

PERFORMANCE COMPARISON OF BETWEEN CITRUS AURANTIFOLIA(LIME) SEED & MOMORDICA CHARANTIA (BITTER MELON) IN REDUCING BLOOD GLUCOSE

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Abstract

Momordica Charantia (Bitter Melon) and Citrus Aurantifolia (Lime) seeds have been used as a traditional remedy in diabetics due to its hypoglycemic activity. However, its anti-hyperglycemic effect and anti-glycation activity has been demonstrated in vitro and for type-1 diabetics patient, but not in long term clinical study. The study aimed to develop plant based insulin from Momordica Charantia (Bitter Melon) and Citrus Aurantifolia (Lime) seeds in reducing blood glucose. To determine the functional group presence in the both plant based insulin, a qualitative study using Fourier-transform Infrared (FTIR) Spectroscopy was conducted. To compare the effectiveness of Citrus Aurantifolia (Lime) Seed and Momordica Charantia (Bitter Melon) compare with Insulin (Biphasic Isophane), an observation on blood glucose level based on patient's dietary for a period of time had been carried out. Methods: Dried Citrus Aurantifolia (Lime) seeds and Momordica Charantia (Bitter Melon). Then, milled and soaked in ethanol for 3 days. Lime seed and bitter melon were filtered and evaporated to get extract product. Both product was run into FTIR to identify the qualitative of functional group. For patient diet, the patient had Citrus Aurantifolia (Lime) seeds and Momordica Charantia (Bitter Melon) drink for 3 days continuously for each. Result: The functional group presence inside the Momordica Charantia (Bitter Melon) and Citrus Aurantifolia (Lime) seeds was 1,2,4-trisubstituted, Sulfoxide, Primary Alcohol, Phenol, Sulfonyl Chloride, Sulfate, Alkane, Alkene, Alcohol. Based on patient's dietary, Momordica Charantia (Bitter Melon) shows the highest reduction on blood glucose level compared to Citrus Aurantifolia (Lime) seeds and Insulin (Biphasic Isophane).

Keywords: Momordica Charantia (Bitter Melon), Citrus Aurantifolia (Lime), Fourier-Transform Infrared (FTIR) Spectroscopy, Active compound, Patient's Dietary

1.0 INTRODUCTION

Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies. Despite the presence of known antidiabetic medicine in the pharmaceutical

market, diabetes and the related complications continued to be a major medical problem. Antihyperglycemic effects of these plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin

output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes.[5]

More than 400 plant species having hypoglycemic activity have been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which demonstrate alternative and safe effects on diabetes mellitus [5]. The occurrence of both type 1 (juvenile-onset) insulin dependent diabetes and type 2 (maturity-onset) non-insulin dependent diabetes is increasing in most communities [6]. In this research, we will be focusing on the functional group presence and the effectiveness of two plant based insulin, Citrus Aurantifolia(Lime) seed and Momordica Charantia (Bitter Melon).

2.0 EXPERIMENTAL

Objective

- To identify functional group presence in plant based insulin from Momordica Charantia (Bitter Melon) and Citrus Aurantifolia (Lime) seeds in reducing blood glucose.

- To compare the effectiveness of Momordica Charantia and Citrus Aurantifolia in reducing blood glucose.

Materials and Methods

Preparation of Bitter Melon Extract

The bitter melon was sliced into small pieces and then was dried using oven at 65 degree Celsius for 24 hours. Then the dried bitter melon was milled and crushed and then was kept in air tight container. Then the dried bitter melon was weighed and ratioed based on their weight to get the volume of ethanol for soaking purpose. The dried, crushed, powdered bitter melon was kept in the air tight plastic bag in room temperature until further used. The bitter melon was then soaked in 95% of ethanol for 72 hours (3 days). Then, the extract was filtered using muslin cloth and Whatman filter paper. Next, extracted solvent have been separated from the solid by using rotary evaporator to remove remaining ethanol and collect extracted oil. Lastly, the pure extraction was tested using Fourier-transform infrared spectroscopy (FTIR) to identify the functional group presence in Momordica Charantia (Bitter Melon).

Preparation of Lime Seed Extract

The seeds were washed thoroughly with distilled water, air-dried, powdered using a commercial mill and soaked in 95% ethanol for 72 h (3 days). Milled seed (117 g) was soaked in 235 ml of ethanol and was stirred intermittently. It was then sieved using a fine muslin cloth, after which the mixture was filtered

through Whatman filter paper. The extract was concentrated using a rotary evaporator (Stuart, Germany). A golden brown, oily semi-viscous fluid was obtained. It was stored in an air tight container and refrigerated at 2 °C–8 °C until required for use. Then, the extract was filtered using muslin cloth and Whatman filter paper. Next, extracted solvent have been separated from the solid by using rotary evaporator to remove remaining ethanol and collect extracted oil. Lastly, the pure extraction was tested.

Approach Considerations

Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform Infrared Spectrometer are commonly use in organic synthesis, polymer science, pharmaceutical industry as well as food analysis [9]. The main role of FTIR is to verify the structures of compound as the functional groups are differ from each other [8]. To identify the functional groups presents, the sample undergoes a technique known as Infrared Spectroscopy by applying IR radiation to measure the frequencies at which radiation is absorbed. Then, the absorption peak was compared to a data bank of spectra to identify the functional groups [7].

Anti-diabetic Studies

Diabetes Patient

A 4 years diagnosed Young Diabetes Mellitus Type 1 patient with 22 years old of age was selected to be a tester. Was firstly diagnosed at the age of 18 years old and was an insulin dependent and metformin taker. Two times injection per day in the morning and night with 22 units in the morning and 28 units in the night as well as 2 gram of metformin per day (tablet form). Both will be taken before (insulin) and after meal (metformin tablet). Blood glucose range without taking insulin and medication is 13 to 30 mmol/mmol while with insulin and metformin is 5.0 to 9 mmol/mmol. The blood glucose was taken 4 times a day using the glucose meter (diabetes scanner).

Preliminary Studied on Lime Seed and Bitter Melon as Plant Based Insulin

Dietary Design

Breakfast

Taken sharply at 8.00 am with 2 pieces of regular bread and slightly smooth pineapple jam with a cup of less sugar tea.

Lunch

Taken sharply at 12.00 pm with two scoops of rice with fried chicken and some vegetables with less sugar drink.

Pre-Dinner

Taken sharply at 5.00 pm with a piece of fried shrimp ball with mineral water.

Dinner

Taken sharply at 6.00 pm with two scoops of rice with fried chicken and some vegetables with less sugar drink.

Supper

Taken sharply at 9.00 pm with 2 pieces of biscuits with a glass of plane water.

3.0 RESULTS AND DISCUSSION

FTIR Analysis

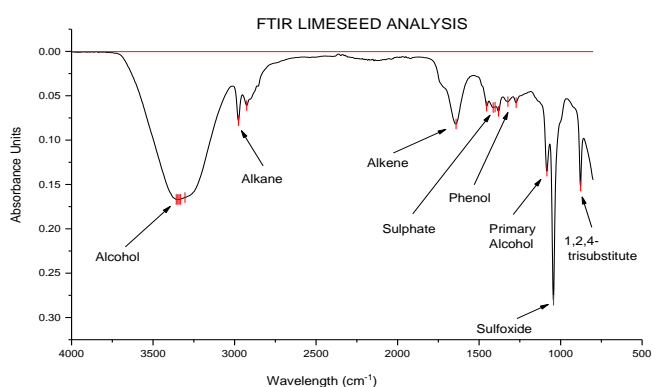


Fig. 1. The functional group presence in Citrus Aurantifolia (Lime) seed in reducing blood glucose.

Table 1. Peak Centres identification for Citrus Aurantifolia (Lime) seeds.

No.	Peak Centres A	Peak Centres B
1.	877.58156	0.15165
2.	1043.45412	0.28046
3.	1083.95788	0.13506
4.	1380.98549	0.06752
5.	1402.20175	0.06275
6.	1413.77425	0.06333
7.	1454.27802	0.06174
8.	1641.36683	0.08184
9.	2925.91479	0.06103
10.	2976.0623	0.07804
11.	3303.94992	0.16475
12.	3332.88118	0.16677
13.	3340.59618	0.16689
14.	3348.31119	0.16686
15.	3357.95494	0.16694

Table 2. Infrared spectroscopy absorptions by frequency regions for Citrus Aurantifolia (Lime) seeds.

Characteristic Absorptions (Cm ⁻¹)	Intensity	Functional Group	Type Of Vibration	Functional Class
880 ± 20	Strong	C-H	Bending	1,2,4-trisubstituted
1070-1030	Strong	S=O	Stretching	Sulfoxide
1085-1050	Strong	C-O	Stretching	Primary Alcohol
1390-1310	Medium	O-H	Bending	Phenol
1410-1380	Strong	S=O	Stretching	Sulfonyl Chloride
1415-1380	Strong	S=O	Stretching	Sulfate
1450	Medium	C-H	Bending	Alkene
1648-1638	Strong	C=C	Stretching	Alkene
3000-2840	Medium	C-H	Stretching	Alkene
3550-3200	Strong	O-H	Stretching	Alcohol

Functional Group. FTIR spectrum indicated the important functional groups present on FTIR Citrus Aurantifolia (Lime) seeds. C-H bending vibrations (880 cm⁻¹ ± 20) of 1,2,4 trisubstituted. Highest peak occur at the range of absorptions from (1070-1030) cm⁻¹ where indicated the existence of S=O stretching vibration of Sulfoxide.

C-O Stretching vibration (1085-1050)cm⁻¹ of Primary Alcohol. O-H Bending vibration (1390-1310)cm⁻¹ of Phenol. S=O Stretching vibration (1410-1380)cm⁻¹ of Sulfonyl Chloride. S=O Stretching vibration (1415-1380)cm⁻¹ of Sulfate. C-H Bending vibration (1450)cm⁻¹ of Alkene. C=C Stretching vibration (1648-1638)cm⁻¹ of Alkene. C-H Stretching vibration (3000-2840)cm⁻¹ of Alkene. O-H Stretching vibration (3550-3200)cm⁻¹ of Alcohol.

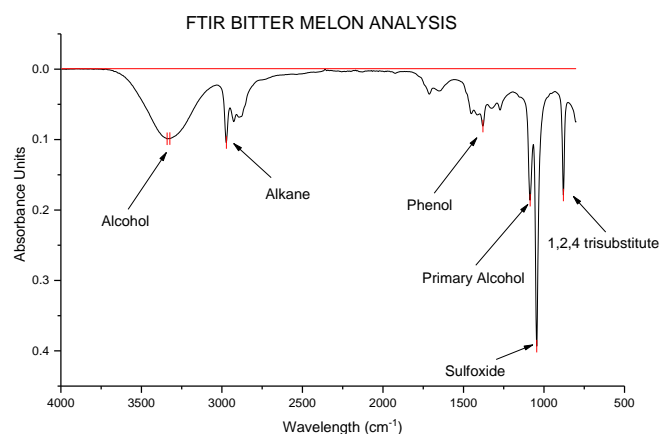


Fig. 2. The functional groups presence Momordica Charantia (Bitter Melon) in reducing blood glucose.

Table 3. The peak identified from FTIR analysis for Momordica Charantia (Bitter Melon).

No.	Peak Centres A	Peak Centres B
1.	879.51031	0.17865
2.	1045.38287	0.39323
3.	1085.88663	0.18601
4.	1379.05674	0.08082
5.	2972.2048	0.1042
6.	3323.23743	0.09853
7.	3340.59618	0.09884

Table 4. Infrared spectroscopy absorptions by frequency regions for Momordica Charantia (Bitter melon)

Characteristic Absorptions (cm ⁻¹)	Intensity	Functional Group	Type Of Vibration	Functional Class
880 ± 20	strong	C-H	bending	1,2,4-trisubstituted
1070-1030	Strong	S=O	Stretching	Sulfoxide
1085-1050	Strong	C-O	Stretching	Primary Alcohol
1390-1310	Medium	O-H	Bending	Phenol
3000-2840	Medium	C-H	Stretching	Alkane
3550-3200	Strong	O-H	Stretching	Alcohol

FTIR spectrum indicated the important functional groups present on FTIR Momordica Charantia (Bitter Melon). C-H bending Vibration (880 ± 20)cm⁻¹ of 1,2,4-trisubstituted. Highest peak occur at the range of absorptions from (1070-1030) cm⁻¹ where indicated the existence of S=O stretching vibration of Sulfoxide. S=O stretching vibration (1070-1030)cm⁻¹ of Sulfoxide. C-O stretching vibration (1085-1050)cm⁻¹ of Primary Alcohol. O-H Bending vibration (1390-1310)cm⁻¹ of Phenol. C-H Stretching vibration (3000-2840)cm⁻¹ of Alkane. O-H Stretching vibration (3550-3200)cm⁻¹ of Alcohol.

Collection of Blood Samples

After 9 days of treatment, the blood glucose levels of the diabetes patient in the positive control was found to be significantly lower.

Table 5. Comparison of level of blood glucose reduction based on patient's dietary design

BSP	DATE	1 (FBS) 0730 HRS	2 (PRE-L) 1130 HRS	3 (PRE-D) 1730 HRS	4 (POST-D) 2130 HRS
Citrus Aurantifolia (Lime) Seeds	14/11/17	8.9	7.8	6.6	8.7
	15/11/17	7.2	7.6	8.0	8.4
	16/11/17	7.3	7.5	7.9	8.2
Momordica Charantia (Bitter Melon)	17/11/17	7.0	6.8	7.3	8.2
	18/11/17	5.1	6.2	7.3	8.2
	19/11/17	5.5	6.8	8.3	8.1
Insulin (Biphasic Isophane)	20/11/17	6.3	7.2	8.3	8.5
	21/11/17	5.0	6.3	7.1	8.4
	22/11/17	5.2	6.5	7.6	8.2

Reference

Low (1.1– 3.8 mmol/L)

SYMPTOMS: Shaking, Fast Heartbeat, Sweating, Dizziness, Hunger, Headache, Fatigues, Irritable, Weakness.

High (10.0 – 13.2 mmol/L)

SYMPTOMS: Increased thirst, Need to Urinate often, Hunger, Blurry Vision, Drowsy

Very High (13.3 – 33.3 mmol/L)

SYMPTOMS: Increased thirst, Need to Urinate often, Hunger, Blurry Vision, Drowsy.

Normal Range

Normal and diabetic blood sugar ranges. For the majority of healthy individuals, normal blood sugar levels are as follows: Between 4.0 to 6.0 mmol/L (72 to 108 mg/dL) when fasting. Up to 7.8 mmol/L (140 mg/dL) 2 hours after eating.

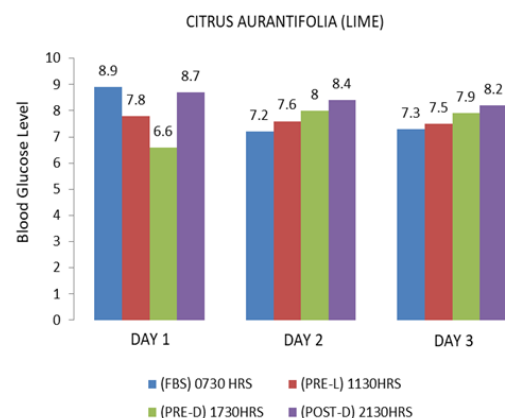


Fig. 3. The Reduction of Blood Glucose by Citrus Aurantifolia (Lime) seeds

The drink was taken for three days continuously. The result of the blood glucose level was tabulated in Table 5.

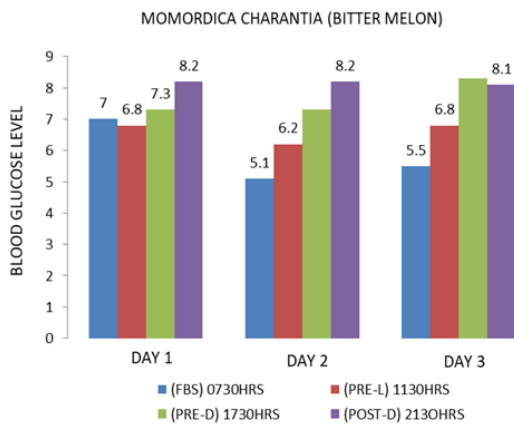


Fig. 4. The Reduction of Blood Glucose by Momordica Charantia (Bitter Melon).

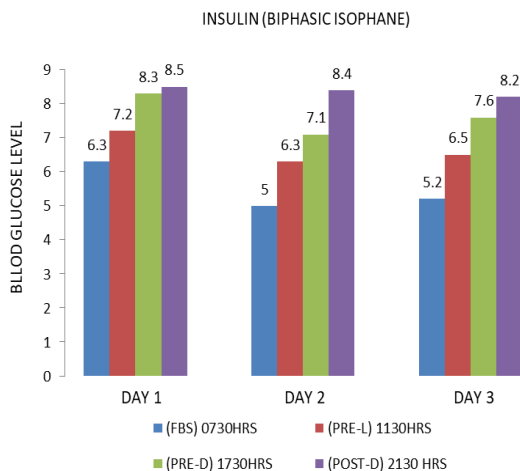


Fig. 5. The Reduction of Blood Glucose by Insulin (Biphasic Isophane).

Effects of treatments

In this research, insulin (Biphasic Isophane) is used as a reference in the role of reduction of blood glucose for a diabetic patient.

Table 5 illustrates the comparison of level of blood glucose reduction based on patient's dietary design between Citrus Aurantifolia(Lime) seed, Momordica Charantia (Bitter Melon) and Insulin (Biphasic Isophane) where the cycle was held three days continuously each at set time, at 0730AM, 1130AM, 1730PM and 2130PM. The reading of blood glucose reading for Day 1 Citrus Aurantifolia (Lime) Seeds

drink, it began with 8.9 mmol/mol , reduced to 7.8 mmol/mol , 6.6 mmol/mol and end with 8.7 mmol/mol. Day 2 showed the reduction to 7.2 mmol/mol , then 7.6 mmol/mol , 8.0 mmol/mol and 8.4 mmol/mol. Day 3 showed 7.3 mmol/mol, then 7.5 mmol/mol, 7.9 mmol/mol at and 8.2 mmol/mol. Day 1 of Momordica Charantia (Bitter Melon) drink showed the first reading is 7.0 mmol/mol, it reduced to 6.8 mmol/mol, 7.3 mmol/mol and 8.2 mmol/mol. Day 2 reading was 5.1 mmol/mol, continue with 6.2 mmol/mol,7.3 mmol/mol and 8.2 mmol/mol . Day 3 showed reduction to 5.5 mmol/mol, carry on to 6.8 mmol/mol ,8.3 mmol/mol and end with 8.1 mmol/mol.

Day 1 showed 6.3 mmol, go on with 7.2 at mmol/mol , 8.3 mmol/mol and 8.5 mmol/mol. Day 2, the reading taken was 5.0 mmol/mol, keep on with 6.3 mmol/mol, 7.1 mmol/mol and 8.4 mmol/mol. Day 3, the reading was 5.2 mmol/mol, proceeded with 6.5 mmol/mol, 7.6 mmol/mol and last reading was 8.2 mmol/mol. The effectiveness of the lime seed drink and bitter melon drink can clearly be seen by the reduced blood glucose level. This study have proven that bitter melon showed significantly higher levels of reduction than Citrus Aurantifolia (Lime) seed and Isulin (Biphasic Isophane).

Citrus Aurantifolia (Lime) seed is rich in anthocyanins. Anthocyanins are powerful plant pigments that have been characterized as some of the most powerful antioxidant sources in lowering blood sugar by increasing endogenous insulin secretion and conferring additional beneficial health effects.Thus, the ability of Citrus Aurantifolia (Lime) seed to lower blood sugar concentrations may be linked to the presence of this compound. Contributing to the high antioxidant properties is the richness of the antioxidant vitamin C. [1]

Anti-diabetic effect of Momordica Charantia

There are many traditional herbal remedies that have been used to treat diabetes in Asia and other developing countries Momordica Charantia is one of the plants that has been investigated thoroughly for the treatment of diabetes. With the traditional use supported by modern scientific evidence of the beneficial function Momordica Charantia, it is one of the most promising plants for diabetes today.

Investigation of the traditional uses of Momordica Charantia in India revealed that it is one of the most important plant for lowering blood glucose levels in patients with diabetes. Today, over 140 different studies worldwide have investigated anti-hyperglycemic and hypoglycemic effects of the different extracts and ingredients of Momordica Charantia in both human and animal models [4].

Table 6. Comparison between this research and other research.

In this research		Other research study	
Lime seed	Bitter melon	Lime seed	Bitter melon
FTIR spectrum indicated the important functional groups present on FTIR Citrus Aurantifolia (Lime) seeds. Those are : 1,2,4-trisubstituted, Sulfoxide, Primary Alcohol, Phenol, Sulfonyl Chloride, Sulfate, Alkane, Alkane, Alcohol	FTIR spectrum indicated the important functional groups present on FTIR Momordica Charantia (bitter melon). Those are: 1,2,4-trisubstituted, Sulfoxide, Primary Alcohol, Phenol, Alkane, Alcohol	A total of 46 compounds were identified from the Citrus Aurantifolia oil. Most of these were terpenes, which were found in greater amounts than sesquiterpenes, aldehydes, ketones, phenols, and free acids. Alcohols and some terpenes show higher percentage areas in the maturity stages [3]	The fruit is also rich in minerals including potassium, calcium, zinc, magnesium, phosphorus and iron, and is a good source of dietary fiber. Medicinal value of bitter melon has been attributed to its high antioxidant properties due in part to phenols [4]

4.0 CONCLUSION

Citrus Aurantifolia (Lime) seeds and Momordica Charantia (Bitter Melon) have the ability to reduce the blood glucose in our body. From our study Momordica Charantia (Bitter Melon) are more effective than Citrus Aurantifolia (Lime) seeds but both of them give same effect as Insulin. From our result the active compound functional group founded in this 3 samples and the compound can reduce blood glucose. Therefore, both plant based insulin have the potential to be used as diabetic substituted plant based insulin alternative to common insulin provided in the market. We recommend further investigations to explore the use of Citrus Aurantifolia (Lime) and Momordica Charantia (Bitter Melon) as adjuvant therapy in the management of diabetic condition.

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