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Antioxidant and *In-vitro* Antidiabetic Activities of Fermented Peels of *Citrus* x *Sinensis* (l.) Osbeck (Rutaceae)

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ABSTRACT

Introduction: The various parts of Citrus sinensis plant have been employed over the years for medicinal purposes. As part of the concept of waste to wealth, the medicinal importance of the peels was exploited. This study aimed at determining the antioxidant and in vitro antidiabetic activities of fermented peel extracts of C. sinensis.

Method: Fermentation of the peels was carried out, and phytochemical tests were done. DPPH free radical scavenging activity, anti-lipid peroxidation and Antidiabetic assay method were performed in vitro using standard procedures.

Results: C. sinensis were rich in polyphenols and reducing sugars. Radical scavenging assay showed an IC50 of 0.57µg/ml and 1.60µg/ml for fresh peels and dried peels respectively for DPPH assay, and 0.67µg/ml (fresh) and 0.72µg/ml (dried) for anti-lipid peroxidation assay. The α-amylase inhibition assay showed that the fermented fresh peels and fermented dried peels had IC50 values of 2.23µg/ml and 2.73µg/ml respectively. The α-glucosidase assay showed better inhibition by the extracts. Fermented fresh and dried peels had IC50 values of 0.03µg/ml and 0.03µg/ml respectively.

Conclusion: Fermented fresh peels of C. sinensis expressed relatively stronger protection in the antioxidant and antidiabetic assays than the fermented dried peels. This study revealed that extracts can be considered as an important addition to the therapeutic management for diabetes.

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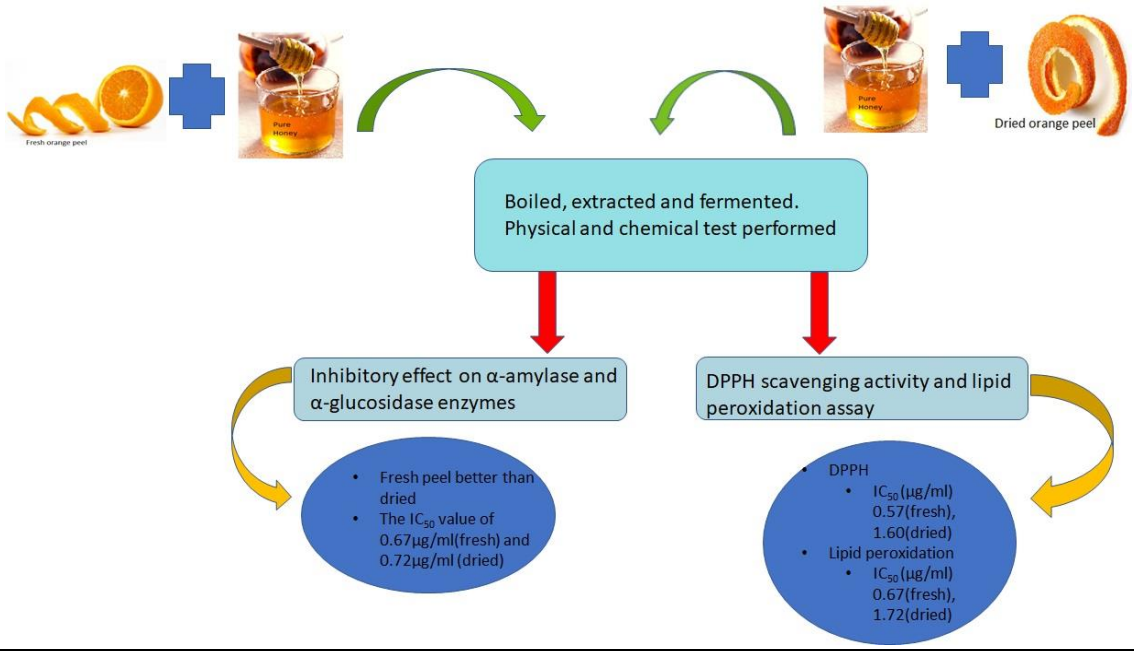
GRAPHICAL ABSTRACT



1- Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by an increase in glucose levels due to absolute or relatively low insulin deficiency. It is associated with several complications such as eye, renal, cardiovascular, and neurological problems over a long term. This disease is associated with symptoms such as polyuria, polydipsia, polyphagia, fatigue, weight loss, delayed wound healing, blurred vision, increases in urine glucose levels, etc. (1). Reports show that Africans present with about 80% of cases of diabetes which are undiagnosed. This could be because diabetes is not symptomatic or produces because it only shows mild symptoms that are either ignored or attributed to other causes (2).

1.1 Management of Diabetes in Traditional Medicine

It has been reported that 75 - 90% of the populations in developing countries rely on traditional remedies for treatment of various diseases. Patients prefer their services because they perceive herbal treatments as affordable, easily available, and acceptable compared to orthodox medicines. Traditional Medicine Practitioners (TMPs) are also easily accessible and offer more personalized care compared with conventional doctors (3).

A study carried out by Kasole *et al.,* 2019 showed some of the commonly used traditional medicines in the management of diabetes by people. Moringa leaves and seeds (*Moringa oleifera*) (25.2%), sour sop leaves (*Annona muricata*) (11.5%), black plum bark *(Syzygium cumini)* (11.5%), okra pods (*Abelmoschus esculentus*) (9.2%), avocado seeds *(Persea americana*) (9.2%), and lemongrass (*Cymbopogon citratus*) (8%), and about 25.3% of people reported using other traditional medicines. (2)

1.2 Significance of α-Amylase andα-Glucosidase in Diabetes.

A life-threatening disease such as diabetes mellitus keeps affecting more people daily globally, insulin plays a key role in the regulation of carbohydrate, fat, and protein metabolism. In a case where there is deficiency of insulin, this will impair the metabolisms of carbohydrate, fat, and protein. (4). Certain enzymes are responsible for accelerating the breakdown of carbohydrate into simple sugars, hence increasing the blood plasma glucose levels. Thus, an approach to hyperglycemia therapy will be to inhibit the two carbohydrate hydrolyzing enzymes (αamylase

and α-glucosidase). α-Amylase is responsible for hydrolyzing the starch, which breaks down to glucose before it is absorbed (5). Hence, inhibiting α-amylase will cause a reduction in postprandial hyperglycemia (6). α-Glucosidase is responsible for cleavage of disaccharides into glucose in the small intestine. Many natural plant constituents have better activity for α-glucosidase inhibition than α-amylase inhibition.

Type 2 diabetes (T2D) occurs because of an imbalance between blood sugar absorption and insulin secretion. Postprandial hyperglycemia has been found to play a significant role in the development of T2D and its complications (7). Alpha-amylase is an enzyme responsible for hydrolyzing complex starches to oligosaccharides, while alpha-glucosidase hydrolyzes oligosaccharides to glucose and other monosaccharides. These inhibitors help to reduce postprandial hyperglycemia. Acarbose is a standard inhibitor of carbohydrate metabolic enzymes in the gastrointestinal tract amongst others, but a drawback to these conventional drugs is associated with side effects such as diarrhea and other intestinal disturbances such as bloating, flatulence, cramping, and abdominal pain (8). The increased demand for herbal medicine led to continuous exploitation of novel and effective plant-derived compounds for commercialization. Herbal drugs have been used globally for diabetic treatment over thousands of years due to their traditional acceptance, affordability, and lesser side effects.

This has led to the screening of more α-amylase and α-glucosidase inhibitors in medicinal plants with fewer side effects.

1.3 Sweet Orange (Citrus sinensis (L.) Osbeck (Rutaceae)

Citrus *sinensis* is a small, shallow-rooted evergreen tree about 6-13 m high, leaves are smooth, oval, dark green above, glossy, with a distinctive smell often like the fruit, petiole winged. Its antidiabetic activity is due to bioflavonoids such as hesperidin and naringin present in citrus fruit peels (9). The potential of orange peel and juice having anti-diabetic activity is mediated by anti-peroxidation, inhibition of α-amylase enzyme activity that is responsible for the conversion of complex carbohydrates to glucose, increased hepatic glycogen content, stimulation of insulin secretion, and repair of secretory defects of pancreatic β-cells (10).

1.4 Honey (Apis mellifera Linnaeus, Family: Apidae)

Honey is a natural sweet product made by honeybees (*Apis mellifera* Family Apidae) from the nectar of blossoms or from secretions on living plants, which the bees collect, transforms and store in honeycombs (11). The abundant source of phenolic compounds in honey has been proven to lower the lipid profile, particularly cholesterol levels. The exact mechanism of honey in which this is done is not clearly determined. Presence of Phenolic compounds have been reportedly associated with improvement of coronary vasodilation, prevention of blood clots and protection of LDL-cholesterol from oxidation (12).

1.5 Rationale of Study

A wide range of plants and plant derived compounds are being used in present day for the treatment of various diseases, many of which have been employed as antidiabetic drugs as they have shown to be therapeutically safe and effective in controlling blood sugar levels in humans (6). Oranges are an all-season fruit that grows abundantly in Nigeria, one of the largest producers of citrus in Africa (13). Oranges can be consumed as fresh fruit and are commonly pressed or squeezed to produce orange juice. The peels are taken as waste. One of the concepts of this research is to turn Waste to Wealth.

This study was carried out to investigate the inhibitory activity of fermented fresh and dried orange peels (*Citrus* x *sinensis)* on α-amylase and α-glucosidase and their extent of antioxidant properties. This study was carried out controlled

fermentation for both fresh and dried peels of *Citrus* x *sinensis* using natural honey which was done for a period of 28 days leading to biotransformation of these extracts used for this study. Since metabolic changes had occurred within the initial extracts used, proper identification of the constituents present in the extracts were conducted through phytochemical analysis using standard procedures prior to other analysis.

1.6 Aim of the work

This aim of the work is to carry out in vitro studies on α-amylase and α-glucosidase inhibitory activities and antioxidant properties of fermented peels of *Citrus* x *sinensis* (L.) Osbeck (Rutaceae). The phytochemical screening, antioxidant test and the in vitro activities of the α-amylase and α-glucosidase inhibitory of fermented peels of *Citrus* x *sinensis* (L.) Osbeck (Rutaceae) were evaluated.

2. METHOD AND MATERIALS

2.1 Chemicals, Reagents, and Instruments

Biomate 3 Ultraviolet spectrophotometer (Rochester, New York, USA) was used to run the assays. Absolute ethanol (EMD Milliform Corporation, Germany), Glacial acetic acid (GFS Chemicals, Inc., Columbus), Ethyl acetate, Acetic acid, Benzene, concentrated Sulphuric acid (BDH Binder), Lead acetate, Ferric chloride (Sigma Aldrich Germany), 1% hydrochloric acid, diluted Sulphuric acid (BDH Binder) , Chloroform (Merck), Methanol ((EMD Milliform Corporation, Germany), Acetic anhydride, Fehling's A reagent (Sigma Aldrich Germany), Fehling's B reagent (Sigma Aldrich Germany), Dragendorff reagent (Merck), Wagner's reagent (Merck), Mayer's reagent (CDH Fine Chemical), Distilled water and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Standard, α-Amylase, α-Glucosidase, Phosphate buffer, 1% Soluble Starch, Maltose, A glucose estimation kit (GLUC-PAP) from RANDOX, Dinitrosalicylic acid, Liver homogenate, Acarbose, Ascorbic acid, Trichloroacetic acid, Thiobarbituric acid (Sigma Aldrich Germany), Ferrous sulphate (GHCL, India), Sodium bicarbonate.

2.2 Collection and Preparation of Plant Material

Sweet orange peels were collected on the 12th of July 2019. Collection was done for a period of one week around Idi-Araba environs in Lagos state, Nigeria (N 6° 30' 0", E 3° 21' 0"). A portion was dried in the oven at 50ºC for 24 hours and the other portion was left to prepare the fresh peel extract. After proper drying of the peels, it was then pulverized using the mechanical grinder for adequate size reduction. Pure natural honey was obtained from Department of Pharmacognosy of the University of Lagos Model Herbal clinic, Idi-Araba, Lagos, Nigeria.

2.3 Fermentation of Orange Peels

A total of 2 kg of the pulverized orange peel was extracted in 6.25L of water (containing 0.5L of fresh natural honey). The mixture was boiled for 15 minutes with consistence stirring. The resultant solution was allowed to ferment for 28days. The same process was done with 2 kg of fresh peels of fresh peels.

2.4 Phytochemical Screening

Phytochemical tests were carried out on the liquid extracts of dried fermented orange peels and fresh fermented orange peels of *Citrus sinensis.* Orange peel preparation was investigated for phytochemicals using standard procedures (14).

2.5 Physical and General Test for Pure Honey

The following test for honey were carried out using standard procedure; Physical Test (odour, colour, taste), solubility Test (Water and Alcohol), Ignition Test, Resorcinol Test, Fehling's Test, Flocculation Test, Fieche's test and Molisch's Test (15, 16).

2.6 DPPH Radical Scavenging Assay

The DPPH free radical scavenging activity of fermented orange peel extracts were determined using a slightly modified method of Hua, *et al*., 2014, Pękal & Pyrzynska 2015 (17,18). DPPH

solution (0.01%w/v) was prepared in 95% methanol. The crude extracts were mixed with 95% methanol to prepare stock solutions of (5%v/v) by adding 5ml of each of the samples to a beaker. Final volumes were made up to 100ml with methanol. From this stock solution different concentrations of extracts were obtained by serial dilution (1%, 2%, 3%, 4% and 5%). To 3ml of each of the solutions, 1ml of methanol solution of DPPH (1mM) was added, the mixture was shaken and left to stand at room temperature in a dark closet for 30mins and absorbance was measured at 517nm using a UV-visible spectrophotometer. A stock solution of Vitamin E at the concentration of 5%v/v was prepared in 95% methanol. From this stock solution different concentrations ranging from 1%-5%v/v was prepared and used as the reference standard. To 3ml of each of the solutions, 1ml of methanol solution of DPPH (1mM) were added, the mixture was shaken and left to stand at room temperature in a dark closet for 30mins and absorbance was measured at 517nm using a UV-visible spectrophotometer. All determinations were carried out in triplicates. The blank solution was prepared ed containing only methanol and DPPH (17,18).

The percentage inhibition of DPPH was calculated using the equation below.

$$Percentage\ inhibition = \frac{Ac-Aa}{Ac} X\ 100\% \qquad [1]$$

Where Ac is the absorbance of the control sample and Aa is the absorbance of the extract sample

The same procedure as with the ascorbic acid standard was carried out with each of the two extract samples (19).

2.7 In vitro anti-Lipid Peroxidation Assay of Fermented Dried and Fresh Citrus x sinensis

Standard method for estimation of thiobarbituric acid reactive substances was used to assay the degree of lipid peroxidation (20), with slight modification of the method. Freshly excised liver homogenate was grinded and mixed with cold phosphate buffer saline (pH 7.4). Extract concentrations of (25 ug/ml, 50 ug/ml, 100 ug/ml, 200 ug/ml, and 500 ug/ml) were made and 100 µl of (15 mM) ferrous sulphate was added to each followed by the addition of 3 ml of homogenate. After which they were incubated for 30 mins, 1.5 ml of 10% trichloroacetic acid was added into each tube and incubated for 15 mins, then filtered and each supernatant was added into tubes having 1.5 ml of 0.67% TBA (in 50% acetic acid) and placed in a boiling water bath for 30 mins. The same procedure was carried out for Ascorbic acid as the standard. Concentration of chromogen formed was measured at 532nm. Anti-lipid peroxidation was assessed by using the Equation [1] above.

2.8 In Vitro Studies of Dried and Fresh Fermented Citrus x sinensis on α-amylase and α-glucosidase inhibition

α-Amylase inhibitory activity of the extracts was carried out according to the standard method of Ademiluyi and Oboh, 2013 with minor modification (21). Briefly 0.1g of aqueous extracts of fermented *Citrus sinensis* peels were dissolved in 50ml of distilled water (2mg/ml). Serial dilutions of the extracts were made, having concentrations of (25, 50, 100, 200, and 500 ug/ml). 1ml of 1% soluble starch (in 20 mM phosphate buffer pH = 6.8) was added as a substrate into each tube then preincubated at 37°C for 20 min. 0.01g of α–amylase was weighed and dissolved in phosphate buffer (20 mM, pH = 6.8), and 1ml was added to the tubes containing the extracts, they were incubated for 10mins. 1ml of 100 µl Dinitrosalicylic acid color reagent was then added into each tube to stop the reaction and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm using a multiplate reader (Multiska thermo scientific, version 1.00.40). Acarbose at various concentrations (25-500 ug/ml) was used as a standard using same procedure. A concentration containing all reagents except the extract was prepared as the control and each experiment was performed in triplicates.

All experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using equation 1. The 50% inhibition concentration (IC_{50}) of plant extracts against lipid peroxidation, α-amylase and α-glucosidase were calculated (21). Similar procedure was carried out for the α-glucosidase assay, but the enzymes were replaced with α-glucosidase.

Table 1: Phytochemical Evaluation of Fermented Dried Peels of *Citrus* x *sinensis (L.*)

Phytochemical test	Fresh peel preparation	Dry peel preparation
Saponins	–	–
Alkaloids	–	–
Triterpenoids	–	–
Tannins	+	+
Shinoda	+	+
Fehling's	+	+
Keller killiani	+	+
Anthraquinone	+	-
Kedde	+	+
Molisch	+	+
Shinoda's Test	+	+

Key: + means Present, - means Absent

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

The results of the phytochemical analysis of the dried fermented sweet orange peels and fresh fermented sweet orange peels of *Citrus* x *sinensis* are presented in table 1. The extract contains similar phytochemicals except anthraquinone which is absent in dry peel preparation.

The phytochemical analysis suggested that the dried and fresh fermented aqueous extracts of *Citrus* x *sinensis* peels do not contain saponins, terpenoids, alkaloids and steroids. However, both aqueous extracts were found to contain reducing sugars, cardiac glycosides, phenolic compounds, tannins, and flavonoids. However, the difference in the presence of anthraquinones in only the fermented fresh peels and not the fermented dried peels maybe attributed to the application of heat to form the dried peels.

Evaluation of the pH for the aqueous extracts were carried out, both extracts had acidic pH (4.8 for dried extract and 5.63 for the fresh). The variation in pH between the two extracts could be because of differing metabolic processes that occurred in them by action of the enzymes in the honey.

3.2 Physical and General Test for Honey

Natural honey was tested to observe its characteristics by carrying out physical and general tests. Result of these tests is as shown in table 2 and 3. These results confirm the originality of the honey and further prove that it is devoid of adulterants.

Honey gave positive results to both the physical and chemical test as seen in the tables above. This indicates that honey used in his research is pure and unadulterated.

3.3 Percentage inhibition of the samples compare to the standard Vitamin E using DPPH Free Radical Scavenging Capacity

Table 2: Physical Test for Pure Honey

Sample	Colour	Odour	Taste
Pure honey	Dark colour	Strong odour	Sweet
Apis mellifera			
Family: Apidae			

Table 3: General Test for Pure Honey

Test	Honey reaction
Solubility in water	Insoluble
Solubility in absolute ethanol	Soluble
Ignition Test	Evolution of gas; Gas turned blue litmus paper red
Resorcinol Test	Presence of Furaldehyde confirmed
Fehling's test	Reducing sugar Present
Flocculation test	Gum/mucilage absent
Fieche's test	Transient pink obtained. Honey is pure
Molisch's Test	Positive test

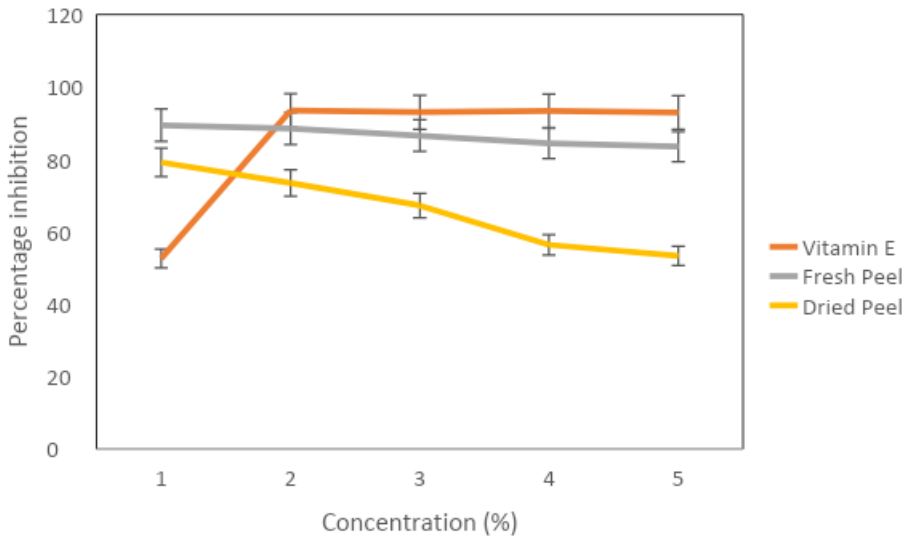


Fig. 1 Percentage inhibition against concentration of the extracts with DPPH free radical.

DPPH is a compound that is a free radical. The following results were obtained for the ability of these extracts to scavenge this free radical (the graphical representation, Fig. 1) as well as the IC_{50} of the extracts (table 4).

The free radical scavenging activity of the extracts was studied by their ability to reduce the DPPH, a stable free radical and any molecule that can donate an electron or hydrogen to DPPH. The molecule should react with it and bleaches the DPPH absorption (22). Figure 1 above show the chart of percentage inhibition of the extract against concentration using the DPPH assay method.

The results of the percentage inhibition for the extracts tested for DPPH free radical scavenging ability showed that the aqueous extracts of fermented dried and fermented fresh *Citrus* x *sinensis* peels did not follow a concentration dependent manner up to the highest concentration rather their scavenging ability decreased with increased concentration. The reason for this could be based on the level of concentration of the extracts used for the assays, since the extracts were in aqueous forms, the active components would have been diluted, this further impacts on the results seen in the different assays. The percentage inhibition of scavenging abilities of fermented dried peels for DPPH showed 53.31% DPPH inhibition at 5%w/v, fermented fresh peels showed 83.48% DPPH inhibition at 5%w/v which was the highest concentration (Fig. 1).

Table 4: IC_{50} of the extracts for DPPH assay

Samples	IC_{50} (%)
Vitamin E	1.25
Fermented fresh orange peels	0.57
Fermented dried orange peels	1.60

The percentage inhibition for the extracts tested for DPPH free radical scavenging ability showed that the aqueous extracts of fermented dried and fermented fresh *Citrus* x *sinensis* peels did not follow a concentration dependent manner up to the highest concentration rather their scavenging ability decreased with increased concentration. The reason for this could be based on the level of concentration of the extracts used for the assays, since the extracts were in aqueous forms, the active components would have been diluted and thus further impacts on the results seen in the different assays.

The percentage inhibition of scavenging abilities of fermented dried peels for DPPH showed 53.31%, fermented fresh peels showed 83.48% DPPH inhibition at 5%w/v which was the highest concentration.

The IC_{50} is the concentration of an inhibitor where the response (or binding) is reduced by half. The lower the IC_{50} value, the better the activity. The IC_{50} value for the fermented dried peels was 1.60µg/ml, which was high, showing a weaker DPPH scavenging ability while the fermented fresh peels showed less IC_{50} value of 0.57µg/ml comparable to the standard Vitamin E value of 1.2µg/ml. Based on these results, fermented fresh peels show higher DPPH scavenging ability than fermented dried peels.

3.4 Anti-Lipid Peroxidation Assay

An assay on the inhibition of lipid peroxidation by the extracts was carried out. The results are shown (the graphical representation, Fig. 2) as well as the IC_{50} of the extracts (table 5).

In the anti-lipid peroxidation assay with thiobarbituric reactive substance method, the ability of the extracts to inhibit the reactive radical peroxide formed from the reaction of one molecule of malondialdehyde (MDA) and two molecules of thiobarbituric acid was tested.

Figure 2 above show the chart of percentage inhibition of the extract against concentration using the DPPH assay method. The percentage inhibitions of the fermented dried and fresh aqueous extracts at 100ug/ml were 72.58% and 72.57% respectively while the standard Ascorbic acid gave an inhibition of 90.82%.

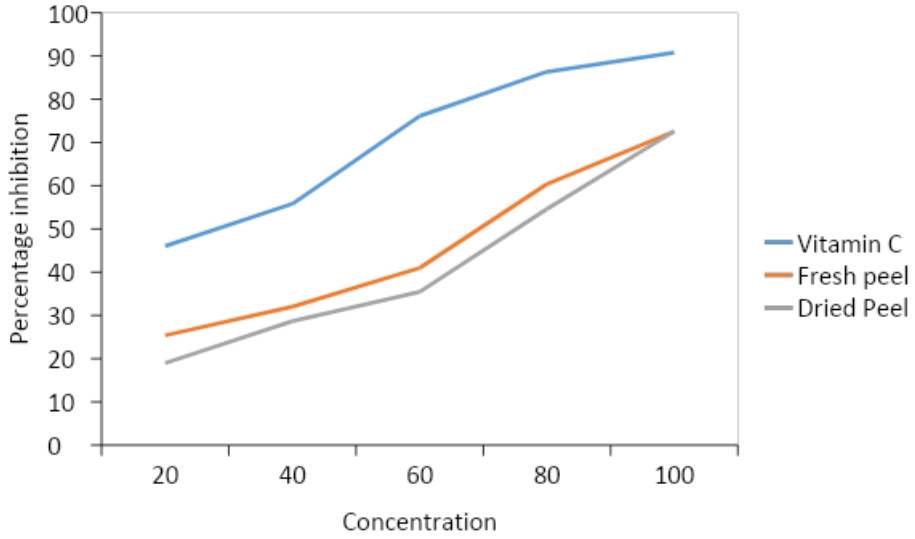


Fig 2. Percentage inhibition against Concentration for Fermented Dried Peels, Fresh peel, and Ascorbic acid for Anti-Lipid Peroxidation Assay.

Table 5: IC_{50} of the extracts and standard for inhibiting Lipid Peroxidation

Samples	IC_{50} (µg/ml)
Ascorbic Acid	0.46
Fermented Dried Orange peels	0.72
Fermented Fresh Orange peels	0.67

Table 6: Concentrations and Percentage Inhibition of Acarbose against α-Amylase.

	Percentage Inhibition (%) against α-Amylase				
Concentration	25	50	100	200	500
Acarbose	51.4±0.08	67.09±0.04	70.67±0.05	76.56±0.06	87.91±0.03
Fresh peel	47.51±0.05	66.45±0.05	64.12±0.08	75.75±0.04	83.72±0.05
Dried peel	23.59±0.04	36.88±0.03	57.14±0.09	56.48±0.03	72.09±0.03
	Percentage Inhibition (%) against α-glucosidase				
Concentration	25	50	100	200	500
Acarbose	41.37±0.07	46.48±0.05	51.59±0.06	68.99±0.08	88.75±0.05
Fresh	47.53±0.04	52.11±0.03	71.58±0.03	73.16±0.05	81.58±0.04
Dried	56.32±0.05	64.74±0.05	72.11±0.03	73.68±0.08	77.37±0.03

Table 7: IC_{50} of the extracts and standard for inhibiting α-amylase

Samples	α-amylase	α-Glucosidase
	IC_{50} (µg/ml)	IC_{50} (µg/ml)
Acarbose	2.13	0.024
Fermented Dried Orange peels	2.73	0.034
Fermented Fresh Orange peels	2.23	0.031

The IC_{50} value for the fermented dried peels was 0.72µg/ml which was higher and showed a weaker antioxidant activity in binding to the free radicals compared to the fermented fresh peels IC_{50} value of 0.67µg/ml that was closer to the standard Ascorbic acid value of 0.46µg/ml.

3.5 In Vitro Inhibitory Activity of The Extracts On A-Amylase & A-Glucosidase

The ability of the extracts to inhibit α-amylase and α-glucosidase (table 6) and IC_{50} of the extracts (table 7) were presented as follows.

This study was carried out to investigate the inhibitory activity of fermented fresh and dried orange peels (*Citrus* x *sinensis)* on α-amylase and α-glucosidase and their extent of antioxidant properties. The rationale behind this study was to carry out controlled fermentation for both fresh and dried peels of *Citrus* x *sinensis* using natural honey which was done for a period of 28 days leading to biotransformation of these extracts used for this study. Since metabolic changes had occurred within the initial extracts used, proper identification of the constituents present in the extracts were conducted through phytochemical analysis using standard procedures prior to other analysis.

From the study findings, it can be said that the inhibitory activity of the extracts relatively increased with increasing concentrations against α-amylase and α-glucosidase. Acarbose was used as the standard for comparison in both assays. In the α-amylase inhibition assay, fermented fresh peels had a percentage inhibition of 83.72% at 500µg/ml and IC_{50} value of 2.23µg/ml comparable to Acarbose percentage inhibition of 87.91% at 500µg/ml and IC_{50} value of 2.13µg/ml, while fermented dried peels had a percentage inhibition of 72.09% at 500µg/ml and IC_{50} value of 2.73µg/ml. The fermented fresh peels had a better inhibitory effect because it had a lower percentage inhibition of the α-amylase and IC_{50} than fermented dried peels. In vitro α-glucosidase inhibitory assay showed that the percentage inhibition at 500µg/ml for fermented fresh peels was 81.58% with an IC_{50} of 0.03µg/ml and the inhibition of fermented dried peels was 77.37% at 500µg/ml having an IC_{50} of 0.03µg/ml as well, which were comparable to Acarbose with IC_{50} of 0.02µg/ml and a percentage inhibition of 88.75% at 500µg/ml. This means that both aqueous extracts had the same inhibitory activity for α-glucosidase inhibitory assay.

Fermented fresh and dried peels showed less effective inhibitory activities for alpha-amylase enzyme inhibition compared to alpha-glucosidase inhibition. This is in accordance with literature that says, plant extracts have mild inhibitory action for α-amylase and strong inhibitory action for α-glucosidase (23, 24).

In this study, the fermented fresh peels showed a significant inhibition of intestinal α-glucosidase and pancreatic α-amylase than that of the fermented dried peels, thus indicating possible reduction in blood glucose levels by the fermented fresh peels.

4. Conclusion

This study showed that the fermented fresh and dried peels contain bioactive that are responsible for antioxidant activity and inhibitory actions of α-amylase and α-glucosidase. The fresh peels consistently showed significantly better inhibitory action in donating hydrogen atom to the radicals produced in both antioxidant assays and in inhibiting α-amylase and α-glucosidase action. These activities can be due to possible loss of major constituents while drying for the fermented dried peels, which also reflected on the results from the phytochemical screening. Furthermore, since natural honey on its own also has diverse pharmacological benefits due to its medicinal constituents, a synergistic effect may be said to have occurred through the fermentation process of the *C. sinensis* peels responsible for the inhibitory activities in this study. This Study revealed that fermented fresh and dried orange peels can be considered as an important addition to the therapeutic management for diabetes. Further studies can be undertaken at the cellular and molecular level, which may further elucidate its mechanism in detail.

Conflict of Interest declaration

The authors declare no conflict of interest (financial or whatsoever) related to this research article.

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