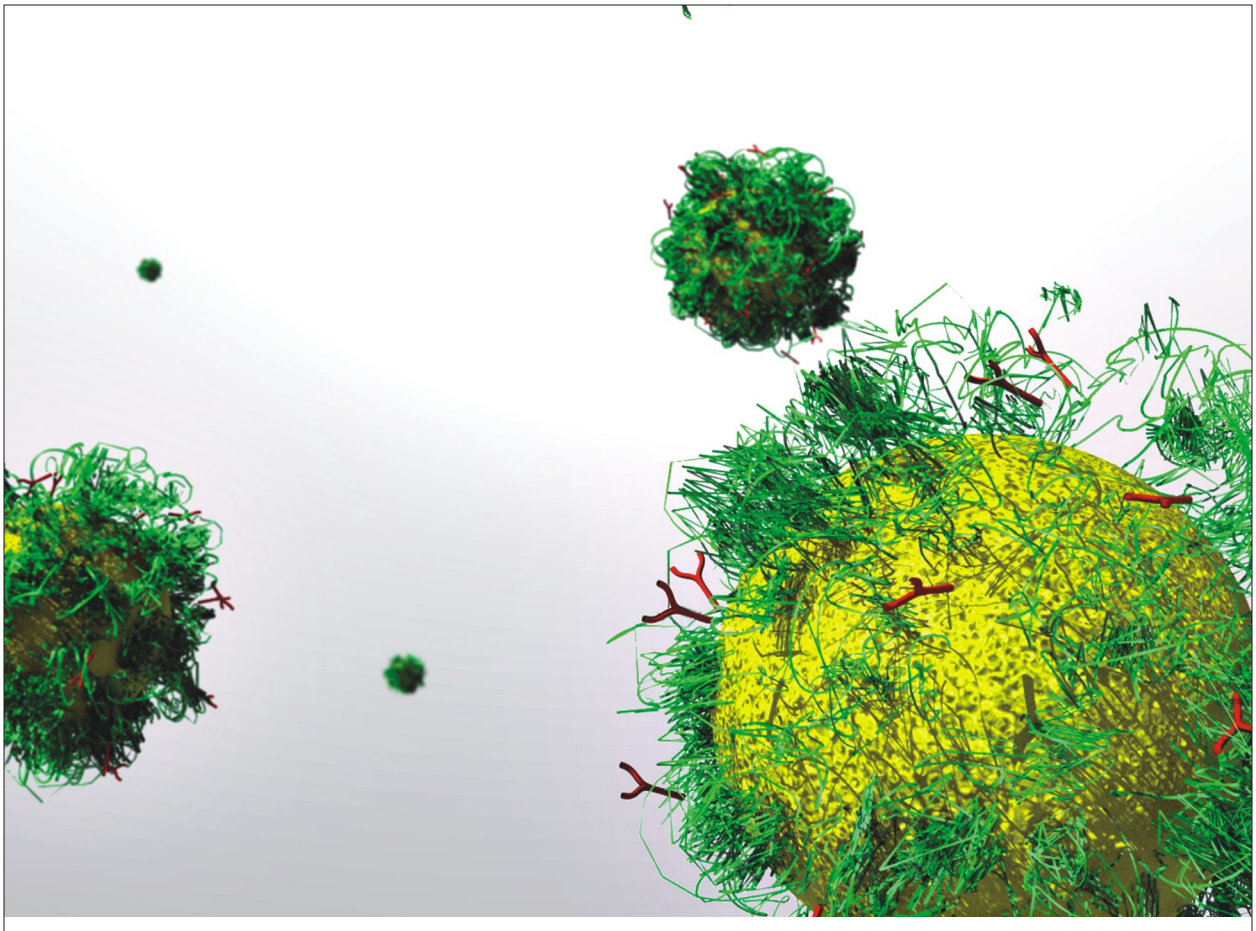

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Extraction of Insulin like Compounds from Bitter Melon Plants

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ABSTRACT

This study was focused on the insulin-like compound, charantin, as alternative drugs in order to reduce the blood. As such, the anti-diabetic compound, charantin was extracted from leaves and fruits of bitter melon. Liquid extraction charantin, from leaves and fruits of bitter melon was proposed using ethyl alcohol under special conditions of temperature and at a suitable pH values. Experiments were conducted to determine the effects of several pH values including (2-12) using different buffer systems on extraction efficiency. In addition, the extraction efficiency was found to be highly influenced by temperature, ethanol/water ratio and time of contact. A purification step was carried out using methanol-water mixture to remove interfering components such as chlorophylls and sugars from the analyte. Chemical analyses as well as identification of charantin were carried out by High Performance Liquid Chromatographic. The results indicate that Charantin were found in the fruit and leaf samples of Bitter Melon Plants. It was observed that bitter melon leaves plants contained higher concentrations of charantin analyzed as compared to fruits. The optimized conditions were 50 and 70% ethanol, 70.24 and 80.34°C and a time of contact of 60 and 70 h at pH is 5.4 for fruit and leaves, respectively. The corresponding predicted values were 55.27 mg charantin equiv. g^{-1} dry fruit and 144.58 mg charantin equiv. g^{-1} dry leaves. This experiment will help to highlight the importance of these valuable organic compounds found in these plant species and their demand in the market will be increased in the future.

Key words: Antidiabetic plants, bitter melon, pH effects, liquid extraction effects, charantia

INTRODUCTION

Medicinal plants are a major source of drugs for the treatment of various health disorders. Nowadays huge number of allopathic medicines also contains plant based ingredients that are used for their preparation by different companies. There are about 400,000 species of higher plants in the world, as compared to animal's species that are about 5-10 million. The plant materials contain thousands of chemicals which act against diseases and infections of humans and animals when properly used (Shinwari and Khan, 1998).

Plants contain different types of compounds such as resins, rubbers, gums, waxes, dyes, flavors, fragrances, proteins, amino acids, bioactive peptides, phyto hormones, sugar, flavonoids and bio pesticides (Khattak *et al*., 1985). Furthermore according to assessment of WHO about 80% of world population depend on medicinal plants for their health care needs and more than 30% of the pharmaceutical preparations are based on plants (Shinwari and Khan, 1998). Where as some reports indicated that there are 90 popular medicinal plants and different Pharmaceutical

companies are using extracts of these plants in various drugs. Scientists throughout the world are trying to explore the precious assets of medicinal plants to help the suffering humanity (Edward, 2001). However, the developed countries mostly import raw material from developing countries and after processing export it back as high priced prepared medicines to developing countries (Shinwari and Khan, 1998).

Bitter melon (Momordica charantia) or commonly as Ma-ra-khee-nok, is an herbal plant grown in Thailand and other tropical regions. It is traditionally known for its medicinal properties such as antidiabetic, antitumorous, anticancer, anti-inflamatory, antiviral and cholesterol lowering effects etc. (Ahmed *et al.*, 2001; Taylor, 2002). The main constituents of bitter melon which are responsible for these effects are such as triterpene, proteid, steroid, alkaloid, inorganic, lipid and phenolic compounds. The protein in bitter melon including protein MAP-30, alpha-momorcharin and beta-momorcharin were shown to have the ability for fighting against HIV (Luetrakul, 1998). A steroid, charantin, contained mainly in the aerial parts, has been proven for its antidiabetic activity (Chanchai, 2003).

Conventionally, isolation of this compound involves extraction Charantin could be used to treat diabetes and can potentially replace treatment by injection of insulin which has not been successful in stimulating the pancreas of the diabetic patients to lower blood sugar to the desired level (Belinda, 2000). In some cases, the injected patient shows signs of side effects. A molecule of charantin consists of aglycone or a steroidal portion, which is highly soluble in relatively non-polar solvent such as chloroform and dichloromethane. However, the glucosides attached to its molecules make it slightly soluble in polar organic solvent such as ethanol or methanol. Conventionally, isolation of this compound involves extraction with mixtures of these solvents.

In this study, we investigated the charantin contents of bitter melon obtained by water ethanol extraction. The effect of extraction temperature and pH values was considered also. Moreover, the quantitative analysis of these extracts was determined.

MATERIALS AND METHODS

Materials and chemicals: The fruits and leaves of bitter melon were obtained from the market in Al Qassiem Saudia in the period between March and May 2004. Ethanol was obtained from Sigma Chemical Co. (St. Louis, Mo, USA). A heater model 21V50-2, serial number 1291A39968, manufactured in Germany it set to work with Natural Gas only. A Beckman Model 332 (Germany) liquid chromatographic system was employed, consisting of a Model 110A pump and a Model 420 system controller. Charantin was detected using a Beckman 155 variable wavelength detector fitted with a 20-p1, l cm optical path cell.

Sample preparation: The cleaning of Bitter Melon was made using distilled water before cutting them into small pieces and then oven dried at 50°C for a day. The dried sample was then pulverized into fine powder in a grinder, which was then stored at 4°C until use. The leaves fragments were further ground under liquid nitrogen using mortar and pestle. A sub sample was weighed, dried at 50°C and reweighed to obtain the dry weight/wet weight ratio.

Water extraction: In order to extract and purify charantin from leaves and fruits samples, following procedures were adopted: About 100 g (each of fruits and leaves) samples were soaked in water/ethanol solvent at different contact time from 1 to 72 h The effect of temperature also carried out from 30 to 80°C. The pH of the mixture was adjusted to several values from 2-13 with

buffer solution. Buffers solution at pH 3.0-6.0 is 10 mM citrate-phosphate); pH 7.0 (10 mM sodium-phosphate); pH 8.0 (10 mM Tris-HCl); pH 9.0-10.0 (10 mM carbonate-sodium bicarbonate); pH 11.0-12.0 (10 mM glycine-NaOH). The extract was cooled in a coil immersed in a water bath and the extract was collected in fractions in sample collecting vials every 3 h in a first day and then was collected every 6 h in the second and third days. These extracts were evaporated under vacuum to remove water ethanol solvent. The mixture was run through a column using silica gel to separate the charantin, which were further identified on High Performance Liquid Chromatographic using reference standards whereas for protein, sephadex (G 20 and G 50) was used. The concentration level of charantin was determined with the help of spectrophotometer at 734 nm.

Sample purification: To purify the crude extract, the protocol was carried out (Chanchai, 2003). Briefly, 5 mL of 50:50 (v/v) methanol-water was added to the crude extract. The mixture was then sonicated for 15 min and centrifuged at 3500 rpm for 15 min to separate the supernatant from the precipitate. The precipitate was then added with 5 mL of 70:30 (v/v) methanol-water and the mixture was again sonicated and centrifuged. The precipitate from this step was added with 3 mL of hexane and the step was repeated. The precipitate from this step was re-dissolved in 200 L of 1:1 (v/v) chloroform-methanol mixture and then adjusted to volume with methanol (to 1 mL volume for extracts obtained with PLE and to 2 mL volume for that obtained with Soxhlet extraction). The purified solution was filtered through a 0.45 m nylon membrane filter (Millipore, USA) before being analyzed by an HPLC.

RESULTS

A chemical analysis of various compounds found in leave and fruits of Bitter melon were detected expressed in percentage (%) (Table 1). The results obtained from Table 1 indicated that concentration of charantin (9.65%) and (3.41%) was found in leaves and fruit, respectively. The utilization of pH effects on extraction of charantin from bitter melon plants was successfully applied using suitable ethanol/water solvent ratio and temperature. Although, Fig. 1 demonstrate that the maximum extraction of charantin (55.27 mg charantin equiv. g^{-1} dry fruit and 144.58 mg charantin equiv. g^{-1} dry leaves) was found to be at pH 5.4 with at 50 and 70% ethanol, 70.24 and 80.34°C and a time of contact of 60 and 70 h for fruit and leaves, respectively.

Present results on the extractability of charantin at pH 4.5 agree with those of Smith and Circle (1938), Wolf *et al.* (1964). The effect of temperature on extraction efficiency of charantin was

Table 1: The percentage of various chemical compounds analyzed from leaves and fruit of bitter melon

Constituent in leaves	Percentage	Constituent in fruit	Percentage
Chrantin	9.65	Chrantin	3.21
Protein	7.50	Protein	15.60
Fat	6.50	Fat	14.50
Sugar	2.10	Sugar	27.50
Fiber	13.50	Fiber	6.50
Palmatine	0.32	Palmatine	0.80
Calcium	1.80	Calcium	0.90
Sulphur	2.70	Sulphur	0.80
Celleulose	40.25	Celleulose	20.20
Berberine	5.60	Berberine	2.50
Vitamin C	1.20	Vitamin C	14.58

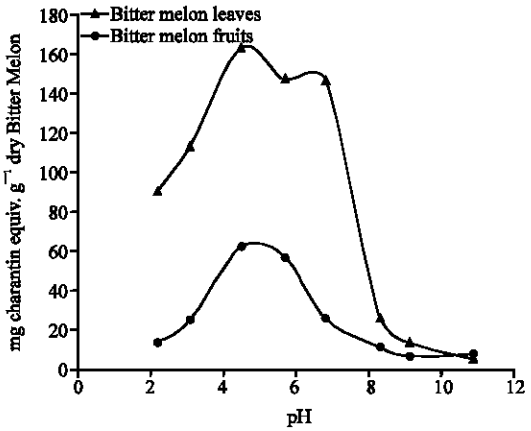

Fig. 1: Effect of pH on charantin extraction at 50 and 70% ethanol, 70.24 and 80.34°C and a time of contact of 60 and 70 h for fruit and leaves, respectively

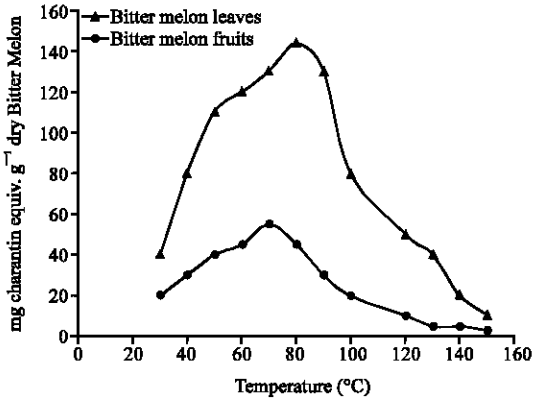

Fig. 2: Effect of temperature on extraction of charantin from bitter melon leaves and fruits at optimized conditions 50 and 70% ethanol, time of contact of 60 and 70 h at pH is 5.4 for fruit and leaves, respectively

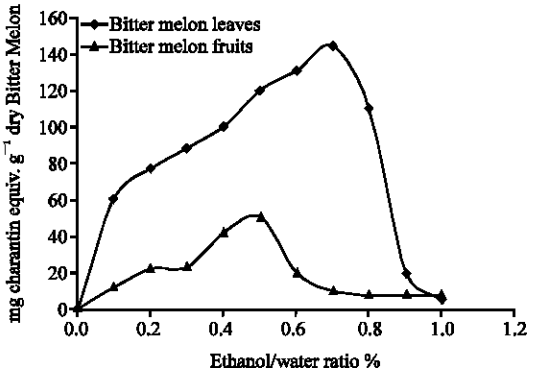

Fig. 3: Effect of solvent composition on extraction of charantin from bitter melon leaves and fruits at time of contact of 60 and 70 h and 70.24 and 80.34°C for fruit and leaves, respectively at pH is 5.4

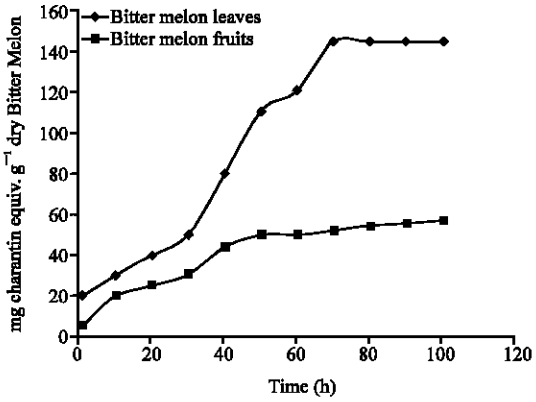

Fig. 4: Effect of contact time on extraction of charantin from bitter melon leaves and fruits at optimized conditions 50 and 70% ethanol and 70.24 and 80.34°C for fruit and leaves, respectively at pH is 5.4

examined over the range of 30-150°C at optimum pH (4.5) and ethanol/water ratio, the results show that 70.24 and 80.34°C are the optimum temp for fruit and leaves respectively (Fig. 2). Also the results of extraction of charantin from bitter melon fruits at different ethanol/water ratio illustrated as shown in Fig. 3. The mixtures of ethanol and water were tested to determine the effect of mixture compositions: 0, 20, 50, 70, 80 and 100% ethanol on the extraction of charantin from leaves and fruits. The conditions used in each experiment were pH = 4.5, at 70.24 and 80.34°C and a time of contact of 60 and 70 h for fruit and leaves respectively. The extraction efficiency increased with increasing percentage of ethanol in the solvent mixture until up to 50 and 70% for fruits and leaves this result. These results were supported also by Pitipanapong *et al.* (2007). Also the effect of contact time are studied and the results are shown in Fig. 4 which show that high concentration of charantin observed in leaves and fruits after 70 and 60 h at the same previous conditions.

DISCUSSION

The major determining factors on charantin solubilization in ethanol/water solvent at a definite temperature are the pH influence. The pH of the aqueous phase was varied between 3.0 and 12.0, using different buffer systems according to their pKa value (Fig. 1). The results showed a high extraction of chrantin from bitter melon within the pH range of 3.0-6.0, with a maximum of value at pH 5.4. A similar situation has been reported for Cratylia mollis seed lectin (Nascimento *et al.*, 2002), with a maximum extraction at pH 5.0. As the pH of aqueous phase increased from 5.5 to 12, the extracted protein decreased, probably due to the proximity of the isoelectric point of charantin compound. This phenomenon was observed for different proteins at distinct isoelectric points (Goklen and Hatton, 1987). Therefore, the selected pH to be used in further experiments was 5.4.

Figure 2 reveals that extraction efficiency was greatly influenced by temperature. As described earlier, the increase in temperature decreases solvent polarity as a result of reduced polar forces and hydrogen bonding, making the solvent more suitable for extraction of charantin. Moreover, at elevated temperatures, the solvent density and viscosity decrease, resulting in increased mass transfer of the solvent into the matrix of plant sample. However, when temperature increases from 80 to 100°C, the percent charantin extracted did not change until the end of extraction. This

suggests that there appears to be an optimal temperature between 60 and 80°C. It is possible that beyond this temperature, further decrease in ethanol polarity may be disadvantageous for extraction of charantin. However the influence of temperature on solvent polarity and thus solubility at subcritical condition is not completely understood as the experimental data near critical temperature are not available. More detailed study is needed to completely understand the behavior of solvent and the solute solubility under these condition these results were supported by (Pitipanapong *et al.*, 2007).

Figure 3 show the effect of solvent composition on extraction of charantin where as increasing ethanol/water ratio (from 0 to 70%) the extraction of chrantin from bitter melon leaves and fruits increase to certain values. Further increase in ethanol composition (up to 70%) did not further increase the extraction efficiency. It is worth noting that when pure water was used, the solubility of charantin was low because water polarity was extremely high. However, the addition of some amount of water to as high as 70% into ethanol was found not to hinder the extraction efficiency. A possible explanation was that the lower viscosity of water allows it to more easily penetrate into the pores of the sample matrix, thus causing the swelling of the plant materials. This increases the contact volume and area between the solvent and the plant porous matrix, thus the internal mass diffusion is increased (Rostagno *et al.*, 2003; Li *et al.*, 2005). Rostagno *et al.* (2003) has indeed reported that addition of water enhances the solubility of some glucoside compounds and thus improves the extraction efficiency (Li *et al.*, 2005). In this study, the extraction efficiency of pure ethanol and ethanol-water mixture up to 50% ethanol are comparable. However, the ethanolic mixture with too high water content has lower dissolving power for charantin as a result of increased polarity.

CONCLUSION

Extracts of charantin obtained by water ethanol solvent at optimum pH had higher total contents from leaves and fruits of Bitter melon. So, it can be used by people to diabetes type 2. Therefore it is recommended that extraction and purification of such charantin are very valuable in the preparations of drugs of diabetes of various types. The assessments of various effects of such compounds on human health are required in the future studies.

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