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PRELIMINARY PHYTOCHEMICAL EVALUATION AND ANTIOXIDANT ACTIVITY OF CITRUS SINENSIS (L.)OSBECK

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Abstract*: Citrus sinensis*, which is commonly known as sweet orange, is a powerful natural antioxidant that intensify body immune system. It is also known to be the source of various phytochemicals. This present study was aimed for the preliminary phytochemical evaluation and antioxidant activity of *C.sinensis* pulp. Phytochemicals were analyzed both qualitatively and quantitatively. The qualitative analysis of *C.sinensis* revealed the presence of alkaloids, flavonoids, tannins, polyphenols and saponins. The quantitative analysis of phytochemicals in methanolic extract revealed that the presence of polyphenols (37.79) were maximum, followed by tannins (21.6), then flavonoid (9.6) and saponin (9.5). Polyphenols were dominant followed by tannins. Alkaloids were present in least amount. The antioxidant activity were assessed by DPPH radical scavenging assay. Six varying concentrations (0, 5, 10, 15, 20 and 25 ppm) of methanol solvent extract of orange demonstrated different percentage of inhibition. Appealingly the scavenging activity of extract increased in a concentration dependent manner. The 25 ppm extract showed the best antioxidant activity in DPPH that is 24.63 %.

Key words: *Citrus sinensis, preliminary phytochemical evaluation, antioxidant activity*

I. INTRODUCTION

Today oranges are cultivated almost all over the world and are well known for its nutrition and health-promoting values. This reputation is gained from the studies on the function of phytochemicals in Citrus fruits and their derived products in the past years. Orange is a powerful natural antioxidant that intensify the body immune system. Important phytochemicals like liminoids, synephrine, hesperidin, flavonoid, polyphenols, pectin, and sufficient amount of folacin, calcium, potassium, thiamine, niacin and magnesium are also present which prevent arteriosclerosis, cancer, kidney stones, stomach ulcers and reduction in cholesterol level and high blood pressure which promotes human health (Etebu *et al*., 2014).

From time immemorial the entire orange plant, including fruits, leaves, flowers, peels and juice is being used as traditional medicine. Oranges are effective against diseases such as arthritis, asthma, Alzheimer's disease, Parkinson's disease, macular degeneration, diabetes mellitus, gallstone, multiple sclerosis, cholera, gingivitis, optimal lung function, cataracts, ulcerative colitis and Crohn's disease. Traditionally orange juice is used as a general tonic that helps to eliminate toxins from the body and maintain hydration. It also used as a Mexican traditional medicine for the treatment of tuberculosis and stomach upsets. It improves appetite and prevents constipation. In Chinese medicine it is used as a cooling agent for cough, cold and respiratory disorders. Orange is the traditional Chinese symbol of good luck and prosperity. In France *Citrus sinensis* is used for the treatment of angina, hypertension, constipation, diarrhea, menstrual disorders and palpitations (Xingqian Ye, 2018). Citrus fruits are consumed as fresh or as processed products. Juice is the primary product obtained from citrus fruits (Braddock, 1999) and it is one of the most important commodities. The juices produced from the citrus fruits can be of different forms such as single-strength or concentrated juice (Ting and Rouseff, 1986).

Antioxidant activity denotes the ability of a bioactive compound to maintain cell function and structure by clearing free radicals and preventing other oxidative damage. There are more than 170 antioxidants from citrus fruits including vitamins, mineral elements, phenolic compounds, terpenoids and pectin (Zhou, 2012). The antioxidant activity of citrus fruits are affected by a wide variety of factors. Thus, a deep study of natural antioxidants from fruits and vegetables is of great importance to human health. Hence, the present study on *C.sinensis* was attempted to analyze preliminary phytochemical studies, quantify the secondary metabolites present in the selected fruit extract and study the antioxidant activity of fruit juice extract of the plant.

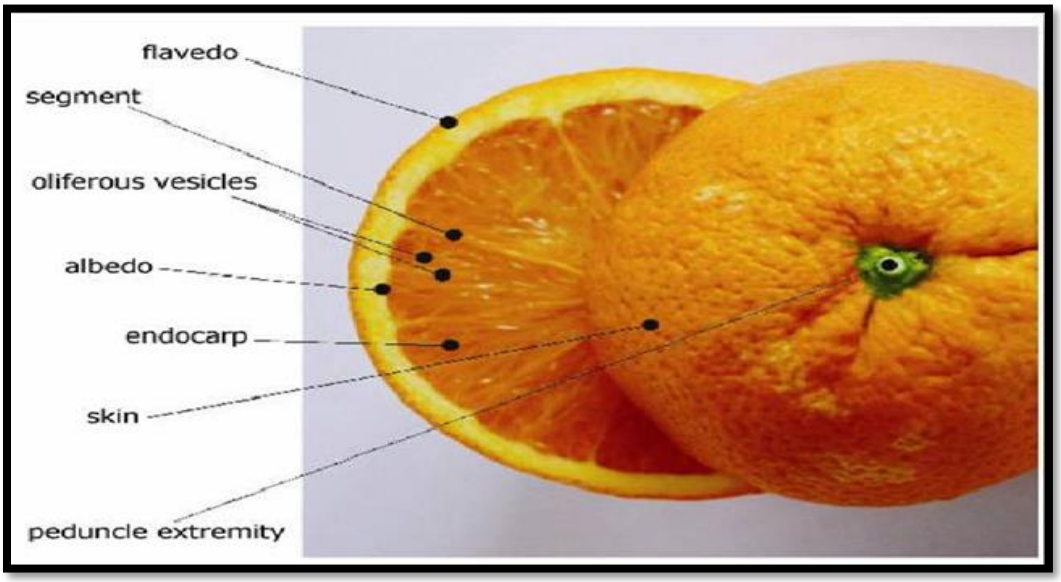
II. MATERIALS AND METHOD

Citrus sinensis is an evergreen flowering tree of Rutaceae family. They emit a strong characteristic citrus odor due to the presence of copious oil. Anatomically, the fruit consists of two distinct regions, the pericarp, also called the peel, skin or rind, and the endocarp or pulp with juice sac glands. The skin consists of an epidermis of epicuticular wax with numerous small aromatic oil glands that give of its particular smell. The pericarp consists of the outer flavedo or epicarp, largely made of parenchymatous cells and cuticle. The albedo or mesocarp lying beneath the flavedo consists of tubular-like cells joined together to constitute the tissue mass compressed into the intercellular area. The fruit usually contains a sweet pulp and several seeds within. The fruit pulp is typically formed of eleven segments of juice filled with flavor that goes from sour to sweet (Goudeau *et al*., 2008).

2.1 Preparation of plant extract

The phytochemical components of *Citrus sinensis* fruit extracts were prepared by using standard procedures as described by Harborne (1998). The extracts from *C.sinensis* are prepared using Soaking method. 25 g of the fresh samples of *C.sinensis* were percolated in 200ml methanol for 24 hours with occasional shaking. The extracts were then filtered using Whatman no. 41 filter paper. The organic solvent filtrates were concentrated in vacuum using a rotary evaporator, and the hexane extracts were dried using water bath to obtain crude extracts. They were collected and stored for further analysis.

Citrus sinensis habit



Anatomy of *Citrus* fruit

2.2 Qualitative analysis

The plant extract was tested for phytochemicals such as test for alkaloids, flavonoids, tannins, polyphenols and saponins.

2.2.1 Test for alkaloids

To 2 ml of extract, 2 ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

2.2.2 Test for flavonoids

To 2 ml of extract, 1 ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

2.2.3 Test for tannins

To 1 ml of extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

2.2.4 Test for phenols

To 2 ml of distilled water followed by few drops of 10% ferric chloride was added to 1ml of the extract. Formation of blue or green color indicates presence of phenols.

2.2.5 Test for saponins

To 2 ml of extract, 2 ml of distilled water were added and shaken in a graduated cylinder for 15 min lengthwise. It resulted in the formation of 1 cm layer of foam that indicated the presence of saponins.

2.3 Quantitative analysis

The fruit extract is tested for phytochemicals using following tests - test for alkaloids, test for flavonoids, test for tannins, test for polyphenols and test for saponins.

2.3.1 Total alkaloid content (Harborne, 1973)

To 40 ml of 10% acetic acid in ethanol was added to 10g of grounded sample, covered and allowed to stand for 4 hours. It is filtered and the filtrate was then concentrated on a water bath to get 1/4th of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle down. The precipitate collected and washed with dilute ammonium hydroxide. Then it was filtered by using a pre weighed filter paper. The residue was dried and weighed.

2.3.2 Total flavonoid content (Cameron *et al.*, 1993)

To 10 g of the fruit sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a pre weighed crucible, evaporated into dryness over a water bath, and weighed to a constant weight.

2.3.3 Total tannin content (Folin-Denis method, 2007)

The amount of total tannin in the extracts was estimated by Folin-Denis method. The principle behind this method is that Tannins like compounds reduce posphotungsto molybdic acid in alkaline solution, which will produce a blue colour complex. Their colour intensity will be proportional to the concentration of Tannin and are measured at 700nm.Tannins were determined by slightly modified Folin and Ciocalteu method. Briefly, 0.5 ml of sample extract is added with 3.75 ml of distilled water, then 0.25 ml of Folin Phenol reagent and 0.5 ml of 2 % sodium carbonate solution added. The absorbance was measured at 725 nm. Prepared a stock standard of 100 mg /100 ml Tannic acid. Pipet out 10 ml stock and made up to 100 ml with distilled water and used as working standard. Tannic acid dilutions (0 to 1 ml) were used as working standard solutions for standard graph preparation. The results of tannins are expressed in terms of tannic acid in mg/ml of extract.

2.3.4 Total polyphenol content (Folin and Ciocalteu method, 1980)

Phenols are estimated based on the principle that phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent to produce a blue-coloured complex in alkaline medium, which can be estimated spectrophotometrically at 765nm.The phenols were determined by slightly modified Folin and Ciocalteu method. Briefly, to the 200µl of the sample extracts, 800 µl of Folin Ciocalteu reagent mixture and 2 ml of 2 % sodium carbonate were added. The total content was diluted to seven volumes with distilled water and finally the tubes were incubated in dark for 2 hours. The absorbance was measured at 765 nm. Gallic acid dilutions were used as standard solutions. A stock standard of 100 mg /100 ml Gallic acid was prepared. Pipet out 10 ml stock, made up to 100 ml with distilled water, and used as working standard. Gallic acid dilutions (0 to 1 ml) were used as working standard solutions for standard graph preparation. The results of phenols are expressed in terms of Gallic acid in mg/ml of extract.

2.3.5 Total saponin content (Obadoni and Ochuko, 2001)

20 g of samples powder was taken in a conical flask and 100 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. A 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight and the saponin content was calculated as percentage.

2.4 Estimation of antioxidant activity (Blois, 1958)

The antioxidant activity of the plant extracts was estimated using the DPPH (2, 2-diphenyl-l-picryl hydrazyl) radical scavenging protocol. DPPH solution (0.004% w/v) was prepared in 95% ethanol. 2 ml of freshly prepared DPPH solution (0.004% w/v) was added in each of the test tubes containing 2 ml extract. The reaction mixture was incubated in the dark for 30 min and thereafter the optical density was recorded at 523 nm against the blank. For the control, 2 ml of DPPH solution in ethanol was mixed with 10ml of ethanol and the optical density of the solution was recorded after 30 min. The assay was carried out in triplicate. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (%IP) of DPPH radical. The capability of scavenging DPPH radical was calculated using the following equation

$$\text{DPPH Scavenged (\%)} = \frac{(\text{A control} - \text{A test})}{(\text{A control})} \times 100$$

where,

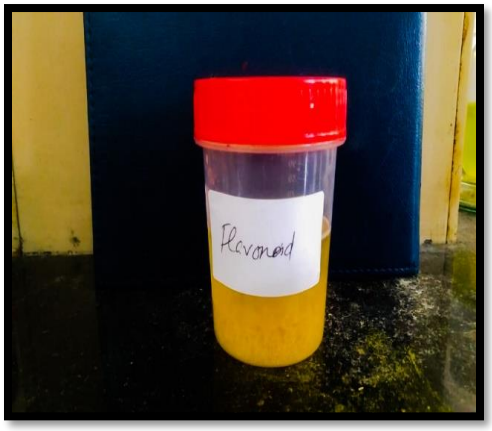
A control - absorbance of the control reaction

A test -absorbance of the sample of the extracts.

The IC_{50} Value of DPPH Radical Scavenging Activity

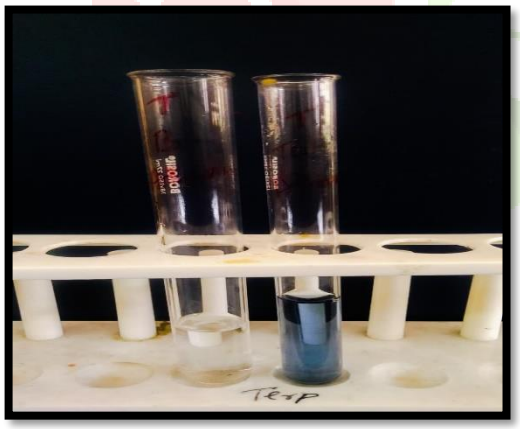
The IC_{50} value calculated to determine the concentration of the sample required to inhibit 50% of radical. The lower the IC_{50} value, the higher the antioxidant activity of samples.

Quantitative analysis of *Citrus sinensis* (L.) Osbeck



Test for flavonoid

Test for Alkaloid



Test for Tannin

III. RESULTS AND DISCUSSION

The present study was focused on the phytochemistry and antioxidant activity in fruit pulp of *Citrus sinensis* (L.) Osbeck. Phytochemicals are compounds that are produced by the plants. Phytochemicals constitute one of the most numerous and widely distributed groups of substances in the plant kingdom. The phytochemicals does not involve directly in the process of growth but acts as deterrents to insects and microbial attack. The qualitative phytochemical analysis of the methanolic extract of *Citrus sinensis* revealed the presence of following phytochemicals (Table 1).

Table 1. Qualitative analysis of Phytochemicals present in methanolic fruit extract

Sl.No.	PHYTOCHEMICAL	METHANOLIC EXTRACT
1	Alkaloid	+
2	Flavonoid	+
3	Tannin	+
4	Polyphenols	+
5	Saponins	+

+ indicates the presence

The qualitative analysis of *C.sinensis* revealed the presence of alkaloids, flavonoids, tannins, polyphenols and saponins. The flavonoids have strong inherent ability to modify the body's reaction to allergens, viruses and carcinogens. Flavonoids are responsible for the bitter taste of some oranges. Quercetin is a flavonoid and it has significant anti-inflammatory activity because of direct inhibition of several initial processes of inflammation (Roger *et al*., 2002). Hesperidin is a flavonoid glycoside found profusely in citrus fruits. Hesperidin reduces cholesterol (Rapisarda *et al*., 1999) and has anti-inflammatory effects (Del *et al*., 1987).

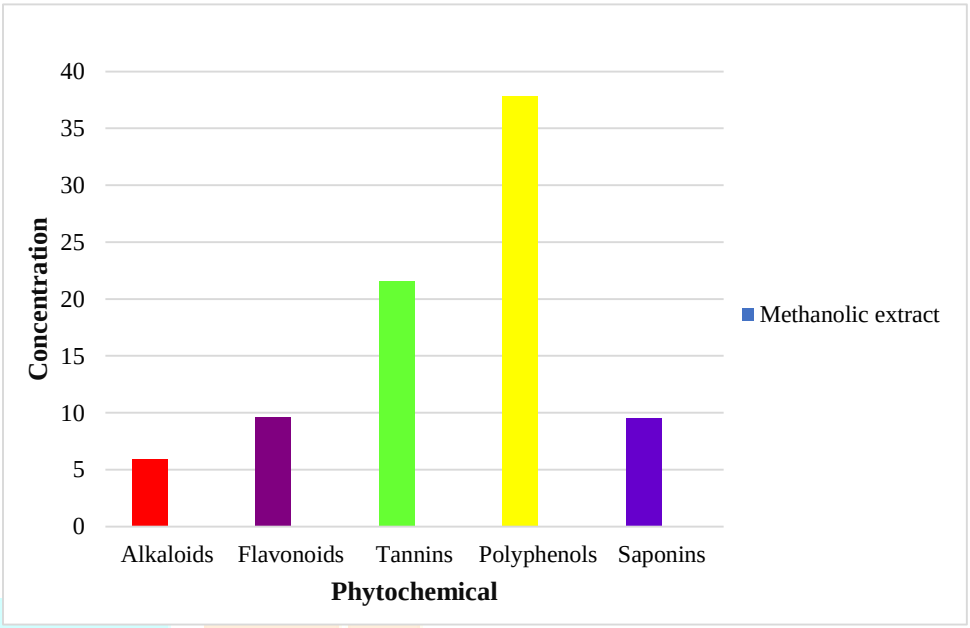
Tannins are convoluted chemical substances derived from phenolic acids and are sometimes called tannic acid. They are classified as phenolic compounds, that found in many species of plants, from all climates and all parts of the globe. Polyphenols constitute a variety of bioactive compounds that are commonly divided into several classes, such as hydroxybenzoic acids, hydroxycinnamic acids, anthocyanins, proanthocyanidins, flavanoids, stilbenes and lignans (Zhou, 2012). Saponins are bioorganic compounds found in particular abundance in the plant kingdom. They are naturally occurring glycosides described by the soap-like foaming, and they produce foams when shaken in aqueous solutions.

Presence of these phytochemicals, such as flavonoids, polyphenols, saponins, tannins and alkaloids indicates the presence of antioxidant activity in *C. sinensis* extract. The quantitative analysis of phytochemicals in methanolic extract revealed that the presence of Polyphenols (37.79) were maximum, followed by Tannins (21.6), then flavonoid (9.6) and saponin (9.5). Alkaloids was present in least quantity. The quantitative phytochemical analysis of the methanolic extract of *Citrus sinensis* (L.) Osbeck is presented in the Figure 1.

Table 2. Quantitative analysis of Phytochemicals present in the methanolic fruit extract

Sl.No.	PHYTOCHEMICAL	METHANOLIC EXTRACT (mg/100ml)
1	Alkaloids	5.9
2	Flavonoids	9.6
3	Tannins	21.6
4	Polyphenols	37.79
5	Saponins	9.5

Figure 1. Quantitative analysis of Phytochemicals present in the methanolic fruit



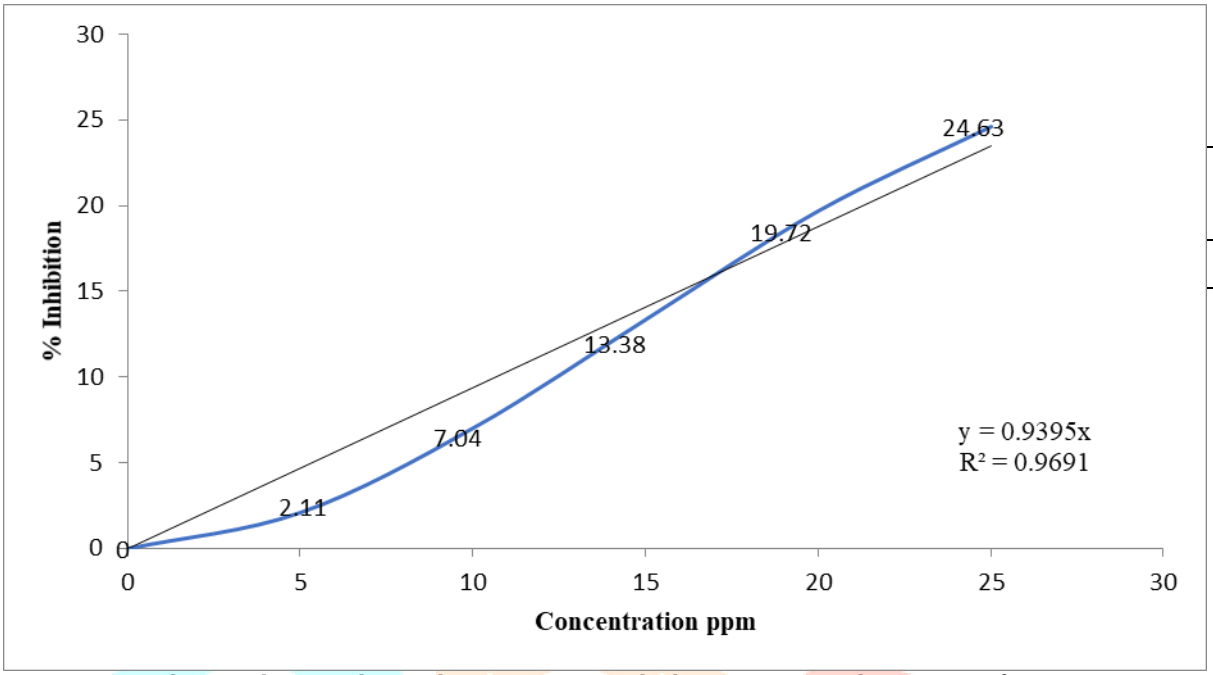
Preliminary phytochemical studies are helpful in the determination of chemical constituents present in the plant material. Extraction of phenolic compounds from plant materials typically depends upon different extraction techniques, temperature and Ph. In present study, antioxidant activity of the sample was determined by DPPH radical scavenging method, and the results obtained as follows (Table 3.).

Table 3. Percentage of inhibition in methanolic extract of *C.sinensis* by DPPH assay

CONCENTRATION (ppm)	**% INHIBITION OF METHANOLIC EXTRACT (DPPH)**
0	0
5	2.11
10	7.04
15	13.38
20	19.72
25	24.63

Six varying concentrations (0, 5, 10, 15, 20 and 25 ppm) of methanol solvent extract of orange demonstrated different percentage of inhibition. Interestingly, scavenging activity of extract was increased in a concentration dependent manner. The 25 ppm extract showed the best antioxidant activity in DPPH that is 24.63 %.

Figure 2.The radical scavenging activity of fruit pulp of *C.sinensis* represented by percentage of inhibition by DPPH



According to Phongpaichit *et al.*, 2007, extracts which possess IC_{50} values ranging from 50 to 100 µg / mL is considered to exhibit intermediate antioxidant activity. Meanwhile, extracts with IC_{50} value ranging between 10 to 50 µg / mL is considered to possess strong antioxidant activity.

Phenolic compounds are classified as primary antioxidants and the antioxidant potential of phenolic compounds depends on the number and arrangement of the hydroxyl groups present (Rio *et al.*, 2013). The antioxidant activity of the phytochemicals are affected by three factors, such as chemical structure of the compounds, pre- harvest and post-harvest factors and finally the processing factors. From the above results, it is evident that the methanol extract of *Citrus sinensis* pulp possess antioxidant activity that might be due to the presence of various phytoconstituents such as phenols and flavonoids in the extracts. From the results obtained, we can conclude that Citrus fruit is a rich source of various phytochemicals, which are great value for human health.

Similar results are obtained for the study conducted on antioxidant activity of citrus fruits (Zhou *et al.*, 2016). The study reveals the antioxidant activity in Citrus fruits. However, the present study reveals information not only about antioxidant activity in *C.sinensis* but also gives the particulars about preliminary phytochemicals, both quantitatively and qualitatively. The knowledge about the secondary metabolites in plants will lead to the discovery of novel varieties of medicines. A major advantage of natural bioactive molecules is that they have milder side effects on the body in comparison to chemically synthesized drugs.

IV. CONCLUSION

The present study was screened to find out the phytochemical and antioxidant activity of an economically important plant species *Citrus sinensis*. Citrus fruits are known to have a variety of health benefits. One of the main benefit is that, they are rich sources of antioxidants, which works as an antiaging agent. The recent research prove that they also work against cardio-vascular diseases. The plant fruit is collected, methanolic extract was prepared and subjected to preliminary phytochemical analysis. The phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins and polyphenols. Polyphenols were present in high amount followed by tannins, then flavonoids. Alkaloid is present in least amount. These phytochemicals are the main cause of antioxidant activity in Citrus fruits. The antioxidant activity is measured using DPPH scavenging assay.

Currently many researchers are interested in medicinal plants for evaluation of antioxidant phytochemicals such as phenols, flavonoids and tannins, which have received more attention for their potential role in prevention of human diseases. Citrus phenolic compounds particularly flavonoids have been reported to possess an important antioxidant activity toward radicals. The citrus flavonoids have the ability to capture electrons, block or scavenge the radicals. The citrus flavonoids prevents the propagating chain reactions of oxygen free radicals.

The knowledge about the secondary metabolites in plants will lead to the discovery of novel varieties of medicines. A major advantage of natural bioactive molecules is that they have milder side effects on the body in comparison to chemically synthesized drugs.

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