

Evaluation of Phytochemical Concentration in Orange and Sweet Lime (Mosambi) Juice and Residues

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Abstract

Citrus fruits and juices are a crucial source of bioactive compounds including antioxidant like vitamin C, flavonoids, phenolic compounds and pectins and dietary fibre that are important to human health and nutrition. Flavanon, flavons and flavonons are three type of flavonoids which occur in citrus fruits especially in lemon, orange and musambi. Orange and musambi were collected from Ghatal (Local market), Paschim Medinipur, West Bengal, India. The present study was carried out to investigate the more potent antioxidative and reducing sugar property present among two portion (raw extract and residue extract) of orange and musambi locally available in the market. Orange and musambi were bring from local market and juice was extracted and separate juice and extract in two separate petridish. The petridishes were kept in hot air oven for two days to prepare extract and ready for different biochemical screening. Main findings and observation: Carbohydrate, protein, flavonoid and phenolic compound test are positive. Orange and musambi appeared to maximum inhibition of orange juice 63.67%, orange residue 59.95%, musambi juice 74.39%, and musambi residue 75.49% in 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. So musambi residue has higher inhibition than Orange juice and residue. The carbohydrate contain in musambi juice is greater than orange juice and residue. In conclusion it is suggested that orange and musambi could be a potential source of natural antioxidants that could have grade importance as therapeutic agent in preventing the progress of aging and age associated oxidative stress related diabetes mellitus (type-II). Further study need to use its pharmaceutical benefits as anti-oxidant on some disease.

Key Words: Citrus, flavonoids, orange, musambi, petridishes, antioxidant.

Order: Sapindales

Family: Rutaceae

Genus: Citrus

Species: C. × sinensis

Binomial name

Citrus × sinensis

(L.) Osbeck

1. INTRODUCTION:

1.1 History about ORANGE (FRUIT):

Scientific classification

Kingdom: Plantae

The orange (specifically, the sweet orange) is that the fruit of the citrus species *Citrus × sinensis* within the family Rutaceae. The fruit of the sweet orange is taken into account a sweet orange, whereas the fruit of the sour orange is taken into account a bitter orange. The orange may be a hybrid, possibly between pomelo (*Citrus maxima*) and mandarin (*Citrus reticulata*), Probably originating in Southeast Asia, oranges were already cultivated in China as far back as 2500 BC. Spaniards introduced the sweet orange to the American continent within the mid-1500s. Orange trees are the foremost cultivated tree fruit within the world since 1987. (Morton et al., 1987)

Orange trees are widely grown in tropical and subtropical climates for their sweet fruit. The fruit of the orange are often eaten fresh, or processed for its juice or fragrant peel. Sweet oranges currently account for about 70% of citrus production. (Citrus Genome Database).

1.1.1. Nutritional Importance of orange

Parameters	Orange (in 100gm)
Energy	48kcal
Carbohydrate	10.9gm
Protein	0.7gm
Fat	0.2gm
Ascorbic acid	10.98 mg
Total phenolics	22.0mg
Flavonoids	9.2 mg
Proanthocyanins	3.4 mg

(Approval for this human study was granted by the Department of Scientific Program Management of the institute.)

1.2 History about Citrus Limetta (FRUIT):

Scientific classification

Kingdom: Plantae

Genus: *Citrus*

Species: *C. limetta*

Binomial name

Citrus limetta

Citrus limetta may be a species of citrus, commonly referred to as sweet lemon, sweet lemon, and sweet limetta. it's native to South- and Southeast Asia and cultivated within the Mediterranean Basin. *C. limetta* may be a small tree up to eight m (26 ft) tall, with irregular branches and comparatively smooth, brownish-grey bark. it's numerous thorns, 1.5–7.5 cm (0.59–2.95 in) long. The petioles are narrowly but distinctly winged, and are 8–29 mm (0.31–1.14 in) long. Leaves are compound, with acuminate leaflets 5–17 cm (2.0–6.7 in) long and a couple of .8–8 cm (1.1–3.1 in) wide. Flowers are white, 2–3 cm (0.79–1.18 in) wide. Fruits are oval and green, ripening to yellow, with greenish pulp. Despite the name sweet lemon, the fruit is more almost like a greenish orange in appearance. *C. limetta* grows in tropical and subtropical climates. It begins bearing fruit at 5 to 7 years old, with peak production at 10 to twenty years. it's propagated by seed. As the name sweet lemon suggests, the flavour is nice and mild, but retains the essence of lime. the flavour may be a bit flatter than most citrus thanks to its lack of acidity.

1.2.1. Nutritional Importance of sweet lime

Parameters	sweet lime (in 100gm)
Energy	180 kJ (43 kcal)
Carbohydrates	9.3 g
- Sugars	1.7g
- Dietary fiber	0.5 g
Fat	0.3 g
Protein	0.7-0.8 g
Water	88 g
Vitamin C	50 mg (60%)

Calcium	40 mg (4%)
Iron	0.7 mg (5%)
Phosphorus	30 mg (4%)
Potassium	490 mg (10%)

Role of fruits in Diabetes:

The increase within the prevalence of type 2 diabetes across the planet has become a crucial public health issue as long as this disease ranks among the leading causes of blindness, kidney failure and lower limb amputation, besides being a big risk factor for coronary heart condition and stroke (American Diabetes Association, 2019). From recent data on the frequency of diabetes in several countries, increasing from 285 million in 2010 (6.4%) to 439 million in 2030 (7.7%) has been estimated (Shaw et al., 2010). There is compelling clinical test evidence that diabetes and its onset are often prevented or its onset is often delayed by lifestyle interventions, thus it's critical to delineate which are the simplest dietary strategies (Gong et al., 2019). The importance of diet within the context of diabetes medical nutrition therapy has been the subject of several reviews, but few have focused on diabetes prevention (Mann et al., 2004). According to the hierarchy of research designs, the results of randomized controlled trials are considered as providing the very best level of evidence, whereas observational studies are viewed as having less validity because they're reportedly amenable to varied biases, including residual confounding. Among observational studies, the very best levels of evidence are often obtained from large prospective cohorts with adequate control of confounders. The potential for bias is higher in case-control and cross-sectional studies. For this reason, a specific effort is formed to differentiate the

studies consistent with the hierarchy of their research designs.

Lifestyle in diabetes prevention:

Several randomized clinical trials involving lifestyle changes with an outcome on incident diabetes that were conducted in individuals at high risk are published. During this study 577 individuals with impaired glucose tolerance (IGT) were randomized to dietary counseling, increased exercise, diet plus exercise, or control. (Gong et al., 2019).

The cumulative 6-year incidence of diabetes was significantly lower within the diet group (44%), the exercise group (41%), and therefore the diet-plus exercise group (46%) than within the control group (67%). The Finnish Diabetes Prevention Study (FDP) randomized 522 overweight volunteers with IGT to usual care or diet plus exercise recommendations. Main dietary goals within the active intervention group were a low-fat diet (<30% energy as fat) with <10% saturated fatty acids (SFA) and dietary fiber intake >15 g/1000 kcal. Participants in this group also received instructions to increase exercise and were targeted for weight loss, which was accomplished to nearly 5% of baseline weight (Tomasova et al., 2019).

Main dietary goals in the intensive lifestyle intervention group were a very low-fat diet (<25% energy as fat) with <10% saturated fatty acids (SFA) and increased fiber intake. In this study, 50% of participants in the lifestyle arm had achieved the weight loss goal at the end of the program and this was associated with a 58% reduction in incident diabetes compared with the usual care group. Despite the success of these landmark lifestyle trials, an important question remains to be answered. Participants in the active treatment arms sustained a significant weight loss, which

appeared to be the driving force to reduce incident diabetes. Thus it is unclear whether diet or exercise alone plays a significant role in preventing diabetes. Nevertheless, post hoc analyses of the FDP study indicated that both diet and physical activity played an important role in reducing diabetes incidence. In this study, a low intake of total fat and a high intake of dietary fiber were identified as significant predictors of a sustained reduction in weight and less progression to diabetes, even after adjustment for other risk factors (Lindstrom et al., 2006).

Also, participants who increased structured leisure-time physical activity to moderate-to-vigorous or strenuous were less likely to develop diabetes (Laaksonen et al., 2005).

That an important proportion of participants in the active treatment arms of these lifestyle trials regained weights during follow-up also raises the question of whether these strategies are effective on the long term. However, it has been shown recently that their benefit in reducing diabetes risk extends beyond the termination of active intervention (Lindstrom et al., 2006),

Demonstrating for the first time the long-term efficacy of lifestyle treatment in reducing the risk of diabetes. Finally, whereas there is firm evidence that lifestyle changes can prevent diabetes in long-term trials, little is known whether they can reduce cardiovascular disease (CVD) morbidity or mortality as well. Recently, the 20-year follow-up results from the Chinese Da Qing Study and results of more than 10 years of follow-up from the FDP Study showed no statistically significant differences in CVD outcomes between the intervention and control groups (Li et al., 2008).

Nevertheless, the results from these trials confirm the long-term safety of lifestyle changes as diabetes prevention strategy.

This kind of long-term evidence is not available for different weight loss approaches. An additional randomized clinical trial conducted in Japan examined lifestyle intervention for the prevention of diabetes while attempting to achieve and maintain ideal body weight (Kosaka et al., 2005).

A non-calorie-restricted traditional Mediterranean diet enriched with high-fat foods of vegetable origin decreased the incidence of diabetes after a median follow-up of 4.0 years. Weight loss was a major driving force in reducing the incidence of diabetes. Of note, in our study diabetes risk reduction occurred in the absence of significant changes in body weight or physical activity, stressing the importance of the food pattern alone in preventing diabetes (Salas et al., 2011).

The role of carbohydrates

Large observational studies have provided conflicting results, showing both positive and negative associations of total carbohydrate intake with diabetes risk (Park et al., 2001). Instead, the quality of carbohydrates ingested may be of extreme importance in determining the ability to raise glucose levels, which depends to a great extent on its influence on gastrointestinal transit and the velocity of nutrient absorption, and the long-term risk of diabetes (Barclay et al., 2008).

Four important qualitative features of dietary carbohydrates relevant to diabetes are fiber, wholegrain seeds, glycemic index (GI), and simple sugars in beverages. Dietary fiber is the indigestible component of complex carbohydrates. Observational studies almost unanimously suggest that high intakes of fiber or fiber-rich wholegrain foods are independently associated with a reduced risk of obesity and diabetes (Mohan et al., 2009). Residual confounding is possible, however, because high intake of

fiber or wholegrain may also be markers of a healthier lifestyle (Li et al., 2008) Soluble viscous fiber plays an important role in controlling postprandial glycemic and insulin responses and satiety, which is attributable to its effect of slowing gastric emptying and intestinal nutrient absorption (Pepa et al., 2018). Most prospective studies have found that insoluble fiber, but not soluble fiber, relates inversely to incident diabetes (Schulze et al., 2007).

Although the mechanisms are unclear, an increase of gastric inhibitory polypeptide (GIP), anti-inflammatory effects, and even changes of the gut microbiotic can be operational to increase insulin sensitivity (Rahman et al., 2020).

Glycemic index (GI) indicates the glucose-raising effect of a food in comparison with a standard (usually white bread), whereas glycemic load (GL) is the product of the GI and carbohydrate content per serving, and both were associated with an increased risk of diabetes in a meta-analysis of observational studies (Barcalay et al., 2008).

Recent results from the prospective EPIC-Netherlands cohort showed that diets high in GI, GL, and starch and low in fiber were also associated with an increased diabetes risk (Sluijs et al., 2010). Furthermore, a recent systematic review of short-term randomized feeding trials in diabetic patients showed that low-GI diets were associated with improvements of glycemic control, insulin sensitivity, and other intermediate biomarkers (Thomas et al., 2009).

There is also an indication that lean, physically active subjects can adjust to the postprandial glucose challenge following a high-GI meal by increasing insulin sensitivity, while obese, inactive subjects must increase their insulin secretion in order to reestablish glucose homeostasis (Tagi et al., 2019).

Commercial beverages containing simple sugars, such as artificially sweetened beverages (soft drinks, non-diet colas, sodas) and natural or commercial fruit juices, which are oftentimes sugar-enriched, are prototypes of high-GI foods that are consumed in significant amounts worldwide. Observational studies have consistently shown that their consumption relates to an increased risk of diabetes after adjustment for various confounders (Schulze et al., 2007).

All the desirable features of being high-fiber, wholegrain, and low-GI are inherent to some high-carbohydrate foods, like dietary pulses (dried leguminous seeds, including chickpeas, beans, peas and lentils). It is not surprising, therefore, that feeding trials assessing the effect of pulses, alone or as part of low-GI or high-fiber diets, on markers of glycemic control in persons with and without diabetes have generally shown a considerable benefit (Sievenpiper et al., 2009).

The present study was to evaluate the phytochemical screening, flavonoid and carbohydrate content and other nutrients also as DPPH antioxidant activity during which part is more beneficial for human health.

2. Method and Materials

2.1. *Collection of Fruits (orange & lime sweet musambi):*

Orange and Musambi were collected from ghatal town (local market).

2.2. *Preparation of Juice from orange and musambi :*

The orange and musambi were picked from nearest market and edible portion are cut into small pieces. The materials were weighted and extract juice by mixer grinder and allowed to stand at room temperature for 24 hours before filtration. The filtrates were diluted to produce extract needed for

the assay and preserved in the refrigerator for use.

2.3. Preparation of residue from orange and musambi:

The orange and musambi were purchased from nearest market and washed the fruits with water. Then cut the fruits by knife. The juice was extracted by mixer grinder. Residue separated from juice and peel and dried to hot air oven upon the petri-dish.

2.4. Phytochemical Screening

Phytochemical Screening were performed using standard procedures

2.4.1. Test for Tannins

About 0.5g of the extract was taken in 10 ml of water in a test tube(Borosil) and then filtered. A few drops of 10% ferric chloride (Merck) was added and then filtered. Observation of Brownish green or blue-black colouration.

2.4.2. Test for Alkaloids

About 0.5g of extract was dissolved in 5 ml of 1% HCl (Qualigen) and was kept in boiling water bath. The 1 ml of filtrate was treated with drops of Mayer's reagent (Merck). A radish brown precipitate indicates due to the presence of deoxy sugars.

2.4.3. Test for Cardiac glycosides

About 0.5 ml of the extract was dissolved in 2 ml of glacial acetic acid(Merck) containing one drop of 1% FeCl_3 along with conc. H_2SO_4 (Qualigen). A brown ring obtained at the interphase indicated the presence of deoxy sugar which is that the characteristic of cardiac glucoside. A violet ring appeared below the ring while within the ethanoic acid layer a greenish ring appeared just above ring and gradually spread throughout the layer.

2.4.4. Test for Flavonoids

2 ml of dil. NaOH (Merck) was added to 2 ml of the extract and shake well. Yellow colour indicates presence of Flavonoids.

2.4.5. Test for Saponins

0.52 ml distilled water was added to 1 ml extract and shaken vigorously. A stable persistent froth indicates the presence of Saponins.

0.53

2.4.6. Test for Phenols

Equal volumes of extracts and FeCl_3 were mixed very carefully. Deep bluish green solution indicates presence of phenols.

2.4.7. Test for Protein

0.5g of the extract was added to 10 ml distilled water. Mixture was left for 3 hours. Then the solution was filtered. Then 2 ml portion of the filtrate was added to 0.1 ml millon's reagent(stanbio). Then yellow colour precipitation appeared.

2.4.8. Test for Carbohydrate

0.5g of the extract was shaken vigorously with water and then filtrated. Then Molisch reagent (Stanbio) and 1.0 ml concentrated H_2SO_4 (Qualigen) added. A brown ring was appeared at interphase.

2.4.9. Test for Phytosterol

0.5 ml chloroform extract is added with 1 ml of concentrated H_2SO_4 . Then a reddish brown colour is appear in choloform layer which indicate the presence of phytosterol.

2.5. Carbohydrate estimation:

At first collect bitter gourd dust and 1 gm dust is weighted by weighing balance. Then it's transfer to a 250 ml conical flask. 6(N) HCl (50ml) is added thereto and mixed thoroughly and capped the conical flask tightly with cotton. The mixture is then hydrolyzed for 30 min during a reflex condition. After 30 min conical flask is cooled in water. Then the mixture is neutralized with anhydrous Na_2CO_3 is

added still to off effer.10ml of 10% Znso4 and 10ml of 10% Ba(OH)₂ are added thereto and mixed thoroughly. the entire amount is then transfer to a 100ml volumetric flask. the quantity is then made upto the mark with distil water and mixed properly. The mixture is then filtered and filtrate is then transferred to the burette.10ml Benedict quantitative reagent(BQR) is taken in 10ml conical flask and a pinch of Na₂co₃ is added thereto . Titrate with the filtrate against BQR and end point is recorded a minimum of 2 times (Laboratory Note Book on Biochemistry,2013)

2.6. DPPH antioxidant assay

IC50 values were calculated from the plotted graph of scavenging activity against the concentrations of the samples. IC50 is defined because the total antioxidant necessary to decrease the initial DPPH radical by 50%. IC50 was calculated for all the extracts supported the percentage of DPPH radicals scavenged. Freshly prepared extracts of the dried plant material were subjected to screening for their possible antioxidant activities.



3. RESULTS & DISCUSSION :

Table 4.1: Percentage of moisture contain in different parts of orange and musambi

Different parts of fruits	Before extract weight raw (in gm)	Residue weight(in gm)	Moisture(in ml)	Percentage of residue(%)
Orange juice extract(OJE)	260.7	37.2	223.5	14.26
Orange residue extract(ORE)	41.6	8.87	32.73	21.32
Musambi juice extract(MJE)	188.3	22.7	165.6	12.05
Musambi residue extract(MRE)	99.02	20.33	78.69	20.53

Phytochemical screening of human revealed same differences in the constituents of the Orange and musambi. The moisture contain

in orange juice is 14.26%,orange residueb 21.32%,musambi jucie 12.05% ,musambi residue 20.53% (Table 4.1). Its may be the

effect of their water holding capacity of musambi.
presence dietary fibre in orange and

Table 4.2: Qualitative analysis of presence and absence of different phyto compound present in different parts of orange and musambi :

TEST	Orange juice extract(OJE)	Orange residue extract(ORE)	Musambi juice extract(MJE)	Musambi residue extract(MRE)
<i>Foam test for saponin</i>	-	-	-	-
<i>Molisch's test for carbohydrate</i>	+	+	++	+
<i>Lead acetate test for flavonoid</i>	++	+	+++	++
<i>Keller- Killiani test for glycosides</i>	-	-	-	-
<i>Dragendroff's test for alkaloid</i>	+	+	++	+
<i>Ferric chloride test for phenolic compounds and tannin</i>	+	+	++	++
<i>Salkowskis reaction test for phytosterol</i>	++	+	+++	+

(+) indicate present,(-) indicate absent

DPPH, flavonoids, protein, carbohydrate test positive for Orange, musambi and residue (Table 4.2). The presence of flavonoids in Orange and musambi is likely to be responsible for the free radical

scavenging effects observed. Flavonoids is phenolic compounds and phenolic are a major group of compounds that act primary antioxidant or free radical scavengers (Diploc).

Table 4.3 Analysis of Carbohydrate (Reducing Sugar) content of different processed juice and residues.

Samples	Burette reading (ml)		Differential reading (ml)	Amount of total carbohydrate (g %)
	Initial reading (ml)	Final reading (ml)		
Orange Juice extract	0	3.3	3.3	60.60
Orange residue	0	6.2	6.2	32.26

Extract				
Musambi Juice extract	0	3.2	3.2	62.5
Musambi residue Extract	0	5.3	5.3	37.73

The carbohydrate content in Orange juice is 60.60 gm%, orange residue 32.26 gm%, musambi juice is 62.50 gm% and musambi residue 37.73 gm%. Thus the

carbohydrate content in musambi juice and residue is greater than orange juice and residue, (Table 4.3).

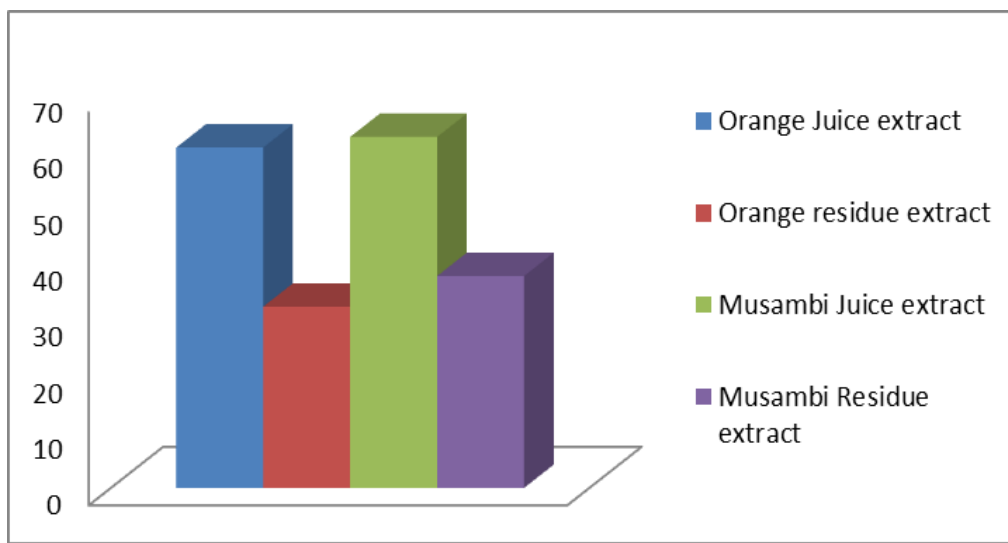


Fig: 1 Bar diagram represent comparison of carbohydrate contain in different parts of orange and musambi.

4.4 Table showing % inhibition by different concentrations of orange and musambi juice and residue extract.

Concentration in (mg/ml)	Percentage (%) inhibition of DPPH			
	% of Inhibition Orange Juice Extract	% of Inhibition Orange Residue Extract	% of Inhibition Musambi Juice Extract	% of Inhibition Musambi Residue Extract
0.25	24.07	23.19	23.85	24.28
0.5	36.76	35.44	38.94	41.79
0.75	48.57	49.67	56.67	59.73
1	63.67	59.95	74.39	75.49
1.2	83.25	71.25	90.55	85.58
1.5	90.21	85.36	99.12	95.68
IC 50 Value	0.73 mg/ml	0.71 mg/ml	0.60 mg/ml	0.52 mg/ml

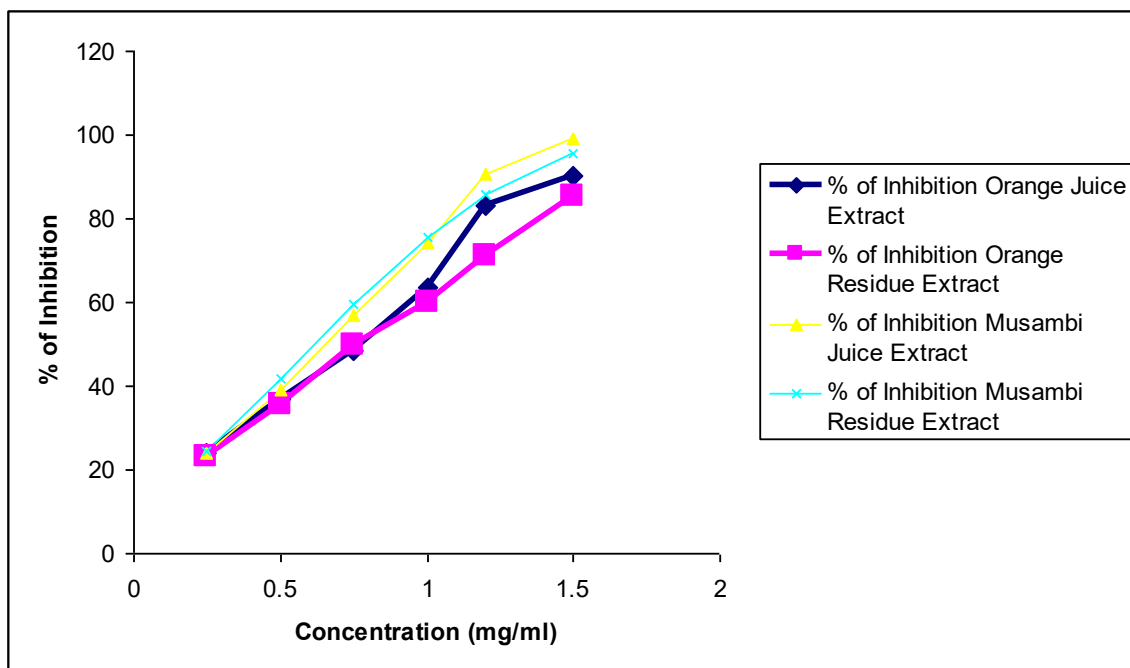
Oxide scavenging methods using UV- VIS spectrophotometer were employed. DPPH radical scavenging test is predicated on the exchange of hydrogen atoms between

the antioxidant and therefore the stable DPPH radical . DPPH may be a stable radical at temperature which accepts an electron or hydrogen radical to

make a stable diamagnetic molecule. DPPH radical is reduced to the corresponding hydrazine, a color change

of the answer from violet to yellow is observed which is monitored spectrophotometrically.

Fig: 2 Showing Percent inhibitions of DPPH activity of Orange Juice Extract, Orange Residue Extract, Musambi Juice Extract and Musambi Residue Extract



The carbohydrate content in fruit juice is 60.60 gm%, orange residue 32.26 gm%, musambi juice is 62.50 gm% and musambi residue 37.73 gm%. Thus the carbohydrate content in musambi juice and residue is bigger than fruit juice and residue, (Table 4.3). Oxide scavenging methods using UV-VIS spectrophotometer were employed. DPPH radical scavenging test is predicated on the exchange of hydrogen atoms between the antioxidant and therefore the stable DPPH radical. DPPH may be a stable radical at temperature which accepts an electron or hydrogen radical to make a stable diamagnetic molecule. DPPH radical is reduced to the corresponding hydrazine, a color change of the answer from violet to yellow is observed which is monitored spectrophotometrically. More reduction of

DPPH radical is said to the high scavenging activity of the actual extract. The reduction capability of DPPH radicals decided by the decrease in its absorbance at 517 nm, which is induced by antioxidants. the many decrease within the concentration of the DPPH radical is thanks to the scavenging ability of Orange and musambi. The half maximal inhibitory concentration (IC50) may be a measure of the effectiveness of a substance in inhibiting a selected biological or biochemical function. This quantitative measure indicates what proportion of a specific drug or other substance (inhibitor) is required to inhibit a given organic process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half. it's commonly used as a measure of antagonist drug

potency in pharmacological research. consistent with the FDA, IC50 represents the concentration of a drug that's required for 50% inhibition in vitro. it's like an EC50 for agonist drugs. EC50 also represents the plasma concentration required for obtaining 50% of a maximum effect in vivo (Earp et al.,2004). Antioxidant activity greater in musambi juice and residue than fruit juice and residue.(Table-4.4)

Summary

Including antioxidant like vitamin C, flavonoids, phenolic compounds and pectin's that are important to human health specially on diabetics mellitus. Orange and musambi contain outstanding phytochemicals that are high in antioxidant and anti-cancer and also anti diabetic properties also. In all over India, folk consume Orange and musambi which are available in local market. Orange and musambi mainly contain great deal of reducing sugar, Vit-c but also contain great deal of antioxidant properties on Orange and musambi juice and residue. The main finding of the study was administered to look out the stronger antioxidative properties among Orange and musambi juice and residue. The main finding of this study are protein, flavonoids, carbohydrate test positive. The related antioxidant property of Orange and musambi juice and residue may provide potential therapeutic intervention against oxidative stress and DM (type-II).

Conclusion

Here I analysis the residue a part of orange and musambi and shown there was high amount of reducing sugar, high fiber and DPPH level in fruit crush and also residue. So, I suggest that not only the fruit crush but also the entire fruit is beneficial to regulate Type- II DM and

stress factors. last it's suggested that orange and musambi might be a possible source of natural antioxidants that would have grade importance as therapeutic agent in preventing the progress of aging and age associated oxidative stress related DM (type-II). So, consumption of total fruit has great implication of health.

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