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Research Article

Determination of Heavy Metals Present in the Hypoglycemic Karela Powder: An Analytical Assay

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Abstract

Background: Diabetes is a common health condition associated with heightened glucose content in the blood due to impaired insulin production/function. Considering current societal trends, the number of patients with this condition is growing fast. To help this subset of the population, researchers are investigating natural products exhibiting hypoglycaemic effects. It is well known that one third of patients with diabetes mellitus use some form of complementary or alternative medicine. One plant that has received some attention for its anti-diabetic properties is bitter melon, or *Momordica charantia*, commonly referred to as bitter gourd, karela and balsam pear.

Methods: Here, we analyze plant powder to identify and quantify some existence of heavy metals, present in karela using atomic absorption spectrophotometry (AAS). Other analytical techniques employed included IR and UV.

Results: AAS was performed and the plant was found to contain 0,019% of zinc, 0,051% magnesium, 0,021% iron and 0,198% calcium.

Conclusion: This plant has a great pharmacological potential, especially within the treatment of diabetes.

Keywords: *Momordica charantia; Karela; Diabetes; Medicinal Plant; Atomic Absorption; Infrared; Ultraviolet*

Introduction

Diabetes is a disease that affects more and more of the population each year; in 2014, 422 million adults had been diagnosed with diabetes, a figure that is predicted to rise to 642 million by 2040 [1]. The significant societal impact of diabetes is driving research into finding new treatments and to improving the quality of life of patients. One of the most important research avenues into drugs and compounds with hypoglycemic properties is the potential of natural products to improve the quality of life of diabetes patients [2]. There are two big types of diabetes: type 1 and type 2. Diabetes mellitus or type 1, occurs when the body is unable to produce insulin (Figure 1), a protein controlling blood glucose levels [3]. Conversely, in type 2, the body does not produce sufficient insulin or its function is impaired. In the first case, the treatment provides insulin for the patient, whereas type 2 offers numerous medicines that are used to control the impact and symptoms of diabetes. Treatment of diabetes also includes a non-pharmacological portion, which encourages practice of physical activities and changes of diet, in order to improve eating habits. Avoiding alcohol, smoking and reducing carbohydrate intake has also been widely noted to improve patient well-being and condition [4].

Numerous plants with hypoglycaemic properties are being discovered, among them, *Momordica charantia* [5-7]. *Momordica charantia* or "Bitter melon" or "bitter gourd" or "balsam pear" is a flowering vine in the family Cucurbitaceae. A tropical plant, widely cultivated in Asia, India, East Africa, and South America, it is an intensely bitter fruit commonly used in cooking and as a natural remedy [8]. This perennial plant that usually grows up to 5 m, and bears elongated fruits with a rough surface, contains a useful medicinal and vegetable extract for human health and has been identified as one of the most promising plants for diabetes treatment [9,10].

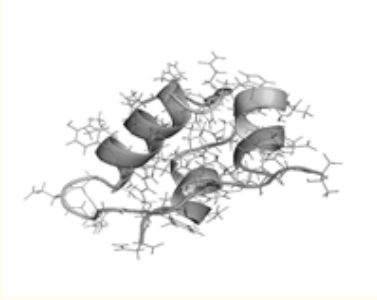

Figure 1: *Protein databank structure of human insulin, 2HIU.*

Besides its effects on diabetes, the bitter melon is also used in folk medicine as stomachic, laxative, antibilious, emetic, and anthelmintic agent and for the treatment of cough, respiratory diseases, skin diseases, wounds, ulcers, gout, and rheumatism [11]. The antidiabetic effects of *Momordica charantia* have been identified as being likely due to: triterpene, proteid, steroid, alkaloid, inorganic, lipid and phenolic compounds contained within the plant [12]. Moreover, the isolated compounds related to hypoglycaemic activity are charantin, polypeptide-p and vicine [9].

Charantin is a cucurbitane-type triterpenoid and a mixture of two compounds: sitosteryl glucoside (Figure 2) and stigmateryl glucoside (Figure 3). However, studies have shown that these substances separated did not presented the antidiabetic effect, which demonstrate that they are not the only compounds in charantin [13].

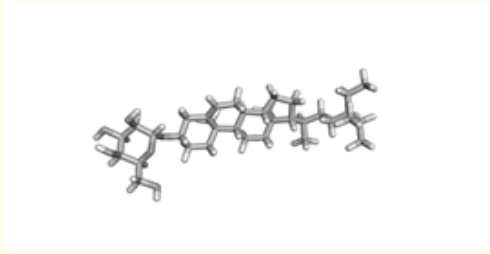

Figure 2: *Structure of sitosteryl glucoside.*

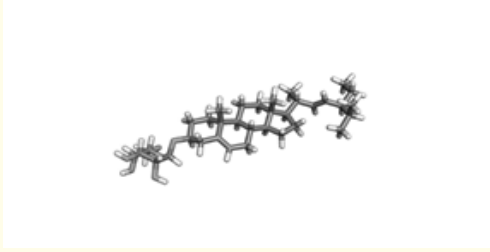

Figure 3: *Structure of stigmasteryl glucoside.*

Polypeptide-p, also known as p-insulin, has an action similar to human insulin when injected subcutaneously and could have promising repercussions in the future treatment of patients with type 1 diabetes [14].

Vicine (Figure 4), a glycol alkaloid, is also present in *Momordica charantia* extract. This substance, also found in fava beans has been shown to induce favism in large quantities, an acute disease characterized by haemolytic anemia [15-17] however there is no evidence that the vicine found in bitter melon can has similar effects.

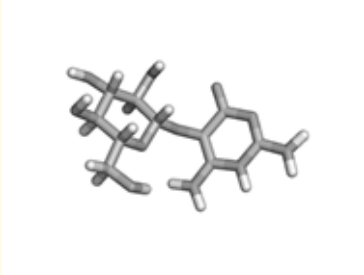

Figure 4: *Structure of vicine.*

Gentisic acid (Figure 5) has also been identified in *Momordica charantia* extracts in large quantities. This antioxidant showed positive results when related to LDL modification, as a consequence of diabetes, inhibiting the formation of atherogenic LDL particles and contributing to the prevention of vascular complications [18, 19].

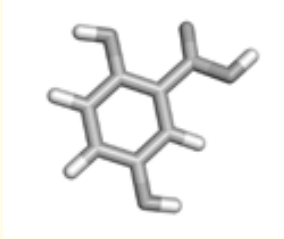

Figure 5: *Structure of Gentisic Acid.*

Our group works in a multicultural context where part of the population is currently using *Momordica charantia* for diabetes purposes. Thus, as a first step, our aim was to investigate its chemical composition. Compounds can be identified and quantified through different analytical techniques, such as Atomic Absorption Spectrometry (AAS), Infrared (IR) and Ultraviolet (UV), these being used in the context of this communication.

Materials and Methods

Sample collection

The powder sample of *Momordica charantia* was obtained from an Indian commercial vendor in Leicester, United Kingdom.

Method of analysis

With 5g of the sample, an extract of 10 mL was produced from the plant, to use in the analyses.

AAS was carried out to identify any metals presented in the plant, and quantify their content. Prior to plant analyses, standards calibration curves were obtained with the five metals: zinc, calcium, lead, iron and magnesium. The final concentrations to determine absorption differed for each metal. Calcium, iron and lead carried equal initial concentrations of 1000 ppm cf. final concentrations of 2, 4, 6, 8 and 10 ppm. Zinc had an initial concentration of 1000 ppm, and lower final concentrations of 2, 1, 0,5, 0,25 and 0,125 ppm. Magnesium began at an initial concentration of 10 ppm and 0,05, 0,1, 0,15, 0,2 and 0,25 ppm as final concentrations. Following the calibrations, the plant extract was analyzed, and the results compared with the standards to identify the presence of metals in *Momordica charantia* and quantify their concentrations.

Infrared (ATR-FTIR) spectroscopic measurements were carried out to identify compound identity through functional groups present in the analyte.

Ultraviolet spectroscopy (UV) was used to identify the components and to quantify them through comparison of calibrations from the sample and standards that can be identified from the literature. The sample was diluted 1:100 prior to analysis. The extract from the same origin of *Momordica charantia* was used in all analyses.

Results and Discussion

Standard calibration curves with five elements: zinc, calcium, lead, iron and magnesium were obtained. The results are presented in the Tables 1-3.

Metal	Metal concentration (ppm)	Absorbance
Fe	2	0,641
	1	0,338
	0,5	0,179
	0,25	0,092
	0,125	0,049
Mg	0,05	0,041
	0,1	0,083
	0,15	0,110
	0,2	0,146
	0,25	0,192

Table 1: *Calibration curve from Iron and Magnesium.*

Metal	Metal concentration (ppm)	Absorbance
Zn	2	0,641
	1	0,338
	0,5	0,179
	0,25	0,092
	0,125	0,049
Ca	2	0,113
	4	0,171
	6	0,258
	8	0,335
	10	0,382

Table 2: *Calibration curve from Zinc and Calcium.*

Pb concentration (ppm)	Absorbance
2	0,007
6	0,018
8	0,025
10	0,032

Table 3: *Calibration curve from lead.*

Using the information from the standard calibration curves, it was possible to determine the presence of metal in the samples. During the analysis of magnesium and calcium it was necessary to dilute the samples. Magnesium was diluted 1:100 and calcium, 1:50. Table 5 shows the quantity of each metal in the *Momordica charantia* extract derived from each calibration curve.

The plant samples do not contain lead, which is harmful to human due its toxic properties. However, zinc, magnesium, iron and calcium are present in accordance with other scientific studies [20], the fruit of *Momordica charantia* is rich in these minerals.

The extract of *Momordica charantia* was analyzed with ATR-FTIR equipment (Figure 6).

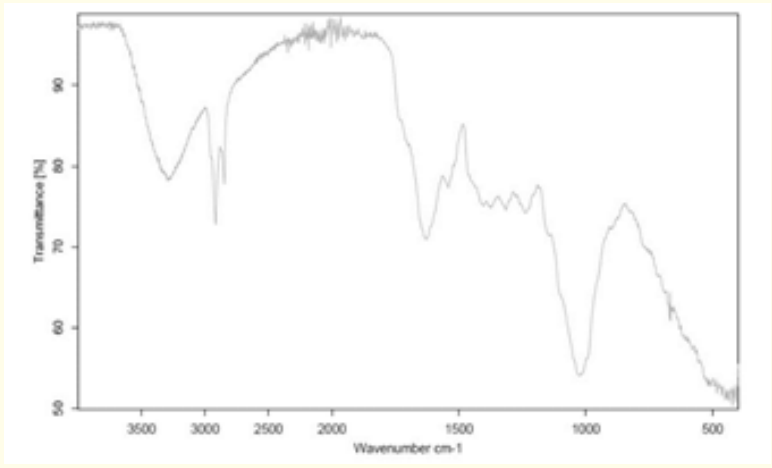

Figure 6: *Infrared spectrum from Momordica charantia powder extract.*

The plant *Momordica charantia* has many components including terpenoids, flavonoids, proteins, saponins, alkaloids and carbohydrates. Figure 7 shows significant similarities with analyses by Supraja., *et al*. (2015), from an alkaloid identified as 13-hydroxy-28-methoxy-urs-11-en-3-one or momordicin [20]. The main peaks that can be identified in the karela spectra are: 3300 cm^{-1}, 2950 cm^{-1}, 1750 cm^{-1}, 1600 cm^{-1} and 1100 cm^{-1}. In the study by Supraja., *et al.* the main peaks were: OH 3278 cm^{-1}, =C-H 2924 cm^{-1}, C=O 1740 cm^{-1}, C=C 1622 cm^{-1}, showing excellent correlation with our analyses [21].

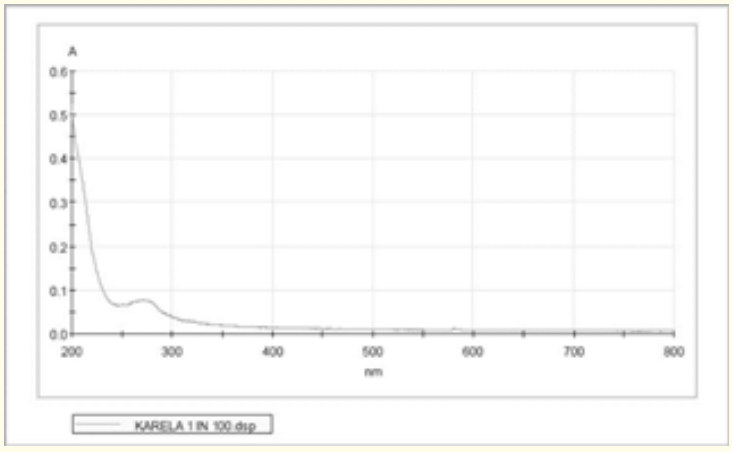

Figure 7: *UV spectrum from Momordica charantia powder extract.*

To analyze the sample of extract with ultraviolet spectroscopy, it was necessary to perform a dilution of 1:100. The results showed an absorbance of 0.070 at 280nm (Figure 7). Zhang and collaborators smartly identified two curcubitane-type triterpenoid compounds related to diabetes [22]. These components were named as charantin A and charantin B and the maximum absorbance of each in methanol were 210 nm, and 208 nm, respectively. It is reasonable to speculate that the compound represented in the UV/Vis spectrum is gentisic acid, an antioxidant present in *Momordica charantia*. This compound is a dihydroxybenzoic acid with a maximum absorbance of 273 nm; a similar value to that have been obtained earlier by the group of Cham [23].

Conclusions

For centuries, *Momordica charantia* has been used as dietary supplements for relieving symptoms and treating conditions related to what we now know in modern days as diabetes [24,25]. Described as a versatile plant, *Momordica charantia* has been extensively studied worldwide for its medicinal properties to treat a number of diseases.

The importance of the present study is the useful information given in compounds composition of the powder which most of the population finds in commerce and use to treat their different harmful conditions [26].

In relation to diabetes, charantin, insulin-like peptide and alkaloid-like extracts possess hypoglycemic properties similar to the plant itself or its crude extracts [27]. These different compounds seem to exert their beneficial effects via several mechanisms to control and treat diabetes [28]. Within the ethnic minorities, particularly, *Momordica charantia* is believed to be a feasible option for patients who have a high prevalence of diabetes but prefer treatment based on natural products according to their cultural beliefs. The experiments presented in our study, support the need to observe analytically the commercial powder of this plant that include fruit, leaves and seed. Using atomic absorption spectrophotometry (AAS) on the plant we found the Kerala powder to contain 0,019% of zinc, 0,051% magnesium, 0,021% iron and 0,198% calcium, thus in accordance with the study of Akram [29]. All the values are in the permissible range. Furthermore, because nowadays computational plays a major role in molecular studies, we aim to combine synthetic and natural products research with computational and bioinformatics techniques [30] to derive a clearer picture of *Momordica charantia* molecular aspects to better understand the therapeutic effects in the near future.

Conflict of Interest

No conflict of interest. The manuscript submitted has been prepared according to the journal's "Aims & Scope" and 'Instructions for Authors', and checked for all possible inconsistencies and typographical errors.

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