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Original Article

Gas Chromatogrphy- Mass Spectrometry Analysis and Phytochemical Screening of Methanolic Fruit Extract of *Momordica charantia*

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Abstract

The fruits of vegetable *Momordica charantia* have been used as folklore medicine for the management of ailments such as leprosy, eczema, piles, rheumatism, malaria, menstrual problems, hypertensions, stomach pain, infections, cold, cough and most efficiently for diabetes. The phytochemical screening of methanolic extract of *Momordica charantia's* fruit exhibited the presence of alkaloids, steroids, flavonoids, tannins, saponins, cardiac glycosides, phlobatinnins, carbohydrate and terpenoids. The gas chromatography-mass spectrometry (GC-MS) analysis of methanolic extract identified the presence of phytochemical like Vitamin E, Gentisic acid, 1-Pentadecyne, Cucurbitacin B Dihydro, Cis-9-hexadecenal, Hexadecanoic acid, methyl ester, Pentadecanoic acid14- methyl-, methyl ester, β-sitoserol, Stigmasterol, Oleic acid, Stigmastan-3-ol, Ethyl-4,5-dimethyl-phenol and Linoleic acid. This is first report on phytochemical screening of methanolic extract of *M. Charantia* fruits. GC-MS analysis shows the presence of compounds which have medicinal properties.

Key words: *Momordica charantia*, gas chromatography- mass spectrophotometry, phytochemical analysis

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Introduction

Plants play a prominent role in maintenance of human health and used as medicine, since ancient times. According to estimation of World Health Organization (WHO) plant extracts are used as folk medicine in traditional therapies of 80% of the world's population (Baker et al., 1995) and more than 30% of the entire plant species have been used for medicinal purposes (Joy et al., 1998). The medicinal plants of India accounts nearly two third of the total plants species used in modern system of medicine and in rural area as tea, decocts or extracts. Herbal drugs are widely prescribed, even when their biological ingredients are not known, due to their effectiveness, fewer side effects and low cost (Kumar et al., 2009, Ajayi et al., 2011). The rational design of novel drugs from traditional medicine obtained from plant offers new prospects in modern health care (Manjamalai et al, 2010).

This vegetable plant, *Momordica charantia* belongs to family Cucurbitaceae cultivated in Asia, east Africa, Caribbean country, parts of Amazon and South America as a food, nutrition and medicine. It is branched, climbing annual, monoecious plant with angled and grooved stems, long stalked leaves and yellow solitary male and female flower borne on leaf axil. Fruits are 5- 15cm long with 3-valved capsules, pendulous, fusiform, ribbed and beaked bearing numerous triangular tubercles. Seeds are many or few with shiny sculptured surface. Synonyms of *M.charantia* are *M.chinensis, M.elegans, M. Indica, M. Operculata, M.sinensis* and *Sicyos fauriei*. The species is known by different names in different parts of the world. such as Bitter gourd (in English), Karela (in Hindi and Urdu), Kugua (in Chinese), Parya and Pare ayam (in Javanese and Indonesian) and Nigauri (in Japanese). All parts of plant, especially roots, leaves, fruits and seeds are widely used as traditional medicine throughout Asia, East Africa and South America. The roots are useful in treatment of coloptosis and Opthalmopathy. The leaves are useful in vitiated conditions of pita, helminthiasis, constipation, intermittent fever, burning sensation of the sole and nyctalopia. The fruits are useful in skin diseases leprosy, ulcers, wounds, burning sensations, constipation, anorexia, colic, helminthiasis, rheumatalgia, diabetes, asthama and dysmenorrhoea.Seeds are useful in treatment of ulcers, pharyngodynia and obstructions of the liver and spleen. The leaves and fruits are used for external application in lumbago, ulceration and bone fracture and internally in leprosy, haemorrhoids and Jaundice (Warrier et al, 1995). Fruits and seeds of bitter gourd possess medicinal properties such as anti- HIV, anti-ulcer, anti-inflammatory, anti- leukemic, anti-microbial, anti-tumor and antidiabetic property (Taylor, 2002), besides both are hypoglycemic, cytotoxic and anti-feedent (Hossain et al, 1992) and reduce the blood cholesterol level (Taylor, 2002).

The pharmaceuticals studies of fruit extract of *Momordica charantia* have shown that the plant has antimicrobial (Mwambete, 2009), antioxidant (Kubola and Siriamornpun, 2008) and anti-inflammatory and immunomodulatory (Manabe et al, 2003), hypoglycemic and hypolipidiamic action (Abd El Baky, 2009), antiallergic (Gupta et al, 1993), anthelmintic (Grover et al, 2004) anticancer activity (Sun et al, 2001), antiplasmodial (Kohler et al, 2002), antileshmania (Gupta et al, 2010) and antiulcer activity (Matsuda et al, 1999)

Several active compounds previously identified and isolated from the fruit of *Momordica charantia* include glycosides (momordin, charantin, charantosides, goyaglycosides, momordicosides), terpenoid compounds (momordicin-28, momordicinin, momordicilin, momordenol, and momordol) and ribosome-inactivating cytotoxic proteins such as momorcharin, momordin and cucurbitane type triterpenoids (Lee et al, 2009). The present investigation was carried out to identify active ingredients present in the methanolic fruit extract of this plant by phytochemical screening and (GC-MS) analysis.

Materials and Methods

The sample *M. charantia* fruits were collected from the experimental plot atBotanical garden of Banaras Hindu University. Methanol used for extraction of active ingredient was of HPLC grade (Merck, India).

Preparation of extract

The fruits were taken from the healthy growing plant, washed with tap water two to three times to remove dust particles and cut into small pieces. These were then kept in an oven at 50˚C. The dried fruits were then ground in fine powder by using grinder.

10g of ground sample was exhaustly extracted in 150 ml of methanol with soxhlet extractor and then concentrated using rotary vacuum evaporator. The extract (10 ml) from the concentrate was used for phytochemical screening while the remaining extract was evaporated to complete dryness at 35˚C in a dark brown colour solid residue. The dried extract was stored in air tight container and placed in refrigerator.

Phytochemical screening

The fruit extract was subjected to preliminary phytochemical screening following methods described by Evans (1996).

Alkaloids

For the screening of alkaloids, 1.0 ml extract was mixed in 5 ml dil. Hydrochloric acid on a steambath, filtered and 1.0 ml of Mayer's reagent was added to 1.0 ml of filtrate in separate tube. A cloudy slightly yellow colour indicates the presence of alkaloids.

Steroids

The screening for the presence of steroid in the *M. Charantia* extract was performed by Liebermann-Burchard's test. Extract (0.5 ml) was dissolved in 2.0 ml acetic anhydride and cooled in ice, then 1.0 ml conc. H_2SO_4 was added and formation of blue green ring was considered positive for presence of steroids.

Tannins

In 1.0 ml plant extract, equal volume of Ferric chloride ($FeCl_3$) or bromine water was added and formation of greenish black (reddish brown) precipitate indicated the presence of tannins.

Flavonoids

In methanolic fruit extract of *M. charantia*, screening of flavonoids was done by Ferric chloride test. Extract (0.2 ml) was added to 10% $FeCl_3$ and mixture was shaken. A woody brownish precipitate indicates the presence of flavonoids.

Saponins

Frothing test was used to determine the presence of saponin in fruit extract of *M. charantia*. In the test, 0.2 ml extract was mixed with 5.0 ml distilled water, shaken for 20 min. Persistence of foams indicates presence of saponins.

Cardiac-glycoside:

The screening of cardiac- glycoside was done by using Legal's method in which 1.0 ml extract was dissolved in 5.0 ml pyridine, 2 drops 2% Sodium Nitroprusside and 2 drops 20% NaOH were added. A deep red color faded to brown indicates presence of cardenolide.

In second method 0.5 g extract was dissolved in 2.0 ml chloroform and 1.0 ml conc. H_2SO_4 was carefully added. A reddish brown colour indicates the presence of steroids.

Phlobatinnins test

1.0 ml extract was boiled in 2.0 ml 1% aqueous HCl. A red precipitate indicates the presence of phlobatinnins.

Carbohydrate tests

Extract (1.0 ml) was added to 2.0 ml Fehling's solution and boiled for 5 min. A red precipitate indicates the presence of reducing sugars; 1.0 ml extract was added to 2.0 ml Bradford' reagent and boiled for 1 min. A red precipitate indicates the presence of reducing monosaccharides; 1.0 ml extract was added to 1.0 ml Molisch's reagent and 1.0 ml conc. H_2SO_4 was carefully added. A reddish ring indicates the presence of carbohydrates.

Anthraquinones

Borntrager's test was used for the screening of anthraquinone. 2.0 g extract in 10 ml ethanol, steamed for 5 min and filtered, 2.0 ml filtrate was added to 2.0 ml chloroform, shaken thoroughly, chloroform layer was taken off and 5.0 ml distilled water added which was shaken with 5.0 ml dilute ammonia solution. Absence of red colour in ammonia upper phase indicates the absence of anthraquinones.

Gas Chromatography Analysis

The volatile components were analyzed using Varian 450GC, 240MS (VF-5 MS Column), injector and oven temperature was 250˚C and 200˚C. The heating rate was programmed at 10˚C/minutes. Injection was performed in the split ratio of 200 and the volume was 10µL. The flow of carrier gases was maintained 1.0 ml/minutes during the run.

The identification of the compounds was performed by similarity searches and mass spectra data in the NIST (National Institute for Standard and Technology) MS Search 2.0 Library. The quantification of components was done by relative peak areas calculation. Relative peak areas were calculated by dividing the peak area for compound by the total peak areas for the entire compounds detected and expressing this value as percent.

Results and Discussion

The results of the phytochemical screening of the methanolic fruit extract of *M. charantia* presented in Table 1. This reveals the presence of alkaloids, tannins, steroids, flavonoids, saponins, phlobatinnins, cardiacglycosides and carbohydrates and absence of anthraquinones. The results of the GC-MS analysis identified the various compound present in the plant (Table 2). The major compound present in the methanolic fruit extract of *M. charantia* as identified by GC-MS was Gentisic acid with RT 16.544 and 8.406 %relative peak area. The structure of gentisic acid was given in Fig. 1, molecular formula as $C_7H_6O_4$, molar mass as 150.12 g/mol and biologically active as antioxidant (Table 3). Other compounds also identified from the fruit extract of *M. Charantia* Vitamin E (RT-14.698), 1- Pentadecyne (RT-17.034), Cucurbitacin B Dihydro (RT- 18.498), Cis-9-hexadecenal(RT-20.295), Hexadecanoic acid, methyl ester (20.683), Pentadecanoic acid14- methyl-, methyl ester (RT-21.713), β-sitoserol (21.838), Stigmasterol (24.811), Oleic acid (RT-25.156), Stigmastan-3-ol (25.965), Ethyl-4,5-dimethyl-phenol(26.565) and Linoleic acid (27.355) (Table 2).

There are diverse group of biologically active chemical constituents are presumed to be present in *Momordica charantia* fruit due to this presumption a detailed phytochemical screening and GC-MS analysis was undertaken. In the present study,the phytochemical screening of methanolic extracts of MC fruits showed the presence of alkaloids, steroids, flavonoids, tannins, saponins, cardiac glycosides, phlobatinnins, carbohydrate and terpenoids and GCMS analysis proved the presence of VitaminE, Gentisic acid, Stigma sterol, β-sitosterol and cucurbitacin B which are important medicinal compound. These phytochemicals are secondary metabolites which are the important constituents of medicinal plant. It has been reported that secondary plant metabolites exert a wide range of biochemical activities on physiological systems (Olagunju et al, 2006).

Previously, it was reported that the alkaloids, saponins, glycosides and phenolic constituents are present in Momordica charantia (Dhalla et al,1961; Ullah et al, 2012).

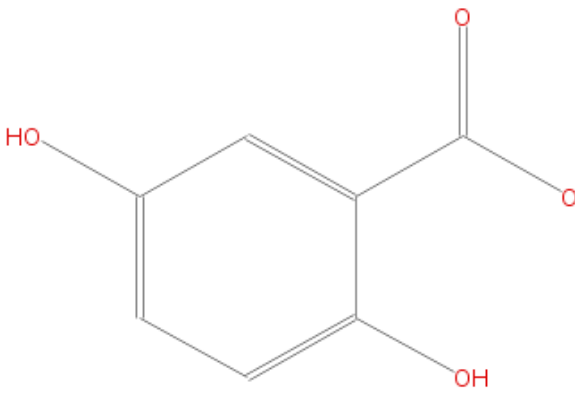

Fig. 1: Structure of Gentisic acid

Table1: Results of phytochemical screening of methanolic fruit extract of *M. charantia*

Phytochemicals	Result
Alkaloids	+
Steroids	+
Tannins	+
Flavonoids	+
Saponins	+
Cardiac- glycosides	+
Carbohydrates	+
Phlobatinins	+
Anthraquinone	-

Table 2: List of chemical compound in fruit extract of *M. charantia*

Components	R.T*. (minutes)	% Relative area peak
Vitamin E	14.698	0.331
Gentisic acid	16.544	8.406
1- pentadecyne	17.034	4.976
Cucurbitacin B Dihydro	18.498	1.674
Cis-9-hexadecenal	20.295	5.082
Hexadecanoic acid, methyl ester	20.683	ND
Pentadecanoic acid14-methyl-, methyl ester	21.713	ND
β-sitoserol	21.838	0.618
Stigmasterol	24.811	2.653
Oleic acid	25.156	ND
Stigmastan-3-ol	25.965	0.928
Ethyl-4,5-dimethyl-phenol	26.565	ND
Linoleic acid	27.355	ND

R.T*-Retention time

Table 3: Properties of Gentisic acid

Properties	Given as
Molecular formula	C7H6O4
Molar mass	150.12 g/mol
Biological property	Antioxidant

The activities of some phytochemicals with compound nature of flavonoids, palmitic acid (hexadecanoic acid ethyl ester and n- hexadecanoic acid), unsaturated fatty acid and linolenic acid (docosatetranoic acid and octadecatrienoic acid) as antimicrobial, anti-inflammatory, antioxidant hypocholesterlemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, antieczimic and anticoronary (Kumar et al, 2010). In *M. charantia* there are various phytochemical compounds present which play major role in the reported biological activities.

Besides Vitamin E, Gentisic acid has also been known as antioxidant agent which acts as free radicals terminators. Thus, Gentisic acid is pharmaceutically important compound which exhibits physiological functions. Cucurbitacin B was also present which has an antiproliferative activity against breast cancer, glioblastoma multiforme and myelid leukemia cells (Thoenissen et al, 2009; Zulbadi et al, 2011). Saponins are natural surfactant. Saponins are amphipathic glycosides of steroids with a distinctive foaming characteristic which have strong biological activity and antifungal and antibacterial properties. Flavonoids are polyphenolic compounds present in plants as secondary metabolites and also have antioxidant activity. It provides many health benefits like protection against damage in blood vessels, thus decreasing the risk of cardiovascular diseases, prevent cancer and enhances immune system of body.

Conclusion

In the present study, the phytochemical screening and GC-MS analysis proved that the *M. charantia* fruits are pharmaceutically important due to presence of different medicinally important phytochemical compound and secondary plant metabolites. The GC-MS analysis of methanolic extract of this plant fruit showed the presence of important bioactive compounds especially, Gentisic acid which has antioxidant activity. Further GC-MS study is required to find out the accurate compound responsible for the plant's medicinal value.

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