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Attenuation of Invigorating Ramification of Citrus aurantifolia Dehydrated Peel Crumb against Hyperuricemia

C. aurantifolia in Reduction of Serum Uric Acid Levels

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Abstract:- ***Citrus aurantifolia*** **(Kaghazi lime) is a fruit from Rutaceae family which is cultivated extensively in tropical and sub-tropical regions. Citrus posess a wide range of phytonutrients serving as anti-oxidants, boosting immune system, inducing protective enzymes in liver and blocking damage to genetic material. As these plants belong to natural origin and their use poses minimum side effects, therefore, they can be used as an alternative or as an assistant to drugs like uricosuria or allopurinol, used to treat hyperuricemia. Therefore, the aim of the study focuses on identifying the nutritional quality of dehydrated peel crumb and evaluating its potential in lessening hyperuricemia.The present study constructed the repercussion of dried peel powder of *Citrus aurantifolia* on serum uric acid concentrations. Hyperuricemic non-smoker, non-diabetic, non-hypertensive males and females of age group 25-60 years were provided with 15g/day of dried lime peel crumb as a ministration for 30 days. Afterwards serum uric acid levels were assessed by blood test at 0^{th}, 7^{th}, 15^{th} and 30^{th} day.The proximate analysis of *C.aurantifolia* showed moisture 8.4±0.08, protein 5.4±0.16, fat 3.88±0.09, fiber 11.99±0.8, ash 4.89±0.16 and minerals to be 3.17±0.01. The efficacy study found a significant decline in serum uric acid concentrations in experimental group with f value to be 0.48. The results of the efficacy study interpreted the fact that lime peel have a potent potential in lessening serum uric acid concentrations and can be administered as supplementation in pharmacological interventions.**

Keywords:- *Uricosuria, Allopurinol, Hyperuricemia, Uric Acid, Pharmacological Interventions.*

I. INTRODUCTION

Gout is a quite prevalent inflammatory disorder which is induced due to the formation of monosodium urate (MSU) crystals in or around joints. Hyperuricemia and high levels of urate crystals are an essential pre-requisite for the development of gout (Edward R, 2008). It affects 1% of Western male population with ratio of gout in males and females to be 7:1 to 9:1. Normal uric acid levels in men and pre-menopausal women are7mg/dl and 6mg/dl respectively, which are too close to the limits of urate solubility being 7mg/dl; hence there is subtle physiological balance of urate. Generally urate levels of 9mg/dl suggest the incidence of gouty arthritis. Excessive production and excretion of urate is associated with high levels of uric acid in blood which further poses risk of calcium oxalate urolithiasis (Robert A, 2003). It is also associated with the risk of cardiovascular diseases and other metabolic disorders (Michael et al., 2010).

Hypertension, drugs for cardiovascular diseases or stroke, obesity, alcohol intake and diuretics are referred to as the predictors of gouty arthritis clinically. Major symptoms are the attacks of acute or chronic inflammation with severe pain. Although the diagnosis of gouty arthritis is quite vast however, it can be detected simply by monitoring blood urate levels (Robert A, 2003).

Long term management for gouty arthritis commonly focuses on maintaining uric acid levels in a delicate range, generally <6mg/dl. Success in achieving this level may result in cessation of urate deposition in body and disappearing the signs and symptoms of gouty arthritis. For the past 40 years, pharmacological approaches involve either enhancement of uric acid excretion through kidneys with uricosuria, or cessation of urate production with the aid of purine based analogues. However, many recent publications document poor clinical outcomes in gouty patients leading to further gouty complications like disabilities and impaired quality of life (Michael et al., 2010).

Identifying various other ways for treatment, especially using remedial and therapeutic plants along with their complements to treat a number of ailments like uricemia, CVDs, Diabetes and hyperlipidemia etc. have amplified over the recent decades in many countries around the world (Bahmani et al., 2015)

Citrus aurantifolia (Kaghazi lime) is a fruit from Rutaceae family which is cultivated extensively in tropical and sub-tropical regions (FAO, 2009). *C. aurantifolia* is rich in a number of nutrients including vitamins, minerals, fiber, essential oils and phenolic compounds and is regarded as the third mot cultivated species around the globe (Gonzalez et al., 2009).

It is probably the most grown fruit for direct human use worldwide. Lime and various products of this species are developed for a number of uses especially medicinal uses, domestic and industrial applications. Its tree is about 5ft in length and 7.5ft in width (Bakare, 2012).

Different components of this plant like leaves, peel, seeds and fruit juice are used for its various medicinal properties like anti-septic, anti-bacterial and anti-fungicidal. It is also beneficial as appetite stimulant, astringent, for stomach diseases, for arthritis, for headache, cough, flu and

sore throats. These properties are due to the presence of flavonoids, coumarins and terpenoids present in lime (Sandovel-Montemayor et al., 2012)

Traditionally, *C. aurantifolia* is incorporated in treatment of a number of health problems especially related to bacterial infections. Efforts are done by scientists in using this plant in medicines of various types to cure not only bacteria related health ailments but also a number of other categories of diseases (Ibukun, 2007). Many citrus plants of Rutaceae family are used as medicine for gout, liver problems, stomach disorders and brain troubles in Asian countries. These plants possess xanthene oxidase inhibitory activity and so are useful for curing these conditions. As these plants belong to natural origin and their use poses minimum side effects, therefore, they can be used as an alternative to drugs like uricosuria or allopurinol (Ajay Kumar, 2014).

In view of these experimental studies, the dehydrated peel should be used in treatment of various other health ailments.

- *Objective:*

This study aimed on identifying biological proficiency of Mexican lime *(C. aurantifolia)* in reduction of hyperuricemia causing gouty arthritis.Moreover, it also paid attention towards identifying the nutritional composition of Mexican lime.

II. MATERIALS AND METHODS

- *Area of Research*

Research was conducted in the school of nutrition and dietetics, with proceeding analysis carried out in post-graduate laboratory, at The University of Faisalabad, TUF. The reseach was done in a duration of 30days.

- *Assortment of Raw Material*

Kaghazi limes, scientifically called *Citrus aurantifolia*, were solicited from fruit market, Faisalabad.

- *Preparation of Dried Peel Crumb*

The peel of *C. aurantifolia* were acquired from the fruit and washed. The peel was then crumbed and dried by conventional drying at 60C to 80C according to the procedure followed by Garau et al. (2007).

- *Chemical Analysis of C. aurantifolia Peel Crumb*

Citrus aurantifolia crumb were evaluated for moisture, ash, crude fiber, crude fat and crude protein according to the standard procedures described by the Association of Official Analytical Chemists (AOAC, 2006).

A. *Moisture Content*

The moisture content of *C. aurantifolia* peel crumb was gauged by drying the specimen in hot air oven at 105±5 till the specimen weight became perpetual as per the method chronicle by AOAC (2006).

$$\text{Moisture \%age} = \frac{\text{Weight of fresh specimen(g)} - \text{Weight of dried specimen(g)}}{\text{Weight of fresh specimen (g)}} \times 100$$

B. *Ash Content*

The ash content of dried peel crumb was appraised conferring to the method defined in AOAC (2006). Ash estimation was conducted by direct incineration of specimen obtained in a crucible. The crucible was heated on the oxidizing flame till it composed no haze. Then, specimen was kindled in a muffle kiln at $550^{\circ}C$, till gray white silt was procured. Percentage ash calculated as:

$$\text{\%age Ash} = \frac{\text{Weight of ash(g)}}{\text{Weight of specimen (g)}} \times 100$$

C. *Crude Fat Content*

Crude fat determination was manipulated utilizing Soxhlet apparatus as per the method described by AOAC (2006). 5g of *C. aurantifolia* dried peel crumb specimen were taken. It was taken in isolated thimbles and implanted in an extraction tube of Soxhlet apparatus post cloaking in filter paper. The modification of temperature of heater was so that incessant drops of ethanol raze on the specimen in the extraction tube. The residuals were relocated into a dry weighted china dish. Then, the china dish was planted in a hot air oven for dispersal of ethanol for 4-5 hours. Afterwards, the china dish was relocated in desiccator to cool it and then weighed again. The crude fat content was ruled out by taking 5g specimen utilizing ethanol as solvent in Soxhlet apparatus conferring to the course given in AOAC (2006).

$$\text{Moisture \%age} = \frac{\text{Weight of specimen(g)} - \text{Weight of fat free specimen(g)}}{\text{Weight of specimen (g)}} \times 100$$

D. *Crude Fiber Content*

The *C. aurantifolia* dried peel crumb specimen after fat extraction was estimated for crude fiber content by following the agendum stated in AOAC (2006). 5g fat free specimen of C. *aurantifolia* dried peel crumb in 500ml beaker was confirmed. The specimen was then heated with 25ml 1.25% H_2SO_4 solution for 30minutes. The contents were then filtered and washed for 2-3 times with distilled water. The washed residue was transferred to a 500ml beaker ad again made the volume upto 200ml utilizing distilled water and was heated with 2.5ml 1.25% NaOH solution for 30 minutes. The contents were drippled and 2-3 washings with hot water were given until it was alkali free. The residue was conscientiously implanted to impugn crucible and dried in an oven at 100˚C for 3-4 hours until constant reading was obtained. The contents were heated on flame until the smoke ceased to come out of the specimen. The specimen was then placed in a muffle furnace at 550˚C for 4 hours till gray ash was obtained, cooled in desiccator and weighed. The difference in weight was calculated as crude fiber by utilizing the maxim:

$$\text{\%age crude fiber} = \frac{\text{Weight of insoluble matter(g)} - \text{Weight of ash(g)}}{\text{Weight of specimen (g)}} \times 100$$

E. *Crude Protein*

The percentage of nitrogen in the specimen was concluded by utilizing kjeldahl method as construed in AOAC (2006). The specimen was digested in digestion tube with the compensation of 30ml concentrated H_2SO_4 in the presence of digestion mixture ($CuSO_4$:$FeSO_4$:K_2SO_4 as 9:1:90) for 5-6 hours or till the digestion mixture reaped light greenish or transparent color. The material was diluted and distilled by taking 10ml of diluted material and 10ml of 40% NaOH. The ammonia discharged was collected in 4% boric acid having methyl indicator. The solution was then titrated against 0.1N H_2SO_4. Crude protein was calculated by maxim:

$$\text{Nitrogen \%age} = \frac{\text{Vol.of 0.1NH2SO4 (ml)} \times \text{vol.of dilution} \times 0.0014}{\text{Weight of fresh specimen (g)} \times \text{Vol.of aliqout specimen (ml)}} \times 100$$

$$\text{Crude Protein} = \text{Nitrogen \%age} \times 6.25$$

F. *Nitrogen Free Extract*

The NFE was calculated by maxim given by (Uraku et al., 2016):

$$\text{NFE \%} = 100 - (\text{\% moisture} + \text{\%crude fiber} + \text{\% crude protein} + \text{\%ash} + \text{\%crude fat})$$

G. *Mineral Profile*

Minerals like Na and K were determined by means of Flame Spectrometry as done by Xu, et al. in 2007.

➢ *Human Study Paradigms*

The bioevaluation was performed to investigate the nutraceutical stature of dried peel crumb of *Citrus aurantifolia* against hyperuricemia. Before the commencement of study, 10 human subjects, non-smoker, non- diabetic, non-hypertensive, from both genders were nominated from Madina Teaching Hospital Faisalabad. The ethical approval was given by the institute.

The selection procedure was carried out on the basis of their; anthropometric evidences, medical history, current medications and life style practices. Afterwards, blood analysis was done to figure out their uric acid levels.

The human trial was conducted into two groups i-e control group with no supplementation given and experimental group which will be given 15g/day of dried lime peel crumb for at least 15days as in Wang's trial with water soluble lemon extract (Table 1). Blood sampling was performed one day before the initiation of trial to get the baseline values of uric acid for both groups. Before the termination of efficacy study, blood sampling was performed again to assess the effect of dried lemon peel crumb and uric acid lowering drugs on the nominated parameter which was serum uric acid concentration.

➢ *Subsumption Precedent for Participants*

- Hyperuricemic, non-diabetic, non-hypertensive and non-smoker males and females of age 25-60 years were chosen and splitted into two troops
- Blood sampling was done before commencement of efficacy trials. Next sampling was done on 30th day

The inclusion criterion for volunteers is mentioned in table 2.

➢ *Uric Acid Test*

Uric acid was assessed from blood test of serum uric acid (SUA test).

➢ *Statistical Analysis*

Data obtained from each group was analyzed utilizing statistical model mentioned by Steel et al., in 1997. A thorough randomized design was applied to evaluate the significance level.

➢ *Considered Criterion*

- Anthropometric measurements
- BMI of all participants
- Dietary history

❖ *Anthropometric Measurements*

Height and weight of all the patients was measured barefooted and in slightly dressed state with body weight being measured on human weighing machine and height, with the aid of height scale. Body weight was assessed again after blood sampling.

*All mock-ups followed a random home diet.

Groups	Description
G_1 (Control Group)	No supplementation given
G_2 (Experimental Group)	15g/day dried lime peel crumb

Table 1:- Human Study Plan

❖ *Body Mass Index*

Changes in body weight were estimated from the BMI values. BMI is characterized as body mass relative individual's weight and height.m^2

$$\text{BMI} = \frac{\text{Weight (kg)}}{\text{Height } (m^2)}$$

❖ *Dietary History*

A detailed dietary history was gathered from all patients as a part of development of standard dietary method (Jain et al., 1980). Major aim for the assessment of dietary history was evaluation of individual's eating pattern and disease risk.

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Inclusion Criteria	
Age Group	25-60 years
Gender	Both male and females
Complication	Hyperuricemia
Others	Non-diabetic Non-hypertensive Non-smoker

Table 2:- Subsumption Precedent for Participants

III. RESULTS

The mandate of study was conducted to evaluate the effectiveness of *Citrus aurantifolia* against hyperuricemia. 15g of dried peel crumb of *C. aurantifolia* was given to males and females within an age range of 25-40 years at 0^{th} ,7^{th} ,15^{th} and 30^{th} day and finally the blood samples were compared for both experimental and control group to identify the effectiveness of *C. aurantifolia* against hyperuricemia. The study was devised in a comprehensive manner; however, for better comparison the results along with their interpretations are allocated into two major portions i-e characterization of *C. aurantifolia* dried peel crumb and efficacy studies.

IV. FINDINGS OF PROXIMATE ANALYSIS

The results obtained from proximate analysis are shown in table 3 with percentages in fig 1 and contains moisture 8.4±0.08, protein 5.4±0.16, fat 3.88±0.09, fiber 11.99±0.8, ash 4.89±0.16 and minerals to be 3.17±0.01.

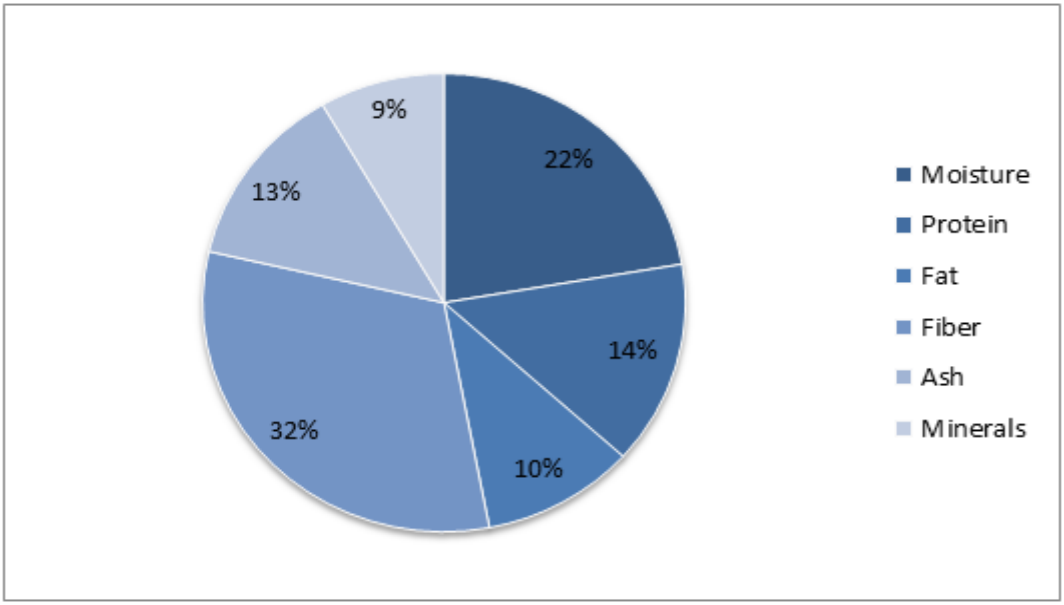

Fig 1:- Percentages of Nutrients in Dehydrated Peel Crumb

Proximate Analysis	Composition
Moisture	8.4±0.08
Protein	5.4±0.16
Fat	3.88±0.09
Fiber	11.99±0.8
Ash	4.89±0.16
Minerals	3.17±0.01

Table 3:- Findings of Proximate Analysis

➢ *Efficacy Study*

The aim of research work conducted was to evaluate the effectiveness of *C. aurantifolia* dried peel crumb against hyperuricemia and for this purpose 20 subjects were selected with the specifications to be non-smoker, non-hypertensive and non-diabetic males and females within an age range of 25-40 years, divided into two groups i-e experimental group, receiving 15g of dehydrated peel crumb as experimental manipulation of 30 days and control group, which were given no manipulations. The blood sampling confirmed the patients to be hyperuricemic and the samples from both groups were collected periodically at 7^{th}, 15^{th} and 30^{th} day. Afterwards, the results from both groups were collected and compared to devise out the potential of *C. aurantifolia* to treat hyperuricemia.

➢ *Findings*

The findings of the presented efficacy study were in a strong favor of *C. aurantifolia* emphasizing the fact that dried citrus peel poses a potential of reducing the serum uric acid concentrations. The uric acid levels of control were increased with time in contrast to serum uric acid concentrations of the experimental group that decline with time. Results also showed that the dried peel crumb of *C. aurantifolia* not only reduces the uric acid levels but also manages them and so can be used as a treatment against hyperuricemia.

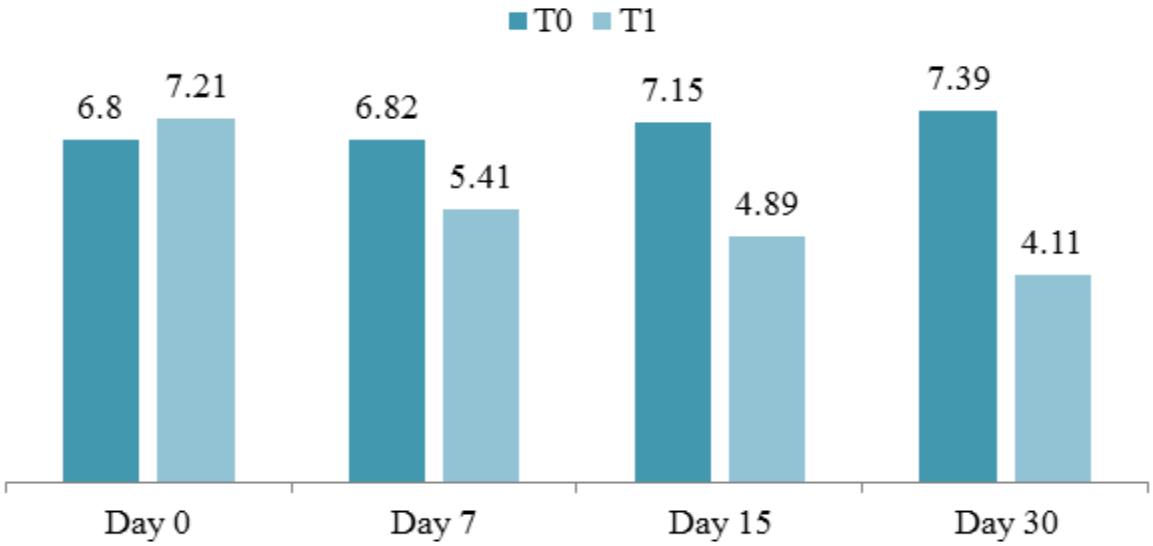

Fig 2:- Comparison of Efficacy Results

*T_0= Control Group
*T_1= Experimental Group

V. DISCUSSION

Hyperuricemia is a medical condition caused by elevation of serum uric acid levels and it is further associated with a number of other health conditions including gout, cardiovascular diseases and renal failure. From the past few decades, many medications are being practiced to control hyperuricemia but now focus id being paid on natural remedies based on providing polyphenols and anti-oxidants to treat hyperuricemia as they pose no side effects to health, unlike other anti-hyperuricemic drugs (Mehmood et al., 2007). Citrus flavonoids have large spectrum of scientific activities and have a potent influence in preventing and curing diseases. Preparations from peel, seeds and leaves can be used for the treatment of various health ailments. Peel of citrus fruits are specifically rich in flavonoid glycosides, coumarins and volatile oils. Polymethoxylated flavones have many favorable biological activities, moreover, the fiber content of citrus fruits have major bioactive compounds like polyphenols and vitamin C. (Mohanapriya et al., 2013).

Therefore, the present study is devised to incorporate *C. aurantifolia* dehydrated peel crumbs in the treatment of hyperuricemia. Moreover, nutritional composition of *C. aurantifolia* has been investigated to identify the quality of product being manipulated in experimental study.

Nutritional analysis showed the moisture content in *C. aurantifolia* peel to be 8.4±0.08, protein 5.4±0.16, fat 1.88±0.09, fiber 11.99±0.8, ash 4.89±0.16 and minerals to be 3.17±0.01. these findings were quite close to the findings of analysis done by Esmail in 2016 where he found the moisture content of peel to be 84.6, protein 5.5, fats 1, fiber 11.3 and minerals to be 3.7 (Esmail, 2013). The slight differences in nutritional analysis may be due to drying of the peel and the environmental influences on fruits in different regions of the world.

Citrus paradis is another fruit from citrus plants and also poses a wide range of phytochemical properties and is rich in flavonoid content protecting against a number of microbial, parasitic and health ailments. The phytochemical analysis of fruit confirmed the presence of anti-oxidants including polyphenols (5.76%), alkaloids (2.42%), glycosides (2.54%), saponins (1.50%), tannins (0.34%) and flavonoids (8.70%). Moreover, the proximate analysis of fruit revealed 11.86% moisture, 3.97% ash, 10.71% protein, 6.64% fat and 7.55% fiber in the peel (Edet et al., 2016). These findings demonstrate that these have high quality of nutritional constituents even more than *C. aurantifolia* and can be used in treatment of various disease in regard of its anti-oxidant activity. I preferred *C. aurantifolia* in my research work as it is economical and easily available in this region, can be grown at home and its high use in Pakistan. Peel is generally wasted and I manipulated the waste product in my study that can be afforded by all economical ranges of region.

Studies done by Kensara O.A. showed a protective effect of vitamin C against hyperuricemia and renal diseases associated with hyperuricemia. Vitamin C show uricosuric properties and have a potent potential in treatment of various diseases. Vitamin C was found to improve gastric functioning and histopathological examinations confirmed improved renal tissues health. Overall results indicated debilitated biochemical indices, oxidative stress markers of hyperuricemia and histopathological outcomes. (Kensara O.A., 2013). These results matches to the present study of incorporating *C. aurantifolia* in treatment of hyperuricemia as it is also rich in vitamin C and have reducing potential. But comparison to Kensara study suggests the use of attenuating *C. aurantifolia* peel in renal diseases, cardiovascular diseases and other health ailments as it will also have a protective effect against many other health ailments.

Citrus unshiu fruit similarly like, *C. aurantifolia*, have uric acid lowering effect especially when focus is paid on immature satsuma orange fruit. It is viewed that the extract did not significantly decrease xanthine oxidase enzyme activity that is the major contributor towards the formation of uric acid in the body. Major effect is due to the presence of flavonoid glycosides having acid lowering effect and also the presence of hesperetin (Nakao et al., 2011). So, overall this research was highly favorable in the use of *Citrus unshiu* in treating hyperuricemia similarly as *C. aurantifolia*. This is due to the fact that *C. aurantifolia* also poses same flavonoids that lower uric acid concentration. Therefore, we can generate an assumption that *C. unshiu* can also be used in place of *C. aurantifolia*. The results from both fruits will be the same.

Hesperidin a well-known flavonoid that is majorly present in citrus fruits including lemon and oranges. Hesperitin being a metabolite of hespridin is also a potent protective unit that has an advantage in cardiovascular diseases, hyperlipidemia, hypertension, diabetes and a number of other health ailments. This study done by Zanwar et al. in 2014 enlightens the path of using citrus aurantifolia peel and fruit in treatment of diseases other than hyperuricemia including cardiovascular diseases, hypertension, diabetes and hyperlipidemia.

Study done by Zasshi in 2005 enlightened the fact that the constituents of citrus plants, from all over the plant including bark, leaves, stem, peel and fruit, have shown to be effective as chemoprotective which leads the scientist towards extracting many of these compounds. The study pays attention towards the effectiveness of citrus peel as a chemopotective agent due to the presence of auraptene and nobiletin. Moreover the peel also have tumor protecting properties having additional effects of P-glycoprotein inhibitor (Zasshi Y., 2005). Hence, *C. aurantifolia* peel can be used for cancer prevention and cure and attention should be focused on employing its peel of such diseases too.

VI. CONCLUSION

This study concluded the fact that C. aurantifolia peel is rich in a number of bioactive constituents and is beneficial for treatment of various diseases along with the prevention of health ailments. This study discovered that the dehydrated peel crumb of C. aurantifolia is beneficial of reduction in high uric acid levels in both male and female subjects.

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