IDENTIFICATION OF AN ALKALOID MOMORDICIN FROM FRUITS OF *MOMORDICA CHARANTIA L.*

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ABSTRACT

Momordica charantia commonly known as Bittergourd belongs to Cucurbitaceae family, widely used in treatment of different diseases. The fruits of *Momordica charantia* were extracted with methanol, ethanol, hexane and water.The present investigation was aimed at to study the presence of phytochemicals in fruits extracts of *Momordica charantia*. Phytochemical screening shows the presence of terpenoids,flavonoids,proteins,saponins,alkaloids,and carbohydrates. The structure of the active compound was determined by interpretation of the spectral data (FTIR and LC- MS) and its structure was elucidated as13-hydroxy-28-methoxy-urs-11-en-3-one which is known as momordicin.

Keywords: Alkaloid, FTIR (Fourier transform infrared spectroscopy)
Momordica charantia, Momordicin, phytochemicals,

1. Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world.*Momordica charantia* is one such plant consists of a number of constituents which contribute to medicinal value of the plant. Some of the important constituents present in the different plant parts are fruits, root, leaf, seed and the entire plant. *Momordica charantia* not only possesses important medicinal components but also contains several other chemical constituents which offer an array of medicinal activities.

Popularity of *Momordica charantia* in various system of traditional medicines for several ailments (antidiabetic, contraceptive, jaundice, abdominal pain, kidney stones, piles, pneumonia, fever etc.) The anticancerous and antileukemic activity of *Momordica charantia* against numerous cell lines including liver cancer, human leukemia melanoma and solid sarcomas have also been documented (Zhu, et al., 1990).

In recent times 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 safer and more dependable than the costly synthetic drugs many of which have adverse side effects (Parekh, 2006). Most recent researches on the plant show that it has ability to inhibit the enzyme guanylate cyclase that is thought to be associated with psoriasis, leukemia and tumor pathogenesis (Grover et al.,2004; Takemoto et al., 1983) crude extracts of the fruit of *Momordica charantia* possess antidiabetic activity (Rathi, 2002; Guevara,1989) and many cucurbitane-type triterpenoids have been isolated from the fruits,(Zhu, et al.,1990 ; Hardman, 1980) seeds,(Rathi,2002., Okabe ,1982) and leaves of *Momordica charantia*.It has been used in treating peptic ulcers, is potential against *Helicobacter pylori*.

2. MATERIALS AND METHODS

2.1 Collection of plant materials

The fruits of *Momordica charantia* were collected from local market of Tirupati. The fruit parts were cut in to small pieces, air-dried and pulverized in to course powder by using a dry grinder and passed through the sieve before being stored in closed vessel for further use.

2.2 Extraction

Shade dried fruits of *Momordica charantia* were powdered and weighed accurately and subjected to extraction in a soxhlet apparatus at room temperature for 48 hours using methanol, ethanol, hexane and water. Alcoholic extract was concentrated under the vacuum in the rotary flash evaporator and successively in hot air oven till solid to semisolid mass. Extracts were stored in an airtight container in refrigerator below 10°C.

2.3 PHYTOCHEMICAL ANALYSIS

2.3.1 Preliminary photochemical investigations

The extract of fruit parts of *Momordica charantia* were subjected to qualitative tests for the identification of various active constituents viz. glycosides, alkaloids, flavonoids, proteins, saponins, carbohydrates, amino acids and tannins, free reducing sugar, saponins using standard test procedures (Roopashree, 2008; Kokate.,2007)

2.3.2 TEST FOR CARBOHYDRATES

To 2 ml of extract, 2 drops of Molisch's reagent was added, then shaken well, and 2 ml of concentrated H_2SO_4 was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates.

2.3.3 TEST FOR CHOLESTEROL

In a dry test tube 2 ml of the extract, 2 ml of the chloroform, 10 drops of acetic anhydrate and 2 to 3 drops of concentrated H_2SO_4 were added. A red rose color changed to blue green color was observed.

2.3.4 TEST FOR PROTEINS

To 2 ml of protein solution 1 ml of 40% NaOH solution and 1 to 2 drops of 1% $CuSO_4$ solution were added. A violet color indicated the presence of peptide linkage of the molecule.

2.3.5 TEST FOR AMINOACIDS

To 2 ml of sample, 2 ml of ninhydrin reagent was added, and kept in water bath for 20 minutes. Appearance of purple color indicated the presence of amino acids in the sample.

2.3.6 TEST FOR ALKALOIDS

To 3 ml of sample, 1% HCl, 6 drops of Mayer's reagent and Dragendroff's reagent were added. An organic precipitate indicated the presence of alkaloids in the sample.

2.3.7 TEST FOR FLAVONOIDS

Five ml of dilute ammonia solution was added to a portion of aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow coloration is observed which confirms the presence of flavonoids and it disappears on standing.

2.3.8 TEST FOR TERPENOIDS

Five ml of each extract was added to 2 ml of chloroform and 3 ml of concentrated H_2SO_4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the terpenoids.

2.3.9 TEST FOR CARDIAC GLYCISIDES

Five ml of each extract was treated with 2 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was underplayed with 1 ml of concentrated H_2SO_4. A brown ring of the interface indicated a deoxy sugar characteristic of cardenolides. A violet ring might appear below the brown ring whereas the acetic acid layer, a greenish ring might form just gradually throughout thin layer.

2.3.10 TEST FOR STEROIDS

Two ml of acetic anhydride was added to 0.5 g of ethanolic extract of each sample with 2 ml of H_2SO_4. The color change from violet to blue or green indicated the presence of steroids.

2.3.11 TEST FOR SAPONINS

The extract with 20 ml of distilled water was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicated the presence of saponins.

2.3.12 TEST FOR TANNINS

A few drops of 1% lead acetate were added to five ml of extract. A yellow precipitate indicated the presence of tannins.

2.3.13 TEST FOR PHLOBATININS

When an aqueous extract of each plant sample were boiled with 1% aqueous HCl, red precipitate was deposited which was taken as evidence for the presence of phlobatinins.

3. EXPERIMENTAL ANALYSIS FOR NMR

All the solvents were analytical grade and purchased from Merck. Melting point was determined in Guna melting point apparatus and is uncorrected. Thin layer chromatography and Column chromatography were performed on silica gel 60 F_{254} and silica 60-120 mesh respectively. ^1H NMR was recorded Bruker 400 MHz spectrometer operated at 400 MHz using $CDCl_3$ and tetramethylsilane as solvent and internal standard respectively. Infrared spectroscopy was recorded on Bruker ALPHA interferometer spectrophotometer. Mass was determined on Shimadzu LCMS-2010A spectrometry.

3.1 Spectral data

Grayish green solid, Mp: 125-128ºC. IR (cm^{-1}) ν_{max}: 3278 (OH str), 2924 (=C-H str), 1740 (C=O str), 1622 (C=C str). ^1H NMR (500 MHz, $CDCl_3$) δ: 0.86-0.91 (m, 6H, CH-$\underline{CH_3}$), 1.25-1.35 (m, 15H, C-$\underline{CH_3}$), 3.54 (s, 3H, O-$\underline{CH_3}$), 6.06-6.19 (m, 2H, CH=CH). LC-MS : 493 (M+Na).

4. RESULTS AND DISCUSSION

The present study carried out on the plant samples revealed the presence of medicinally active metabolites. The photochemical characters of *Momordica charantia* are summarized in the below table. The aqueous extract found to contain carbohydrates, proteins, amino acids, sterols, flavonoids, phlobatanins, terpenoids, cardiac glycosides, and saponins. Ethanolic extract was found to contain carbohydrates, proteins, amino acids, alkaloids, cardiac glycosides, cholesterol, sterol, and phlobatanins. Hexane extract showed the presence of carbohydrates, proteins, amino acids, sterols, alkaloids, cardiac glycosides, saponins, and cholesterol. Methanolic extract was found to contain alkaloids, glycosides, cholesterol, saponins, flavonoids proteins and terpenoids. The secondary metabolites are of immense importance for use as commercially as well as biologically active compounds. The medicinal values of Bitter melon stretch in the bioactive phytochemical constituents that are non nutritive chemicals that produce definite physiological effects on human body and protect them from various diseases. In *Momordica charantia* primary metabolites are common sugars, proteins and chlorophyll while secondary metabolites are alkaloids, flavonoids and tannins. Phenolic compounds, flavinoids are one of the widespread groups also acting as chemotaxonomic markers (Harborne, Shahidul Islam et al., 2011) Phytosteroids are pharmacologically important for human life (Hardman, 1980).

Bio active phytochemical compound was isolated as Grayish green amorphous powder. The LC-Mass showed the molecular ion peak at 493 m/z (M^+ + Na) which corresponds to the molecular formula $C_{31}H_{50}O_3$ (470 m/z). In infrared spectroscopy, broad absorption bands at 3278 cm^{-1} corresponds to OH group. The absorption peaks at 2924 (=C-H) and 1622 (C=C) cm^{-1} suggested that olefin was present in the compound and the band at 1740 cm^{-1} indicates the typical carbonyl group. In proton NMR, the signals at 1.35-1.25 ppm appeared as multiplet corresponds to tertiary methyl containing 15 protons (5x3)

along with a multiplet at 0.91-0.86 ppm (6H) belongs to secondary carbon methyl groups. A multiplet at 6.19-6.06 ppm with 2 protons indicates the olefin protons. From the above data we confirmed that the compound was Momordicin which is also known as 13-hydroxy-28-methoxy-urs-11-en-3-one.

Table – 1: ***Phytochemical screening of Momordica charantia fruit extracts***

Type of extract	Methanol	Ethanol	Aqueous	Hexane
Alkaloids	+	+	+	+
Glycosides	+	+	+	+
Carbohydrates	+	+	+	+
Saponins	+	+	+	-
Amino acids	+	+	+	+
Flavonoids	+	+	-	-
Proteins	+	+	+	+
Terpenoids	+	+	+	+
Phlobatanins	+	+	+	-
Sterioids	+	+	+	+
Cardiac glycosides	+	+	+	+

SPECTRAL ANALYSIS

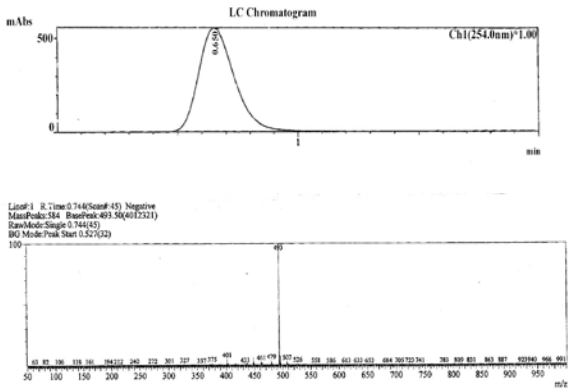

Figure 1: Mass Spectrum

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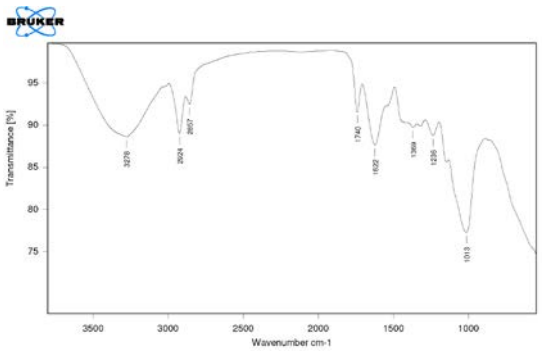

Figure 2: Infrared spectrum

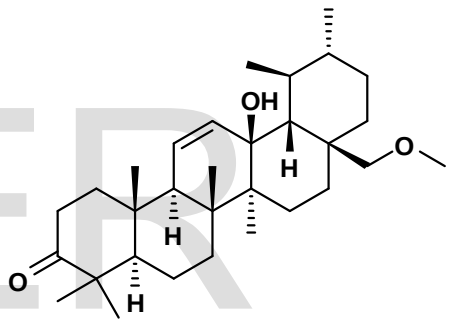

Figure 3: Structure of Momordicin

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