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EXTRACTION, ISOLATION AND CHARACTERIZATION OF CHARANTIN FROM MOMORDICA CHARANTIA FRUIT LINN.

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ABSTRACT

The bitter melon (L), commonly referred as bitter gourd, karela, and balsam pear. Its fruits are used for the treatment of Diabetus and related conditions amongst the indigenous populations of Asia, South America, India and east Africa. The present study deals with an extraction, isolation and characterization of Charantin from Momordica charantia. The Charantin is going to extract and isolated by using following methods like soxhlet extraction, aqueous two phase method and reflux condenser method, and the isolated compound was identified by using TLC, UV-Visible spectrophometric method, by melting point analysis and FTIR Spectroscopy. And concluded that the yield of Charantin is more in reflux condenser method than other methods and Charantin is having antidiabetic activity as more potent like insulin hence can be employed as antidiabetic drug further evaluated clinically for formulating Charantin.

KEYWORDS: Momordica charantia, Charantin, extraction and isolation, characterization.

1. INTRODUCTION

M. charantia (Family: Cucurbitaceae) is of the medicinal plants with hypoglycemic activity being studied extensively. It is a climber widely cultivated as food in Asia, Africa and South America. It is also found all over India and cultivated up to an altitude of 1500 m. The word *Momordica* is derived from the Latin word *Mordeo* which means to bite and the species name is derived from Greek word and it means beautiful flower.

Fruit of this plant is known as bitter melon, bitter gourd, balsam pear or African cucumber. Fruits are traditionally used for hypoglycemic activity. Apart from fruits, leaves and seeds are also used. Several studies on different animal models also proved the hypoglycemic activity of fruits. Main phytoconstituents present in fruits are Charantin, momordicin, momordin, stigmasta-5, 25-dien-3-β-O-glucoside, β-sitosterol-β-D-glucoside, momordicoside G, momordicoside F1, momordicoside F2, momordicoside I, momordicoside K, momordicoside L, etc.

CHARANTIN: Charantin is steroidal glycoside and exist as equal mixture of stigmasterol glucoside and β-sitosterol glucoside. It has got blood sugar lowering property equivalent to insulin. It is a white crystalline, neutral and tasteless compound, sparingly soluble in Water, Highly soluble in chloroform and dichloromethane and soluble in Ether and ethanol.

The seeds and fruits of MC are proved to have antioxidant, hepatoprotective, antiviral, anticancer, antiulcer, analgesic, anti-inflammatory, and antifertility activities. Charantin is one of the bioactive compounds found in all parts of the plant especially in fruits. Charantin improves blood sugar levels by increasing glucose uptake and glycogen synthesis in the liver, muscles, and fat cells. It also enhances insulin release from pancreatic beta cells, and repair or promotes new growth of insulin-secreting beta cells. Alcoholic extract of Charantin was found to be more effective antidiabteic agent than tolbutamide, sometimes used in treating diabetes. Charantin is reported to be an Anti-HIV protein.

2. MATERIALS AND METHODS

2.1. Plant Materials: The unripe fruits of Momordica charantia fruit Linn, were collected from local market of Karnataka, then dried and powdered. The Charantin extracted and isolated from the unripe fruits was used for further studies.

2.2. METHODS OF EXTRACTION AND ISOLATION OF CHARANTIN

a. Soxhlet extraction method
b. Reflux condenser method
c. Aqueous two-phase extraction method

a. SOXHLET EXTRACTION METHOD: (Hot continuous soxhlet extraction method): The dried powder of Momordica charantia fruit Linn(100gm), was subjected to hot continuous extraction with 70% ethanol(350ml) in soxhlet extractor, at 40-50^{0}c temperature for the period of 3 hours. After the complete extraction, the extract was evaporated in hot air oven at 30-40^{0}c, and then concentrated to dry residue in a dessicator over anhydrous calcium chloride.

b. REFLUX CONDENSER METHOD: Fresh unripe fruits of Momordica charantia bought from the market were cut into small bits and dried in hot air oven below 60^{0}c. the dried material was broken into a coarse powdered and the 100gm of unripe fruit powder were mixed with 600ml of petroleum ether(BP. 60-80^{0}c) and refluxed for 6 hours and then filtered and the marc was repeated again for 6 hours with petroleum ether and filtered. The marc was mixed with 80% of ethanol and extracted at reflux temperature for 6 hours and then filtered. The filtrate was basified with potassium hydroxide solution till P^H 10 & kept for 48hours. The resulting solution was diluted with water and extracted with diethyl ether. To the diethyl ether portion add anhydrous sodium sulphate and kept over night. The ether was filtered and concentrated to get residue (crude Charantin). The residue was dissolved in minimum amount of alcohol and kept in refrigerator. The crystals was filtered and the crystals was recrystalized with ethyl alcohol.

C. AQUEOUS TWO-PHASE EXTRACTION: 50 gm of dried powder of Momordica charantia fruit is taken in 1000ml water and boil for 30-40min, then filtered through 100 mesh sieve. The 245ml filtrate(crude water extract) were collected. An aqueous two phase system was prepared by adding various amounts of PEG(1.28gm), K_2HPO_4 (1.4gm) and ethanol(4ml) to various amounts of crude water extract (12ml). The system was stirred to form a homogeneous phase. Then it was centrifuged at 3000g for 5 min to facilitate the phase separation. The Charantin containing salt rich layer was separated and subject to back extraction three times with 95% of ethanol. The ethanol extracts were collected and kept at 4^{0}c over night to allow salt to precipitate. After removing the precipitant by centrifugation, the amounts of Charantin were analyzed by ultraviolet spectrophometry.

1. CHARACTERIZATION OF CHARANTIN

The isolated Charantin was identified and confirmed by following tests,

Chemical Test

a. Libermann-Burchard Test: Giving a play of colours changing from violet to blue to green and yellow with Libermann-Burchard test.

b. Decolourising dilute potassium permanganate & bromine water

Characterization of Charantin

1. Melting point

Melting point of isolated compound was determined by capillary method.

2. TLC identification test

Sample solution: Prepare solution of isolated compound in alcohol.
Stationary phase: Silica Gel G
Mobile phase: Methanol: Benzene (2:8 V/V)
Spraying reagent: Conc. Sulphuric acid
Rf observed: 0.5 (Violet spot)

3. U.V. spectrum of Charantin: A UV spectrum was recorded on Model UV-1601, UV-VIS Spectrophotometer (Shimadzu) between wavelengths 400 to 200 nm. By taking P^H 6.8 Phosphate buffer solution as a blank and preparing 10 µg/ml solution of Charantin in P^H 6.8 a buffer solution.

4. FT- IR Spectrum of Charantin: The IR of Charantin was recorded on Model. FTIR 8400S, Fourier Transform Infrared Spectrophotometer (Shimadzu). The pellets were prepared on KBr press (sun biologics, Bangalore, Karnataka, India) using mixture of sample and KBr in about 1: 10 ratio. The spectra were recorded over the wave number range of 4000 to 400 cm^{-1}.

RESULT AND DISCUSSION

Plant drugs are now receiving great attention for their therapeutics and because of this extensive research are now being carried out in this area.

However, herbal drugs being a complex mixture of several phytoconstituents, it becomes difficult to decide that which component is responsible for activity. The isolation of the various constituents also is a tedious process. Charantin was isolated from fruit by various methods, Characterization of Charantin was done by Melting Point, UV, TLC, FT-IR. Charantin was non-nitrogenous neutral substance, Light Yellowish brown or White in color (Figure 1), melted at 269^0C with decomposition, giving a positive Libermann- Burchard Test, Decolourising dilute potassium permanganate & bromine water. UV Spectrum showed that Charantin absorbs exactly at 339 nm. TLC of Charantin with solvent system Methanol: Benzene (2:8) showed Rf value 0.45 (Figure 2). The FT-IR (Figure 4) of the isolated substance showed the presence of functional groups like 3400 (Broad, Free. OH Stretching), 1663 (C=C), 1042 (C-O str.), 892 (>C=CH2).

Fig. 01: Charantin.

Fig 02: TLC of Charantin.

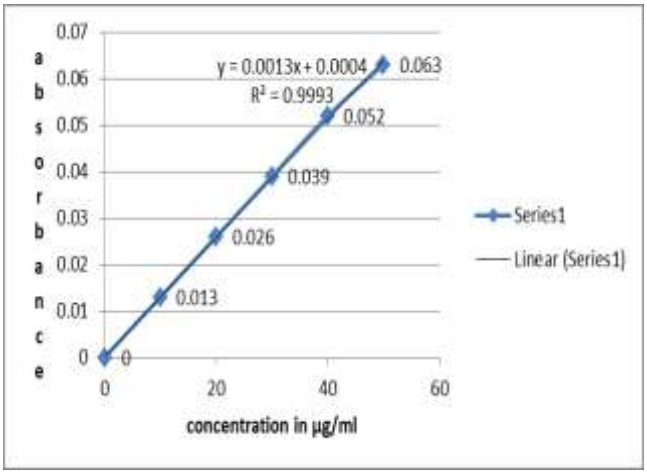

Fig. 03: Standard calibration curve of Charantin.

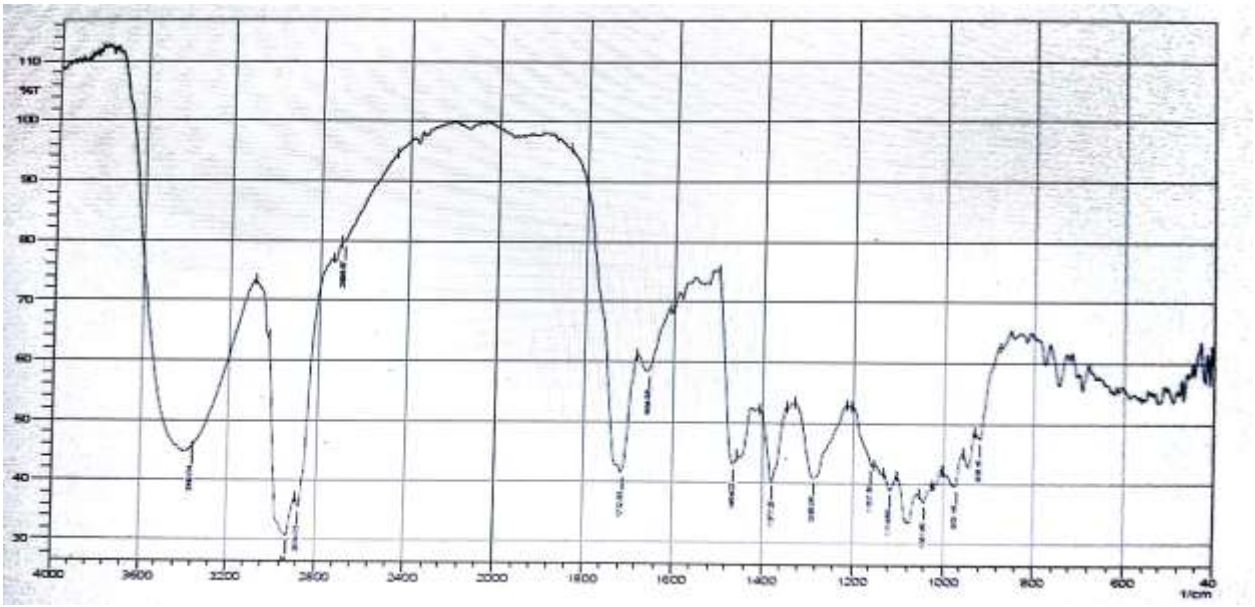

Fig. 04: FT-IR Spectra of Charantin.

CONCIUSION

A more benign alternative of Charantin extraction from fruits of *M. charantia* was proposed. The present investigations provide useful information about extraction and isolation of Charantin from *Momordica Charantia* by using various methods. Among them the reflux condenser method of extraction is can easily conduct and yield higher amount of Charantin than compared to other two methods. The obtained Charantin can use further for formulation. Charantin is having antidiabetic activity as like insulin; hence Charantin can be employed as antidiabetic drug further to be evaluated clinically. The isolated Charantin can be formulated as microspheres, nanoparticle or phytosomes to ensure the control release and to enhance the aqueous solubility in future aspects.

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