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ORIGINAL ARTICLE

The Flame photometric determination of Na^+ and K^+ from selected some citrus fruits and their antioxidant capacity *in vitro* method

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ABSTRACT

Photoelectric flame photometry, a branch of atomic spectroscopy is used for inorganic chemical analysis for determining the concentration of certain metal ions such as sodium, potassium, lithium, calcium, Caesium, etc. Sodium (Na) is the major extracellular cation and it plays a role in body fluid distribution. Concentration of sodium ions inside the plasma (extracellular) is 130-145 mmol/l. Higher and lower concentrations are referred to as hypernatremia and hyponatremia, respectively Potassium (K) is the major cation found inside of cells. The proper level of potassium is essential for normal cell function. An abnormal increase of potassium (hypercalcemia) or decrease of potassium (hypokalaemia) can profoundly affect the nervous system and heart, and when extreme, can be fatal. The normal blood potassium level is 3.5 - 5.0 millimoles/litter (mmol/l). simultaneously conducting research on citrus fruits here would be progressive results on its antioxidant studies. by doing this research on the extracts of this fruit it can use to future drug discovery and essential metabolites with antioxidant effects and also drugs for antimicrobial, anti-inflammatory and antioxidant, etc. Conclusion of this work the proposed flame photometric method was successfully employed to estimate the amount of potassium and Sodium in five different citrus fruits (lemon, orange, pink grapefruit, lime, and pomelos). The proposed method was found to be simple, specific, accurate and precise. This study provided yielded results so as to compare mineral (Na and K) content of different fruits. Shows good antioxidant activity

Key words: *Photoelectric flame photometry, citrus fruit, Na and K.*

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INTRODUCTION

Citrus plants belonging to the family *Rutaceae* which include fruits such as lemon, orange, mandarin, lime, sour orange, and grapefruit appear as a well-known promising source of different useful nutrients for human beings. Citrus fruits are one of the world's most important beneficial fruit crops and are known for their nutritive values and special aroma [1, 2, 6]. Citrus is mainly consumed as fresh fruit or juice. the citrus [11] fruits have high potentially represented a rich source of phenolic compounds and dietary fibres and these fruits had good antioxidant activity agents [3-10].These citrus fruit residues, which are generally discarded as waste in the environment, can act as potential nutraceutical resources. Citrus fruits are good sources of nutrition with a rich amount of vitamin C. Besides, the fruits are abundant in other macronutrients, including sugars, potassium, sodium folate, calcium, thiamin, niacin, vitamin B6, phosphorus, magnesium, copper, riboflavin and pantothenic acid. Citrus fruits contain a number of secondary metabolites, such as flavonoids, alkaloids, coumarins, limonoids, carotenoids, phenol acids, and essential oils. These active secondary metabolites show several bioactivities of vital importance to human health, including anti-oxidative, anti-inflammatory, anti-cancer, as well as cardiovascular protective effects, neuroprotective effects, etc. In addition, Citrus fruits have been used as traditional medicinal plants in several Asian countries, such as China, Japan, and Korea. Nine traditional Chinese medicines have been recorded in the Chinese Pharmacopoeia for appropriate medical use from six Citrus species [5]: *C.reticulata Blanco*, *C.medica L. var. sarcodactylis Swingle, C.medica L., C.wilsonii Tanaka, Citrusaurantium L., and C.sinensis Osbeck*. These peels or whole fruits (mature or immature) are known to treat indigestion, cough, skin inflammation, muscle pain, and ringworm infections, as well as to lower blood pressure. [11]

Sodium (Na) is the major extracellular cation and it plays a role in body fluid distribution. Concentration of sodium ions inside the plasma (extracellular) is 130-145 mmol/l. Higher and lower concentrations are referred to as hypernatremia and hyponatremia, respectively. When a solution containing cations of sodium and potassium is spayed into flame, the solvent evaporates and ions are converted into atomic state. In the heat of the flame (temperature about 1800ºC), small fraction of the atoms is excited. Relaxation of the excited atoms to the lower energy level is accompanied by emission of light (photons) with characteristic wavelength (Na: 589 nm, K: 766 nm). Intensity of the emitted light depends on the concentration of particular atoms in flame [5].
Potassium (K) is the major cation found inside of cells. The proper level of potassium is essential for normal cell function. An abnormal increase of potassium (hyperkalemia) or decrease of potassium (hypokalemia) can profoundly affect the nervous system and heart, and when extreme, can be fatal. The normal blood potassium level is 3.5 - 5.0 millimoles/litter (mmol/l).
Characteristic wavelengths of the elements It is common knowledge that when Sodium is introduced into a flame it emits a radiation in the yellow region of the visible spectrum. Table 1 gives details of the measurable atomic flame emissions of the alkali and alkaline earth metals in terms of the emission wavelength and the colours produced.

Table 1. Details of the measurable atomic flame emissions of the alkali and alkaline earth metals in terms of the emission wavelength and the colours produced.

SN	Element	Emission wavelength (nm)	Flame colour
1	Potassium(K)	766	Violet
2	Lithium (Li)	670	Red (Carmine)
3	Calcium (Ca)	622	Orange
4	Sodium (Na)	589	Yellow
5	Barium (Ba)	515	Lime Green

ANTIOXIDANT EFFECT

Natural phenolic phytochemicals in plants have been receiving increased interest from consumers and researchers for their beneficial health effects on coronary heart diseases and cancers mainly due their antioxidant activity. Free radicals and other reactive oxygen species generated in living organisms leads to many diseases including cancer, cardiovascular diseases, cataracts, asthma, hepatitis, liver injury and immunodeficiency diseases. The use of synthetic antioxidants is an old practice and their safety could be questioned by the consumers. The alternative natural compounds with efficient antioxidant activity have been paid increasing attention.
It is well accepted that reactive oxygen species (ROS), such as superoxide anion (O2 -.), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\cdot}$) formed in vivo are highly reactive chemical species and can be generated endogenously as well as exogenously. Excess production of ROS leads to oxidative stress, which can cause number of diseases. In such conditions dietary intake of antioxidant compounds are needed in assisting the body to neutralize the free radicals to remove the harmful effects of oxidative stress. Fruits, vegetables, grains and medicinal plants are known to contain number of phenolic compounds with strong antioxidant activity. These compounds are found to be well correlated with antioxidant potential.
There is an increasing trend to replace synthetic antioxidants, which are of safety concern, with the natural antioxidants available from plant extracts or isolated products of plant origin. Present study was undertaken to determine antioxidant scavenging inhibitory activity of 5 different citrus fruits from Mumbai of India in order to evaluate their potential as a natural antioxidative source.

MATERIAL AND METHODS

Sample collection (fruits):

Five different common citrus fruits (lemon, orange, pink grapefruit, lime, and pomelos) were collected freshly during the month of June 2019 local market of Mumbai, Maharashtra, India. Following collection, the whole fruits were cleaned and washed with distilled water. Weight of each fruit was recorded and then packed in a polyethylene bag, store at -20°C until analysis.

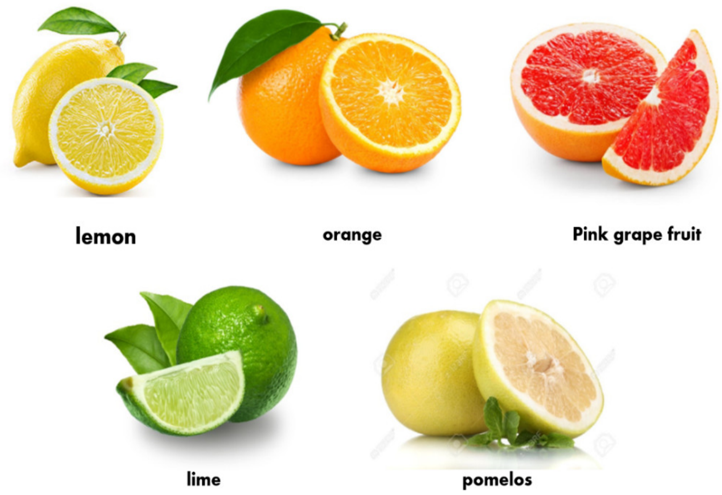

Fig:1 Different citrus fruits

chemicals:
Sodium Chloride (AR), Potassium Chloride (AR), DPPH, DMSO and Distilled water,
Instruments:
Flame photometry and UV-spectrophotometer
Sample preparation
Take The fresh citrus fruits (lemon, orange, pink grapefruit, lime, and pomelos) and slice them in two using a knife. The seeds in the fruit should be removed by hand, then take the all citrus fruits juices using the juicer and filtering by Whatman filter paper using Buchner funnel. The juice is then placed into a sample bottle and used for subsequent experiments.
Preparation of standard solution
Take sodium chloride (NaCl) is weigh about 0.25 gm and transfers it into 100 ml volumetric flask through a funnel. Simultaneously weigh 0.19 gm quality of potassium chloride (KCl) and transfer it into the same volumetric flask through the same funnel. Add double distilled water to the flask, dissolved the crystals and make up the solution to the mark with double distilled water. The stock standard solution contains 1000 ppm/1000 ppm of sodium and potassium. From this stock standard solution 100, 80, 60, 40, 20 and 10 ppm solution of lower concentration was prepared. Aspirate Distilled Water and set the read out 00 by adjusting the zero control. Aspirate the standard solution that has higher concentration adjust the noble to 100. For optimum performance the instrument should be allow 15 min to warm up during this warm up period a blank demonized water sample should be aspirated. Emissions were noted for all standard solution. Lastly, water sample solutions were aspirated and emission was noted.
Procedure
Take 100, 80, 60, 40, 20 and 10 ppm sodium and potassium standards.To 10ml of the fruit juice, add 50ml of deionised water. Ensure that the compressor unit, LPG pipe are properly connected and secured. .Switch ON the main unit and compressor unit .Adjust the air regulator knob to achieve a reading between 0.4-0.6. Open the regulator valve from LPG cylinder .Open the Fuel control valve from main unit.8.Insert the igniter on the burner and ignite the flame. If the flame is not ignited within few attempt increase the fuel flow gradually once the flame is ignited it will be yellow nonoxidizing flame. Slowly reduce the flame so as to get a blue oxidizing flame. Allow the flame to stabilize for 5 min. Take distilled water in the beaker and insert the capillary aspirator insert the required filter. Adjust the zero control to '00' on display. Remove the distilled water and place the beaker containing the repaired sample of known concentration and select appropriate filter. Allow the flame to stabilize for 1-2 min..Adjust the calibration knob to adjust the value of the standard on display. Repeat the operation as given in 8 & 10 above. On pressing freeze switch the reading will be averaged and stored. Remember to aspirate with distilled water between two readings to flush out earlier content. At the end of the experiment shut 'OFF' the fuel valve first when the flame is extinguished close the air valve and shut down the main unit and compressor unit.

pH Determination
The glass electrode pH meter was used to determine the pH of the samples. The electrode was first placed in a buffer solution respectively before it was used/ placed in the samples.

DPPH radical scavenging assay

Take different volume of Citrus fruits (1000,500,200,100 mg/ml) extracts were made up to 40 ul with DMSO and 2.96 ml DPPH (o.1 mM) solution was added. The reaction mixture was incubated in dark condition at room temperature at 20 mins, after 20 mins the absorbance of the mixture was measured at 517 nm .2ml of DPPH was taken as control. All the experiment should carried out in triplicate for suitable statistical calculations. Prepare one blank solution which contains the ml of 9 ml reagent and 1ml 1 ml distilled water. The following equation was used to determine the percentage of the radical scavenging activity of each extract.

Percentage of radical scavenging activity=[(OD control- OD sample)/OD control] × 100

RESULTS AND DISCUSSION

The citrus fruits samples are analyzed for potassium and sodium contents using flame photometry (Table 2). Each value is the mean value of triplicate analysis. the line of regression from Na and K standard graph was used for estimation of Na content and K in different citrus fruits from a standard curve of the sodium and Potassium, line of regression was found to be sodium Y= 0.97x and R2 =0.995 and potassium Y= 1.0073x and R2 =0.993 are representative of the equations for calibration curves for sodium and potassium respectively. using the above equation should be found in ppm of Sodium and potassium. However, the result indicates that the fruits are highly rich in the mineral elements analyzed, hence can be a very good source of mineral nutrients as they are of great importance and contributes to the well being of the body.

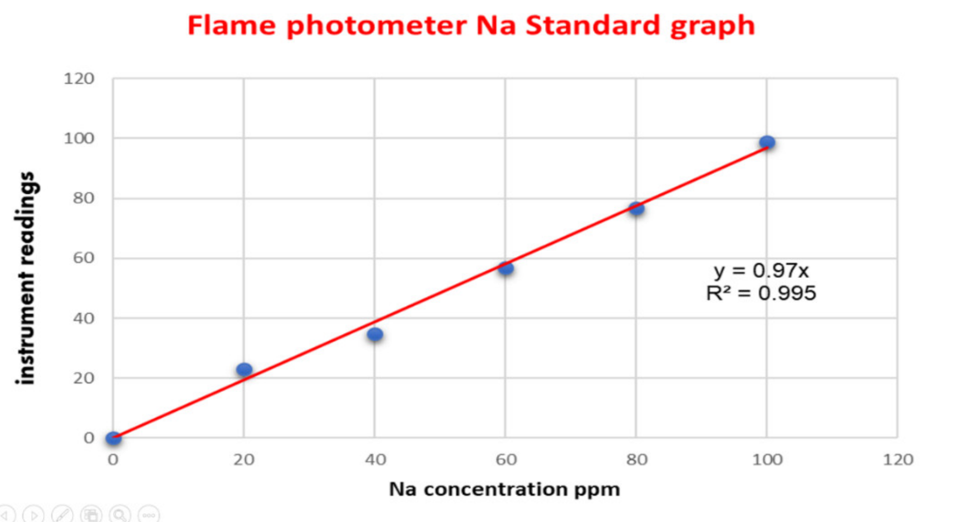

Fig 2: sodium standard graph.

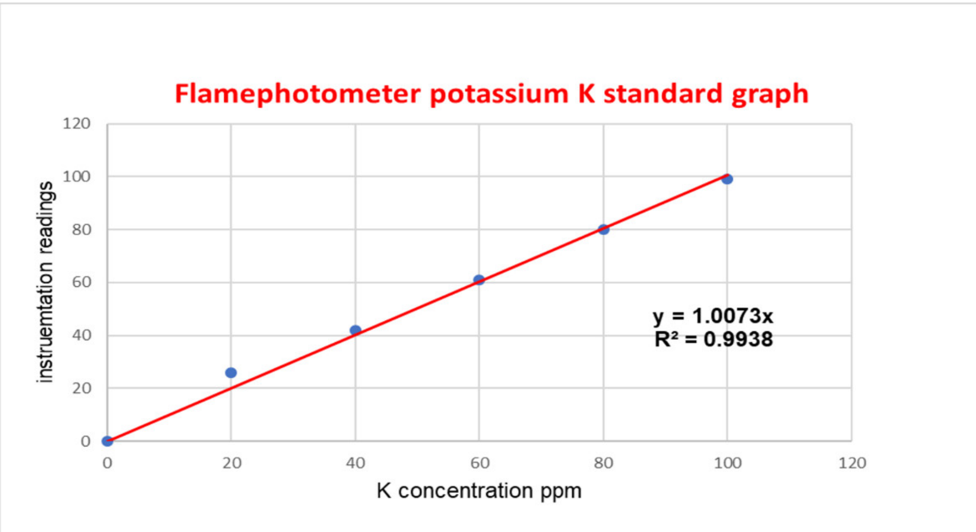

Fig 3: **potassium standard graph.**

S.no	Fruits	pH	Na in ppm	K in ppm
1.	lemon	2.39 ± 0.17	157.67	121.2
2.	orange	4.09 ± 0.03	99.42	56.89
3.	pink grapefruit	3.27 ± 0.08	46.90	29.89
4.	lime	2.07 ± 0.16	86.98	38.0
5.	pomelos	2.27 ± 0.05	55.45	43.78

Table 2 : Na and K element concentration of some different citrus fruit juice

Antioxidant activity

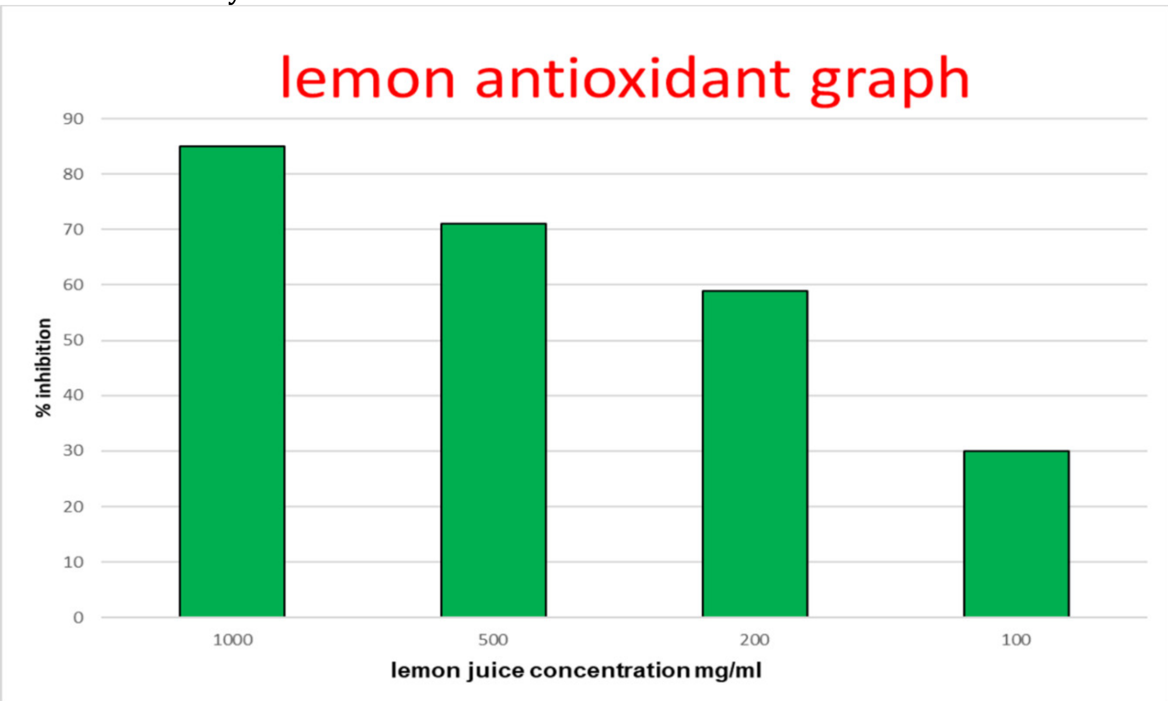

Fig 4: **Antioxidant activity of Lemon juice**

The present study was carried out to analyze the antioxidant activity of the different citrus fruits. The scavenging activity of the fruits juice through the annihilation of the DPPH radicals was investigated. The effect of antioxidant on DPPH is believed to be due to their hydrogen-donating ability. The DPPH assay measures the antioxidant activity of water-soluble phenolics. Figure 4 : shows the dose-response curve of DPPH radical scavenging activity of the different citrus fruit's juices. At the concentration of 1000 mg /mL, the scavenging activity of pomelos juice reached 89%, similarly at the concentration of 1000 mg /mL, the scavenging activity of lemon juice shows 87%, lime juice showing the lowest activity showing at the concentration 1000 mg/ml. As the sample concentration increasing, similarly the % of inhibition also increasing. Although the DPPH radical scavenging abilities of the extracts were significantly lower than that of BHT, it was evident that the extracts showed proton-donating ability and this could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants From this study, we can conclude that all citrus are fruits has a very good antioxidant activity.

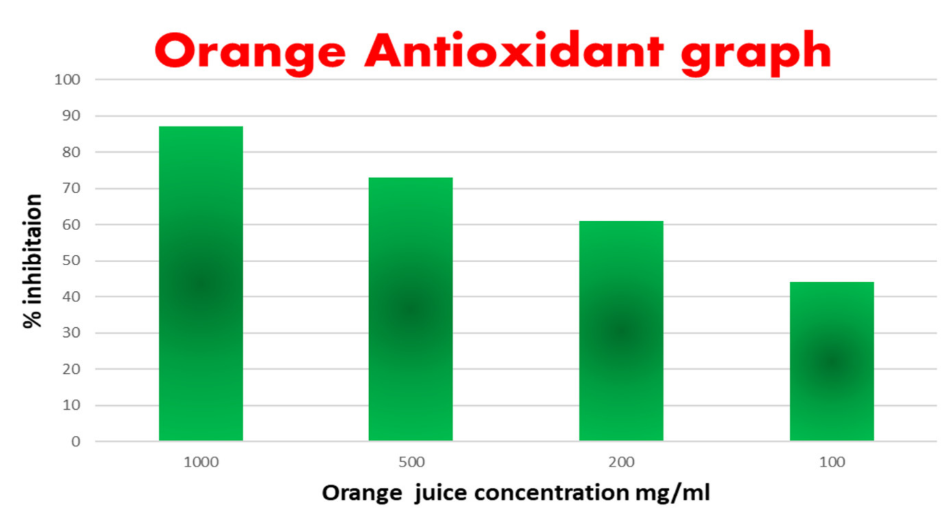

Fig 5: **Antioxidant activity of Orange juice**

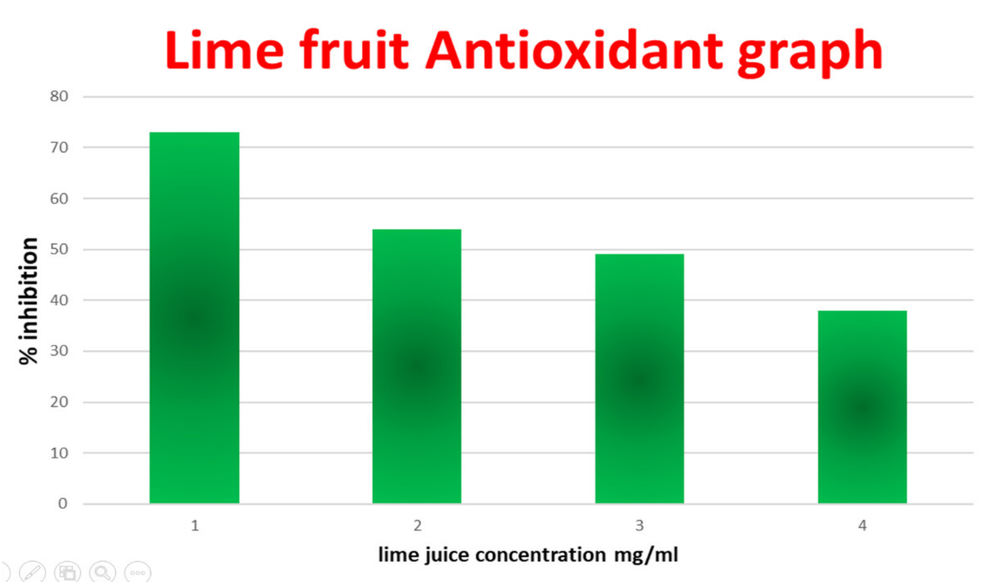

Fig 6: **Antioxidant activity of Lime fruit juice**

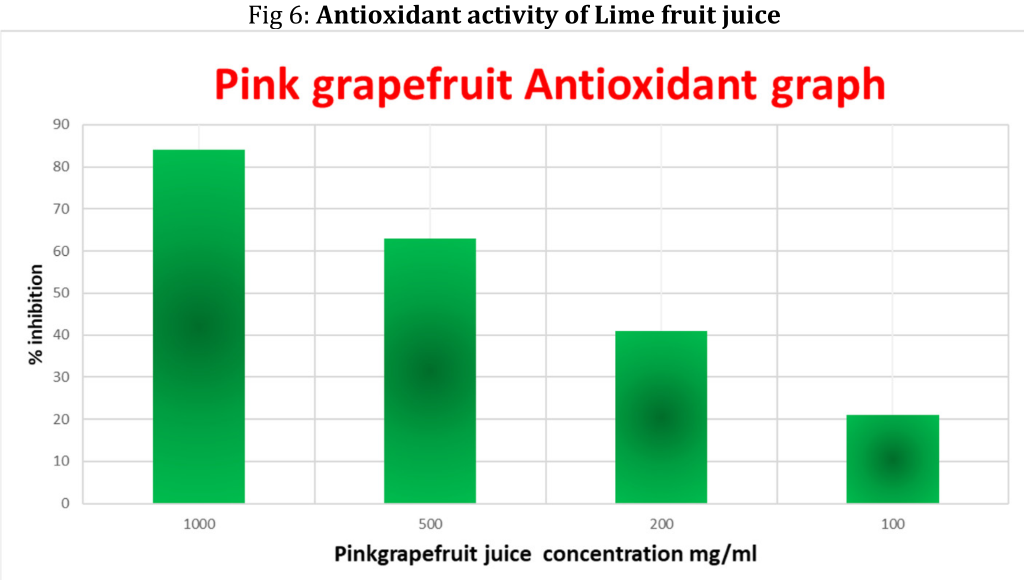

Fig 7: **Antioxidant activity of pink grapefruit**

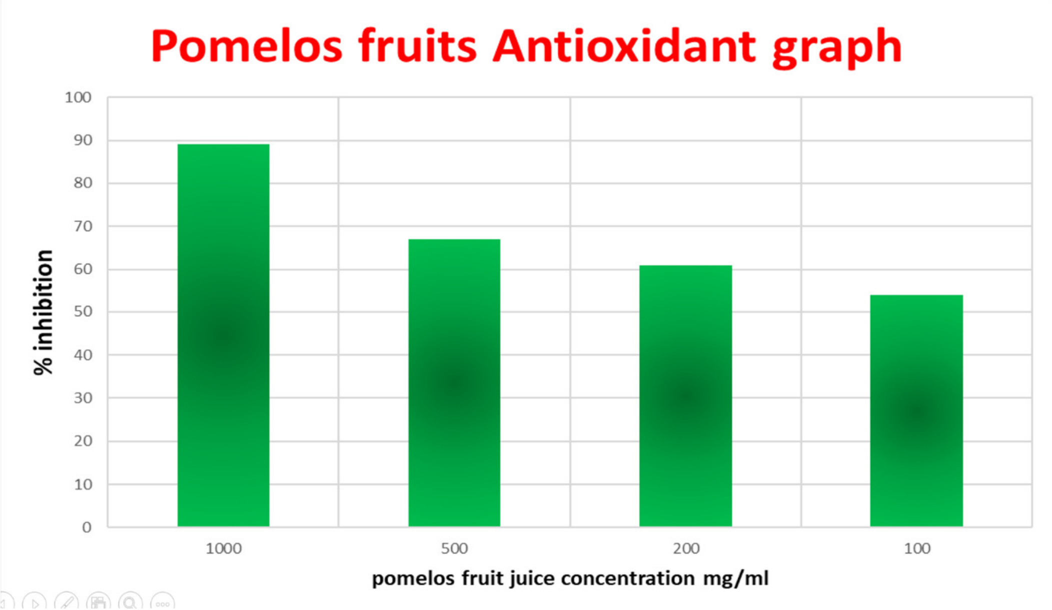

Fig 3: **Antioxidant activity of Lemon juice**

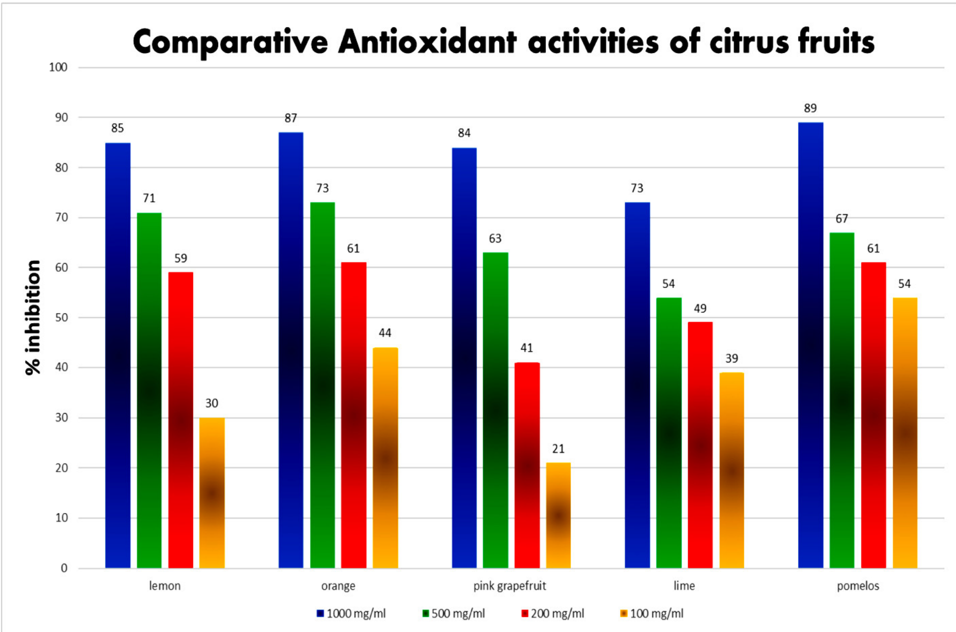

Fig 3: Comparative **Antioxidant activities of citrus fruits**

CONCLUSION

The Conclusion of this work the proposed flame photometric method was successfully employed to estimate the amount of potassium and Sodium in five different citrus fruits. The proposed method was found to be simple, specific, accurate and precise. This study provided yielded results so as to compare mineral (Na and K) content of different fruits. Fruits are generally acceptable as good source of nutrient and supplement for food in a world faced with problem of food scarcity. Citrus fruits have long been valued as part of a nutritious and tasty diet. The determine the antioxidant activity in vitro in order to allow rapid screening of substances since substances that have high antioxidant activity in vitro. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of citrus fruits. Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants due to their scavenging activity are useful for the management of those diseases. All the citrus fruits gave good antioxidant results.

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