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ISOLATION, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF CHARANTIN FROM *MOMORDICA CHARANTIA* LINN. FRUIT

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ABSTRACT

Momordica Charantia (Cucurbitaceae) is commonly known as bitter guard in English and karela in Hindi. The fruit has claimed to contain charantin, steroidal saponin, momordicosides, carbohydrate, mineral matters, ascorbic acid, alkaloids, glucoside, etc. Earlier claim shows that the plant used as stomachic, carminative, tonic, antipyretic, antidiabetic, in rheumatoid arthritis and gout. The present investigation was carried out to isolate, purify and characterize Charantin from fruit of Momordica Charantia Linn. The isolated charantin was further characterized with the help of Ultraviolet Spectroscopy, Thin Layer Chromatography, Fourier Transform Infra Red Spectroscopy, Mass Spectroscopy, Proton- Nuclear Magnetic Resonance Spectroscopy confirmed the identification. The antibacterial activity of charantin was tested by using Agar Diffusion (Cup Plate) method. The minimum inhibitory concentration (MIC) of crude extracts were determined for various organism which was 0.2 mg/ml. The present studies confirm better antimicrobial activity of Charantin when compared with standard, against bacterial species such as gram positive (Bacillus subtilis), gram negative (Pseudomonas aeruginosa) and fungal strains (Saccharomyces cerevisiae).

Key words: *Momordica Charantia, Charantin, Antimicrobial Activity*

INTRODUCTION

In developing countries-all over the world-80% of population continues to use traditional medicine in primary medical problems. In the past decade, therefore, research has been focused on scientific evaluation of traditional drugs of plant origin. *Momordica Charantia* is one such plant that has been frequently used as medicine [1]. *Momordica Charantia* Linn. Cucurbitaceae is a well known to possess antihyperglycemia, anticholesterol, immunosuppressive, antiulcerogenic, anti sperma-togenic and androgenic activities anti-HIV, anti-ulcer, anti-inflammatory, anti-leukemic, anti-microbial, anti-cholesterol, immunosuppresive, and anti-tumor activities [2,3,4]. Unripe fruits of bitter melon have been found to have blood sugar lowering capacity, similar to that of insulin and can be used to treat patients with diabetes. The compound that is responsible for this action is non nitrogenous substances charantin, which is a mixture of two compounds, namely, sitosteryl glucoside and stigmasteryl glucoside. Charantin is 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. In some cases, the injected patient shows signs of side effects. Plant derived compounds that show ant

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diabetic property such as charantin and others are now being widely accepted as an alternative medicine for diabetes mellitus, and they are free from side effects [5,6,7,8]. These chemicals are concentrated in fruits of *Momordica charantia* has shown more pronounced hypoglycemic/antihyperglycemic activity [9]. *Momordica Charantia* shown promising effects in prevention as well as delay in progression of diabetic complication such as nephropathy, neuropathy, gastroapresis, cataract and insulin resistance [10]. Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs. There is an urgent need to systematically evaluate the plants used in traditional medicine. Such research could lead to new drug discovery or advance the use of indigenous herbal medicines for orthodox treatment. Now a day a renewed interest in traditional medicine is observed and there has been an increasing demand for more and more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs many of which have adverse side effects [11].Therefore an attempt has been made to be isolated, purified, characterizes & to find out antimicrobial activity of charantin from *Momordica Charantia* Linn. Fruit.

Material and Methods

Plant materials

The fruits of *Momordica Charantia* Linn. were collected in July 2008, from local market of Modasa, Gujarat, India. The fruit was identified by comparing it morphologically and microscopically with description given in different standard texts and floras. Besides these, the fruits was then identified and authenticated by Dr. H. B. Singh Scientist and Head of Raw Materials Herbarium & Museum, Dept of National Institute of Science and Communication and Information Resources (NISCAIR), New Delhi, and a voucher specimen (SKP 22072008/01) was deposited in Department of Pharmacognosy, shri B. M. Shah College of Pharmaceutical Educcation and Research, Modasa.

Isolation of Charantin from *M. Charantia* Linn.

Fresh unripe fruits of *Momordica Charantia* bought from the market were cut into small bits and dried in a hot air oven below 60^0C. The dried material was broken into a coarse powdered and percolated successively with petroleum ether ($60\text{-}80^0$) and 80% ethanol. The petroleum ether extract was rejected and the ethanolic extract was concentrated in vacuum, then suspended in 95% ethanol and rendered alkaline with KOH to around pH 10. After 48 hours, the suspension was diluted with water and extracted with ether. The ether extract was washed with water, 5% Hydrochloric acid, again with water, and dried over anhydrous sodium sulphate. The ether was distilled off and the residue recrystallized several times from 95% ethanol. The substance thus obtained was referred to here as charantin [6].

Characterization of Charantin

Melting point

Melting point of isolated compound was determined by capillary method.

Chemical Test

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

2. Decolourising dilute potassium permanganate & bromine water

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

R_f observed : 0.5 (Violet spot)

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 methanol as blank and preparing 100 µg/ml solution of charantin in methanol.

FT- IR Spectrum of Charantin

The IR of charantin was recorded on Model – FT-IR 8400S, Fourier Transform Infrared Spectrophotometer (Shimadzu). The pellets were prepared on KBr press (Spectrochem pvt. Ltd., Mumbai, 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}.

Mass Spectrum of Charantin

MS analysis of isolated compound was obtained from Model QP 2010, GC-MS (Shimadzu). The compounds were identified by comparision of their mass spectra with the published mass spectra or NIST-MS library.

^{1}H-NMR of Charantin

^{1}H-NMR analysis of isolated compound was obtained from Model Mercury Plus 300MHz NMR SPECTROMETER, VARIAN, USA by using $CDCl_3$ solvent.

Anti-microbial activity of Charantin

Micro-organisms used

Mother culture for gram-positive *Bacillus subtilis* (ATCC6633), gram-negative *Pseudomonas aeruginosa* (ATCC25619) and Fungi *Saccharomyces cerevisiae* were procured from Department of Microbiology, Sir P. T. Science College, Modasa.

Preparation of Inoculums

Suspension of organism was prepared as per McFarland nephelometer standard (Ellen & Sydney 1990). A 24 hour old culture was used for the preparation of bacterial suspension. Suspension of organism was made in a sterile isotonic solution of sodium chloride (0.9% w/v). The medium was prepared by dissolving all the ingredients in distilled water and subjected to sterilization in an autoclave at 121^{0}C for 15 minutes. The Petri plates were washed thoroughly and sterilized in hot air oven at 160^{0}C for 1½ hours. 30 ml of sterile molten agar medium was seeded by organisms (about 2 ml according to McFarland's standard), in semi hot conditions (40^{0}C) was poured aseptically in sterile Petri plate and allowed to solidify at room temperature. Bores were made on medium using sterile borer and 0.1 ml of charantin solution were added to respective bore and 0.1 ml of the standard at a concentration 0.5, 1.0 and 1.5 mg/ml. The Petri plates were incubated at 32-35^{0}C for 24 hours in a BOD incubator and zone of inhibition was observed and measured using a scale. The same procedure was repeated for fungi strain by using Sabourad's dextrose agar medium for fungus dextrose agar medium; where Petri plates were incubated at 29-31^{0}C for 48 hours in a BOD incubator and zone of inhibition was observed and measured using a scale [12,13,14,15]. All the tests were performed in triplicate.

Determination of zone of Inhibition

The charantin were dissolved in DMSO to get the concentration of 0.5, 1.0, and 1.5 mg/ml. Evaluation of the activity was carried out by cup-plate technique using nutrient agar medium for bacteria and Sabourad's dextrose agar medium for fungai strain. Antibacterial activity was measured in terms of zone of inhibition.

Results 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 method suggested by Lolitkar M.M. and Rao R. Characterization of charantin was done by Melting Point, UV, TLC, FT-IR, MS, ^{1}H-NMR. Charantin was non-nitrogenous neutral substance, Light Yellowish–White in colour(Figure 1), % Yield was 0.091 of the dried fruits, melted at 269^0 C with decomposition, giving a positive Libermann-Burchard Test, Decolourising dilute potasium permanganate & bromine water. UV Spectrum showed that charantin absorbs exactly at 278 nm. TLC of charantin with solvent system Methanol: Benzene (2:8) showed Rf value 0.45 (Figure 2). The FT-IR (Figure 3) 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=CH_2). The MS of the isolated substance showed presence of M^+ at m/e 435, $C_{35}H_{58\text{-}60}$ and fragment ions at m/e 389, 375, 299, 279, 253, 229, 213, 161,147, 121, and 119. The ^{1}H-NMR (Figure 4) of isolated substances showed Chemical Shift (δ) value at 7.5 (3H), 5.591 (*s*, 6H), 5.49-4.51 (*m*, 4H), 5.446-5.437 (*d*), 4.335-4.464 (*m*), 2.193-2.537 (*m*), 0.888, 0.908, 0.986, 1.15, 1.192, 1.309 (methyl signal, 18H). *Spathodea campanulata.*

Fig 1: Powder of Charantin

Fig 2: TLC of Charantin

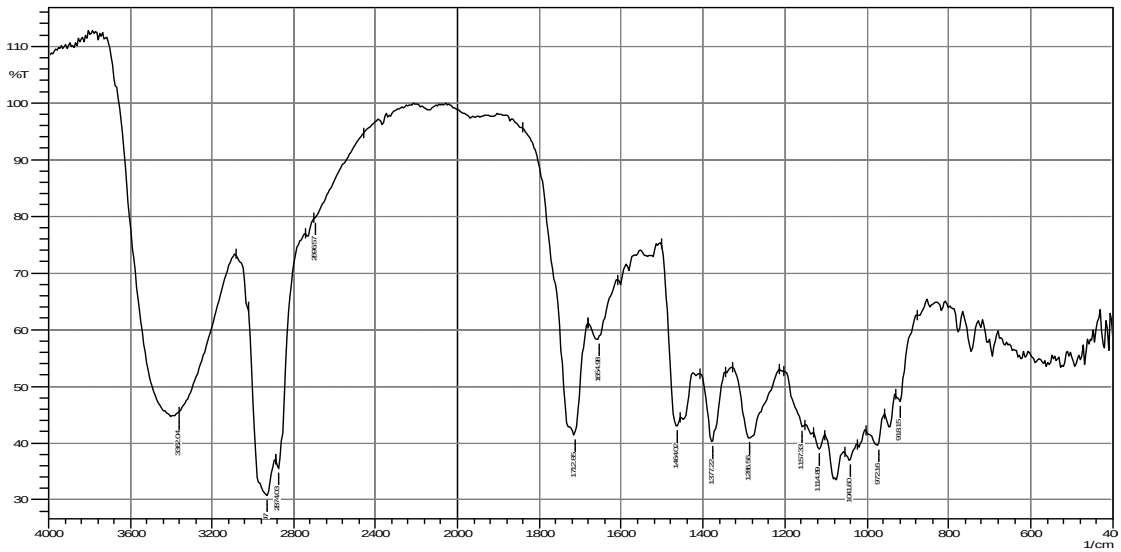

Fig 3: FT-IR Spectra of Charantin

FT-IR showed the presence of important functional group like –OH Stretching, C=C, C-O stretching, >C=CH_2.

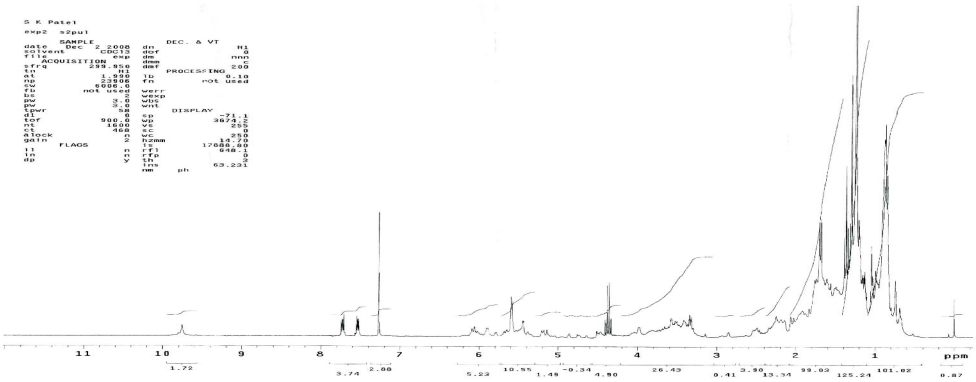

Fig 4: ^{1}H-NMR Spectra of Charantin

The ^{1}H-NMR of isolated substances shows Chemical Shift (δ) value at 7.5 (3H), 5.591 (*s*, 6H), 5.49-4.51 (*m*, 4H), 5.446-5.437 (*d*), 4.335-4.464 (*m*), 2.193-2.537 (*m*), 0.888, 0.908, 0.986, 1.15, 1.192, 1.309 (methyl signal, 18H).

Streptomycin (for *Bacillus subtilis* gram +ve organisms) showed 12.56, 13.13, 14.1 mm, Ampicillin (for *Pseudomonas aeruginosa* gram -ve organisms) showed 12.83, 15.53, 16.76 mm, and Fluconazole (for *Saccharomyces cerevisae* fungi) showed 13.1, 14.56, 16.16 mm zone of Inhibition with concentration 0.5, 1, 1.5 mg/ml respectively. The results were mentioned in Table 1.

Table 1: Anti-microbial activity of Standard

Microorganism	Diameter of zone of Inhibition in mm*		
	B.subtilis	***P. aeruginosa***	***S. cerevisae***
Concentration mg/ml	Streptomycin	Ampicillin	Fluconazole
0.5	12.56 ± 0.033	12.83 ± 0.033	13.1 ± 0.057
1	13.13 ± 0.066	15.53 ± 0.033	14.56 ± 0.066
1.5	14.1 ± 0.057	16.76 ± 0.033	16.16 ± 0.088

*Values are in terms of Mean ± SEM of results done in triplicate.

Charantin isolated from *M. Charantia* Linn. fruit showed good Antibacterial activity 5.1 ± 0.057 (gram +ve), 8.06 ± 0.066 (gram -ve) & 8.96 ± 0.033 (Fungal strain) with concentration 1.5 mg/ml as compared to Standard. (Streptomycin for *Bacillus subtilis* gram +ve organisms, Ampicillin for *Pseudomonas aeruginosa* gram -ve organisms and Fluconazole for *Saccharomyces cerevisae* fungi). The results were mentioned in Table 2.

Table 2: Anti-microbial and Anti-fungal activity of isolated Charantin

Charantin Concentration (mg/ml)	Diameter of zone of Inhibition in mm*		
	B.subtilis	***P. aeruginosa***	***S. cerevisae***
0.5	3.96 ± 0.033	6.16 ± 0.088	5.03 ± 0.033
1	4.56 ± 0.033	7.53 ± 0.033	6.1 ± 0.057
1.5	5.1 ± 0.057	8.06 ± 0.066	8.96 ± 0.033

Conclusion

The present investigations provide useful information on antimicrobial activity of Charantin isolated from *Momordica Charantia*. Among the organisms tested P. *aeruginosa* (gram -ve) & *Saccharomyces cerevisae* (fungal strain) was more susceptible to the Charantin. Further pharmacological and clinical studies are required to

understand the mechanism and the actual efficacy of the charantin in treating various infections and skin diseases like psoriasis.

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