



Research Article

Physiochemical and Phytochemical profiles of Fresh *Citrus limon* (L) JuiceOyebadejo S.A.¹ and Solomon I.P.²¹Department of Biotechnology Faculty of Biosciences Shri Jagdish Prasad Jhabarmal Tibrewala University Rajasthan 333001 India.²Department of Animal Physiology Faculty of Agriculture University of Uyo Akwa Ibom Nigeria.

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Abstract: Considering the potential benefits of plants regarding their medicinal values in the area of ameliorating effect of some diseases conditions, Physiochemical and phytochemical profile of *Citrus limon* juice were carried out. Physiochemical screening such as total solids, temperature, pH value, total titratable acidity and specific gravity were evaluated using physical and chemical method while physiochemical profile, alkaloids, tannins, flavonoids, saponins, steroids, cardiac glycosides, reducing sugar and anthraquinones respectively were evaluated by chemical methods. Result, Physiochemical parameters were found to be within the normal range while physiochemical, saponin, tannins, Anthraquinones and reducing sugar were present in the juice. Alkaloids, steroids and glycosides were present while flavonoids were present in the juice at higher concentration. In conclusion, it is commonly observed that *Citrus limon* juice exhibit relevant and important physiochemical and phytochemical properties which could be associated with medicinal effects.

Keywords: Physiochemical, phytochemicals, *Citrus limon* juice.

INTRODUCTION

People use medicinal plants in accordance to their nutritional components and values. In addition, they are used for treating a wide spectrum of diseases and they have been tested for their potential uses as alternative remedies and to reduce the toxic and oxidants of foods (Sabahat and Perween, 2007). Many plants have been found to have components that have anticancer, antifungal and antibacterial, antiprotozoal, and antiviral activities (Manners, 2007 and Majunder et al., 2011)

Uses of *Citrus Limon* juice include brightening of fingernails, preservation of food, deodorant production, destroying of odor, deterrent of insect, lightening of Age spots and freckles, cleaning, treatment of Dandruff, of glasses, removal of stain soothing of sore throat, cleaning of toilets, boosting of metabolism, polishing of metals, brightening of laundries, treatment of facial disorders, itchy skin smoothening and hangovering.

The administration of Fresh juice of *Citrus limon*, is reported to be effective at reducing the sizes of tumour, quality of live of breast cancer patients, it is well tolerated in the body without any side effect such

as vomiting, alopecia, diarrhea and loss of weight as common side effect known by other chemotherapies when compared to the other active pharmacological and chemotherapeutic obtained from plants which proved to be useful in clinical trials as therapy.

The juice is the liquid part of the *Citrus limon* fruit which can be extracted manually or by the use of automation has historical use as treatment for various diseases, yet there is little or no scientific evidence which shows the physiochemical and phytochemical profile of the juice extracted from the *Citrus limon*.

MATERIALS AND METHOD

Chemicals, Reagents and Equipments

All the chemicals, reagents and equipments used in this study are of international standard organization grade standardized by ISO and analytical grade without any form of impurities.

Citrus Limon fruit Collection

The *Citrus limon* fruit samples of the same species and varieties were collected from a local farm in Uyo, Akwa Ibom, Nigeria within the month of October and December 2016 in sterilized conditions from the same set of trees in sterilized polythene bags,

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stored at 4°C in a refrigerator until use, they were authenticated by Botanist at the Agricultural Biotechnology unit, Derindam Research Institute of Biotechnology, Voucher specimen number DRIB ABU005.11 was created for the fruit and deposited in the herbarium.

Determination of the physicochemical properties of *Citrus Limon* Juice

Specific gravity and density

The Specific gravity was determined using Specific gravity bottle. The empty bottle was weighed, filled with distilled water and reweighed after filling it with the juice sample and weighed, and then the Specific gravity (SG) of the juice was calculated according to Ishiwu and Oluka (2004) as follows:

$$SG = W_j / W_w$$

Where **W_j** and **W_w** are weight of a known volume of juice and weight of an equal volume of water respectively in gram (g) then the Density; P, in kg/m³ was given as **P = 1000SG**

Total Solids

Total solid was determined using the method of Osborne and Vought (1979). An empty petri dish washed, over dried for 15min, cooled in a desiccator for 20 minutes the dried and cooled, petri-dish weighed empty and the reweighed after the addition of 10ml of juice using pipette. The petri-dishes with the content (poured juice) were oven-dried at 100 degree celcius for 6 hours cool in the desiccator and re-weighed. This procedure was repeated until a constant weight was obtained. The total solid, **S₁** in % was calculated as follows:

$$S_1 = 100 \times W_2 / W_1$$

Where, **w₁** and **w₂** are weight of fresh juice and weight of dried sample, respectively in gram (g).

Citrus limon Juice Temperature

The temperature of the juice will be measured using the bulb thermometer. It will be done immediately after the extraction process because of the volatile nature of vitamin C content of the juice (Ishiwu and Oluka, 2004)

Determination of the Ascorbic acid content

According to the method of Ishiwu and Oluka, 2004, 20 ml of juice was added to 80 ml of distilled water and 10ml of 1M H₂SO₄. The resulting solution was titrated against 0.05m, iodine solution (using 6 drops of starch mucilage as indicator). The vitamin C content was calculated as follow.

$$Vc\ 100g = TV \times F \times 100$$

Where **TV** (titre value), factor are volume of iodine used and concentration (0.00886g) of acid used respectively. Each volume of iodine should be equivalent to 0.00886g of Vitamin C.

PH Determination

The juice was dissolved in distilled water and kept in a water bath for 20mm, filtered and the pH of the filtrate was measured using pH meter.

Total Titratable Acidity

A 10ml sample of juice was pipetted and stirred vigorously to remove carbon (iv) oxide, the sample were titrated using 0.1N standardized sodium hydroxide (NaoH) and phenolphthalein as an indicator. The total titratable acidity was calculated as percentage citric acid. (1ml represent = 0.0070g citric acid). The total titratable acidity. TTA in % is mathematically expressed by Ishiwu and Oluka (2004) as follows:

$$TTA = M \times 0.0070g \times 100 \times N/100 \times V_s$$

Where **M** **N** and **V_j** are made of 0.1N NaoH used, normality and value of juice sample used, respectively

Phytochemical screening of *Citrus Limon* Juice Extracted

Aliquots portion of the juice extracted was subjected to the Phytochemical screening (Trease and Evans, 1983).

Test for alkaloids

Determination of Alkaloid was carried out by dissolving *Citrus limon* juice of 500µl in 1% solution of hydrochloric acid at 37°C in the water bath, followed by addition of reagent, Dragendroff producing precipitation (Trease and Evan, 2003).

Test for Tannins

Tannin was determined by dissolving *Citrus limon* juice of 5 mls in distilled water at 100 degree Celcius, green colour of precipitation was observed following the addition of ferric chloride solution of 6% at room temperature (Trease and Evan, 2003).

Test for Flavanoids

Flavonoid content was determined by use of heat on the *Citrus limon* juice of 0.5ml and methanol, orange colour was observed when hydrochloric acid (concentrated) was added drop by drop following introduction of metal (Trease and Evan, 2003).

Test for Saponin

Persistency of frothing formation in the tubes containing 0.5 ml of the *Citrus limon* juice stirred in the water bath confirmed saponin in the juice.

Test for Steroids

Reddish brown at the lower layer of the tube confirmed the presence of steroid when 0.5ml of *Citrus limon* juice was dissolved in 3 ml of chloroform and filtered followed by addition of concentrated Tetraoxosulphate (VI) Acid.

Test for Cardiac glycosides

Cardiac glycoside was analysed by addition of 0.5g of *Citrus limon* juice with 2ml of glacial acetic acid that has been laid underly with Tetraoxosulphate (VI) acid by the use of ferric chloride to produce a brown ring forming like on the surface of the mixture indicating sugar appearance which later turned to violet ring appearance and greenish sugar appearance on top of the ring appearance and later appear through the surface of the solution.

Test for reducing Sugars

Brick red colour formed confirmed the the presence of reducing sugars when Fehling solutions was added to the *Citrus limon* juice in the tubes placed in water bath for 2 minutes.

Test for Anthraquinones

5ml of the juice was hydrolyzed with dil/conc. H_2SO_4 . 1 ml of dilute ammonia was added to it. Rose pink colour confirmed the presence of anthraquinones.

RESULT**Physiochemical screening of *Citrus limon* (L) Juice**

Physiochemical screening of citrus limon juice revealed specific gravity in mg/ml of values ranges between 1.01-1.04g/ml, pH value of the citrus limon showed mild acidic concentration between 6.3-6.8, the total solid values of the *Citrus limon* juice content showed very low percentage of constituents ranging between 5.9-6.2%, Titrable acidity concentration also showed very low percentage at the range of 4.0-5.2%. Ratio of Total Titrable acidity and Total acidity concentration lower at the range of 1.9-2.1%, while the ascorbic acid in mg/100ml showed 23-45mg/100ml (Table 1)

Table 1: Showing Physiochemical Screening of Fresh *Citrus Limon* (L) Juice

S/N	Parameters	Values in Range
1	Specific gravity (g/ml)	1.01– 1.04
2	PH Value	6.3– 6.8
3	Total solid (%)	5.9– 6.2
4	Titrateable Acidity (%)	4.0 – 5.2
5	TSS/TA	1.9 – 2.1
6	Ascorbic Acid (mg/100ml)	23– 45

Phytochemical screening of Fresh *Citrus Limon* (L) Juice

Phytochemical screening of fresh *Citrus limon* juice as illustrated in Table 2, revealed the presence of Steroid, Alkaloid and glycosides at very low concentration, Saponnin, Tannin, Antraquinone and

Reducing sugar at moderate range, while there was increase in the presence of Flavonoids content of *Citrus limon* juice.

Table 2: Showing Phytochemical Screening of Fresh *Citrus Limon* (L) Juice

S/N	Phytochemicals	Fresh Citrus Limon juice
1	Saponins	++
2	Flavanoids	+++
3	Tannins	++
4	Alkaloids	+
5	Steroids	+
6	Glycosides	+
7	Anthraquinones	++
8	Reducing Sugar	++

Keys:- not detected, + Present in low concentration, ++ Present in high concentration, +++ Present in high concentration

DISCUSSION

Series of conventional therapies have been employed and utilized in orthodox, traditional and herbal medicine, yet no clear-cut possible cure have been identified.

Clinical wise, the use of chemotherapy has produced slight significant but the end product still result in dead or irreversible lost of life, even when it has caused the patient huge lost of money fortune and profile in the society (Sofowora,1993). Radiotherapy as another form of cure have been employed in many cases, they appeared working initially but at later end causes pain, wasteful and lost of life and general lost in the society.

According to Hsia and Liu, 2010, variety of conventional therapies for cancer based on chemotherapy, radiotherapy and surgery are limited in efficacy. Most current cancer chemotherapy regimens are normally associated with very high significant levels of toxicity and drug resistance.

The *Citrus limon* juice contain high levels of flavonoid, which is known to posses strong anti-oxidative capacity thereby protective and enhance the cellular profile of the animals based on its inhibit action on free radicals, enabling cell to thrive and survive successfully without any disturbance.

Physiochemical properties, specific gravity for the *Citrus limon* juice were found to be normal in range, the pH value. Total solid, Titrable acidity and its ratio were found to be in the normal range as this contribute to the digestible of the juice in the experimental animal during the study. Ascorbic acid content was high indicating the flavonoid capacity content, which has been tagged to have strong antioxidant capacity, which is line with good anticancer properties.

Phytochemical studies of *Citrus limon* revealed the present of tannin which impacted taste and yellowish colour to *Citrus limon* has been found to be present at very low concentration, containing saponin which is helpful in lowering cholesterol level and help alleviate the risk of heart disease was present at very high concentration, alkaloid which is present in plant as defence against herbivorous or insecticide was present in very little concentration, stood as stimulating precursor organic substances was also present in very small concentration, glycosides concentration was much due to its activities in stimulating the muscle, connective tissue and internal arrangement of an organ, anthraquinone is a natural pigment found in plant and herbs and the concentration in *Citrus limon* juice is very high while the reducing sugar which act as reducing agents being as aldehyde group or fucose ketones are found in high concentration and the flavonoid which poses antioxidant capacity was found in extreme high concentration which gave the *Citrus limon* juice antioxidant capacity.

CONCLUSION

From this study, it was discovered that in search for safe and effective drugs for the treatment or cure of breast cancer and other tumours could utilise the antioxidant capacities of fresh juice of *Citrus limon* in the production of medical drugs that have great values as indicated by its physiochemical and phytochemical effects.

CONFLICT INTERESTS

The authors declared that they have no competing interests.

AUTHORS' CONTRIBUTIONS

All the Authors contributed equally.

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