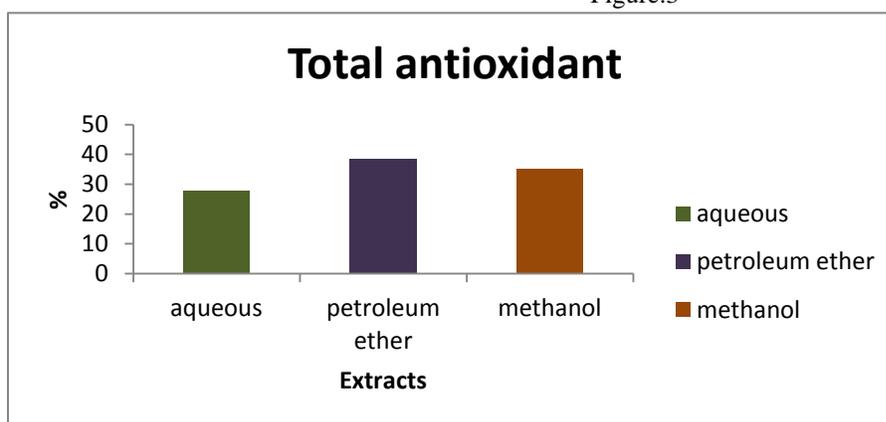


Figure3 and **Table 4** indicate the total antioxidant activity of *Citrus maxima* in comparison with standard of Ascorbic acid. It shows that the total antioxidant activity of *Citrus maxima* which is

moderately increased in petroleum ether and methanolic extracts than aqueous extracts.

The phosphomolybdenum method used for total antioxidant activity is based on the reduction of Mo

(VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximum absorption at 695 nm. The antioxidant activity is determined by the regression equation of calibration curve and expressed as ascorbic acid equivalents (AAE). Among both extract of the plants, the petroleum ether extract of *Citrus maxima* showed the highest antioxidant activity. The extracts of *Citrus maxima* were found to possess considerable antioxidant capacity. The antioxidant activity of plant extracts may be due to their phenolics and flavonoid contents [13].

CONCLUSION

The following investigations were done in this study. The determination of Hydrogen peroxide radical scavenging activity, DPPH activity, total antioxidant activity of *Citrus maxima* shows elevated level of antioxidant activity due to the presence of

phytochemicals such as steroids, phenol, tannins, flavonoids, alkaloids, glycosides, saponins, cardiac glycosides, carbohydrate in aqueous, methanol and petroleum ether extract.

From the present study it can be concluded that all the Citrus fruits are rich source of various nutrients and can be helpful in fighting against malnutrition problem in developing countries. *Citrus maxima* contain different type of phytochemicals and higher activity of antioxidant. Most of the biologically active phytochemicals were present in the methanolic extract, petroleum ether extract and aqueous extract of *Citrus maxima* fruit juice. *C. maxima* is a good source of vitamin C and phenolic compounds. It is available in every season and very economical as compared to other studied fruits. Therefore, daily consumption of the juice of *C. maxima* with food may reduce malnutrition and the risk of cardiovascular and cancer diseases as well.

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