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Original Paper

Phenolic Acid Profiling of the Hydro-ethanolic Extract of *Momordica charantia L.*

Nitesh Kumar Sah*

Assistant Professor
Faculty of Agriculture, Far–west University, Nepal

***Corresponding Author Email:** niteshkumarsah5@gmail.com

ABSTRACT

Momordica charantia L. (Family- Cucurbitaceae)is the most famous and highly appraised species loaded with a number of beneficial aspects in terms of health and disease, capable of exhibiting several therapeutic activities that have been utilized in the ancient mode of medication for treating health-related problems. Although many active compounds like Charantin derived from spices has been shown to have promising effect on therapeutics in terms of preventing and treating several diseases. The aimof this study is to obtain the most active phenolic acid compound of M. charantia using LC-MS method. To carry out this study 50% ethanolic extract was obtained from 50 g dried powder of M. charantia fruits. The solution was then concentrated, filtered, stored, and used for LC-MS analysis. The extracts were analyzed by the LC-MS method which identified different functional compounds present in the extract whose molecular mass did not match with other compounds when compared with other literature and on Pubchem. Therefore, the newly found structures were considered as novel compounds in M. charantia which can be studied for their structure and anticancer activities.

KEYWORDS: *Momordica charantia,* Medicinal plant, Phenolic acid, LC-MS, Phytochemicals

INTRODUCTION

Phenolic or phenol carboxylic acids are natural versatile compounds present in bound form in different parts of plants. Phenolic acid serves as precursors for many important bioactive molecules that are not only required for therapeutic purpose but also for cosmetic and dietary purpose to make people healthy and active. They are loaded with higher chemo-preventive properties than antioxidant vitamins; hence they are significant to prevent inflammation and cellular damage caused by free radicals in the human body[1,2,3].

Momordica charantia L. (MC) is one of the vegetable plants which is rich in phenolic acids. Though *M. charantia* is commonly consumed as a vegetable, traditionally the whole parts of the plants are used as herbal medicine. Extract obtained from the whole part of the plant is termed as 'Vegetable insulin' which has been proved as hypoglycemic. The fruit of MC is attributed with anti-carcinogenic and hypocholesterolemic effects, while the leaf has been reported as antiviral, antibacterial, and insecticidal, anthelminthic[4,5] and the seed extract has been reported as antileukemic[6]. A part from various pharmacological activities, adverse reactions of *M. charantia* (such as hypoglycemic coma in children and abortion) also have been reported which limits clinical application of *M. charantia*[7,8].

Many studies have been done for analyzing the extracts of *Momordica charantia* by using different methods for solvent extraction such as water extraction studies by LCMS, GC-MS, and Subcritical Water Extraction

(SCWE). In aqueous extracts of *Momordica charantia*, the phenolic compounds are commonly found among them Gallic acid was found to be more active. Studies on the *M. charantia* extract done by using combined solvent as ethanol and water have reported higher cytotoxic activity, whereas extracts obtained from only water shows higher cytotoxic activity than ethanolic extraction. Though bioactivities of *Momordica charantia* extract have been reported, little scientific information is available on the phytochemical components that may relate to its nutraceuticals and pharmaceutical health benefits. The paper seeks for determination of phenolic acid constituents present in the 50% ethanolic extract of *Momordica charantia.* The study is based on a qualitative research design applying instrumental data related to the area of research which can be an invaluable tool to study the Immunomodulatory property of plant products against the number of diseases.

Nature is full of life saving as well as poisonous plants. Variety of plant extracts have been used for thousands of years as folk medicine. They are the fundamental basis of sophisticated traditional medicine systems due to their unrefuted efficacy as a phytomedicine. As per the report released by World Health Organization (WHO)- the consumption of herbal medicine is safe and effective so it has been dramatically increased in developing and industrialized countries for the treatment of several diseases and disorders[9]. Strong religious and mythical beliefs have been also associated with the healing property of many plant products. The immunomodulatory property of plant products is the increasing area of interest for the investigators that could be considered as an invaluable tool for preventing and treating various infectious and non-infectious diseases in the upcoming era. Although many active compounds derived from spices has been shown to have advantageous effect on therapeutics in terms of preventing and treating several diseases, we have proposed to investigate the most active phenolic acid compound of *Momordica charantia* by Liquid chromatography–mass spectrometry (LC-MS) method.

Momordica charantia is an herbaceous plant that belongs to Cucurbitaceae family, is more typical of South Asian countries. It is familiar with different local names indifferent languages. The fruit have different varieties, substantially in the shape and bitterness of the fruit. Generally, two varieties of bitter melon are common, namely *karela* (*Momordica charantia* L.var.charantia) and ucche (L. var. muricata (Wild.). The unripen fruit is covered with jagged, triangular "teeth" and ridges, green to white in colour, bitter in test and is commonly used in traditional dishes. In developing and poor countries the plant has been traditionally used for treatment of diabetes, wounds, gout, leprosy, scabies, constipation as well as a tool for the management of worms and parasites[10,11]. Medicinal and nutritional application of MC oil also has been reported[12].

Nutrient Profile of *Momordica Charantia*

Momordica charantia plant is composed of bioactive chemicals, vitamins, minerals, and antioxidants. Research has been shown that *Momordica charantia* plant are rich in vitamin A, thiamine, riboflavin, niacin, folate, vitamin B6, vitamin C, vitamin E, minerals (potassium, calcium, zinc, magnesium, phosphorus, and iron), and dietary fiber. The caloric values for leaf, fruit, and seed are reported as 213.26, 241.66 and 176.61 Kcal/100g respectively[13-16].

Phenols, flavonoids, isoflavones, terpenes, anthroquinones, and glucosinolates contents of MC attribute high antioxidant properties to the Mc[17].

Phytochemistry of *Momordica Charantia*

Around 50 cucurbitacins have been isolated from the *Momordica charantia* plant. The cucurbitane type triterpenoids found in plants and which belong to the cucumber family (Cucurbitaceae) are the main chemical constituents of M. charantia. Many cucurbitane-type triterpenoids are isolated from different parts of *M. charantia*[18-22].

Phenolics are chemical components that are synthesized by phenylalanine ammonia-lyase from phenylalanine and cause 91.25% lipid peroxidation inhibition of linoleic acid emulsion[23]. They are applied in the control of human pathogenic infections[24]. Caffeic acid is regarded as the most common of phenolic compounds distributed in the plant flora followed by chlorogenic acid known to cause allergic dermatitis among humans[25]. Phenolics are the host of natural antioxidants that have enormous ability to combat cancer, and are also thought to prevent heart ailments to an appreciable degree and sometimes are anti-inflammatory agents. Till now the cucurbitane type triterpenoids are reported as the key phytochemicals in the *M. charantia*.

MATERIALS AND METHODS

Preparation of Plant Materials

Dried unripe fruits of *M.charantia*, collected from local market were coarsely powdered using regular house hold grinder which was then dissolved in the appropriate solvent for efficient extraction.

Extraction of *Momordica charantia*

Fifty grams of coarsely powdered *M. charantia* fruits were subjected to extraction by the process called maceration (steady-state extraction) using 50% ethanol. A magnetic stirrer was used for the gradient extraction of hydro-ethanol soluble phytoconstituents.

Method

Pastes made from coarsely powdered of *M. charantia* fruits without adding water by using grinder was lyophilized by freeze-dryer (Alpha 2-4 LD Plus from Christ, GmbH). Fifty

grams of the powder were mixed with 350ml of ethanol + 350 ml of distil water in a stoppered container to obtain 50% ethanolic extract of *M. charantia* which was then allowed to stand overnight for complete percolation of soluble phytoconstituents. After overnight incubation with 50% ethanol, *M. charantia* was subjected to maceration using a magnetic stirrer. After two hours of magnetic stirring, the solvent mixture was then centrifuged at 5500 RPM for 15 minutes at 25°C. The liquid was strained off and the pellet that remained at the bottom of the centrifuge tube, i.e. "marc" (the damp solid material) was again collected into another stoppered container and subjected for extraction using a magnetic stirrer. Repeated extraction was done three times with concentration of solvent and after each round; solvent mixture was collected after centrifugation. Finally, the complete solvent mixture was filtered using Whitman's filter paper number-1 and stored at -20 degree celsius.

The Concentration of Plant Extracts

Fifty percent of ethanolic fruit extract was evaporated to remove excess amount of solvent. The obtained solution of ethanolic extract of *Momordica charantia* (EEMC) was concentrated using Rotovapor R-215 (Buchi, Switzerland) which was later filtered and stored in brown bottles at 4 degree celsius. The obtained extract of *M. charantia* was subjected to LC-MS for the analysis. Liquid Chromatography (LC) showed peaks which is followed by Mass Spectrometry (MS). The MS is done to find the mass of the compounds. MS Development was run in Positive Mode.

RESULT AND DISCUSSION

In this study, hydro-ethanolic extracts of *M. charantia* were prepared from its fruits. The extracts were further subjected for LCMS and the data obtained were analyzed. Various methods for solvent extraction of *M. charantia* have been employed in different studies for analyzing the extracts of *M. charantia.* Water extraction of *M. charantia* shows phenolic acid (mostly gallic acid) as well as other compounds such as flavonoids, triterpenoids and fewer amounts of biologically active compounds like momordicoside L, momordicoside1 and momordicoside K[26]. Gallic acid and its derivatives have been shown to have different pharmacological activities (such as antimicrobial, anticancer) in different diseases[27].

Likewise, in methanolic extraction of *M. charantia* done by GC-MS method the compounds mostly seen are alkaloids, steroids, flavonoids, tannins, saponins, cardiac glycoside, phlobatannin, carbohydrate and terpenoids. Studies also indicate the presence of phytochemicals like Vitamin-E, gentisic acid, 1-Pentadecyne, etc in the extracts. Gentisic acid which is present in the extract has shown antioxidant activity[28]. The studies done by others show that ethanolic extraction followed by HPLC analysis shows presence of phenolic compounds, especially gallic acid in *M. charantia*[29].

Since, LC-MS method provides a clear separation of the component therefore, in this study; the extracts were analyzed by LC-MS to determine the peak and molecular weight. The molecular mass obtained is compared with the same molecular mass from the Pubchem structure and from other Literature of *Momordica charantia.*

Based on the data obtained by MS, a total 10 compounds were present in *M. charantia*. Among those, four compounds that differ in molecular weight, were not recognized from the *M. charantia* and literature to date and are predicted to be novel compounds. Further, the extract was subjected to TLC to get the number of peaks and spots and to determine the polar and non-polar compounds. Then, the fractions were introduced to column chromatography in which the compound having high molecular weight appeared first and that with low molecular weight appeared later.

Several functional components of *M. charantia* have been identified. Such types of functional compounds on *Momordica charantia* obtained in the present study are cucurbitane, kuguacin E, Karavilagenin-A, Karavilagenin- D, Karaviloside-IV and Ferulic acid.

Phytochemicals obtained from M. charantia by LC-MS

Cucurbitane:

The cucurbitane-type triterpenoids and their aglycones have been shown to have biological effects beneficial in treating diabetes, obesity, and possess anticancer, anti- HIV, anti-inflammatory and antifeedant properties[30]. Major Cucurbitane compounds obtained from *M.charantia* are Charantin, Kuguacins A-S, Momordicine I, II and III, karavilagenin A, B, C, D and E and karavilosides I, II, III, IV, and V15. Among these, compounds Kuguacin E, Karavilagenin-A, Karavilagenin-D are the cucurbitane-type triterpenoids and Karaviloside-IV is cucurbitane-type triterpenoids glycoside found in presentstudy.

Kuguacin E:

Kuguacin includes Kuguacin A to H type. These are the compounds that belong to cucurbitane type triterpenoids. Among this type of Kuguacin, Kuguacin J type is most studied[31]. The detailed structure of Kuguacin E was established by NMR data as C27H42O4. Its IR spectrum showed absorption at 3536 cm-1, indicating the presence of hydroxyl group. The UV spectrum displayed no conjugated group based on the absence of absorption from 230 nm to 350 nm. The biological activity of the compound Kuguacin E, which is found in this study, has not been shown by any studies in detail to this date.

Karavilagenin-A and Karavilagenin-D are another cucurbitane type triterpenoids obtained in our studies which are studied together with other triterpenoids but haveyet to be studied for their biological activity separately. Likewise, Karaviloside-IV also needs to be studied in detail for biological activities.

Ferulic Acid:

Ferulic acid is an antioxidant and photoprotective agent used in biomedical and cosmetic formulations to prevent skin cancer and senescence. Ferulic acid is a phenolic compound that exhibits anti-inflammatory, antimicrobial, and anticancer properties[32]. As a photo protective agent and antioxidant in biomedical and cosmetic formulations, Ferulic acid also prevents harmful radiation effects both as UV absorber and free radical scavenger[33].

QUALITATIVE ANALYSIS OF THE FRACTION OF *MOMORDICA CHARANTIA* BY LC-MS METHOD

In the figure:1 the main peak shown has molecular weight 437.40 which corresponds to the molecular weight of the compound, kuguacins E (430.61)[34]. Another peak in Figure:1 shows molecular weight 652.50 which corresponds to the molecular weight of Karaviloside-IV (650.88)[34]

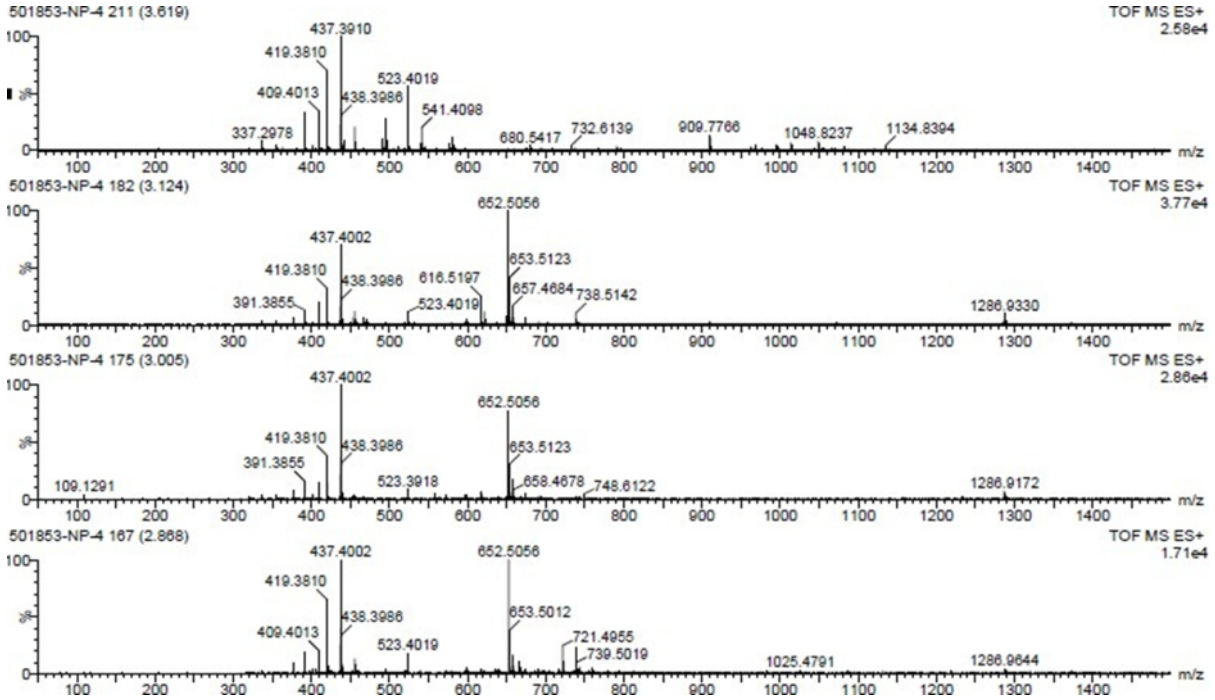

Figure 1: Qualitative Analysis of the Fraction of *M. charantia* by LC-MS Method

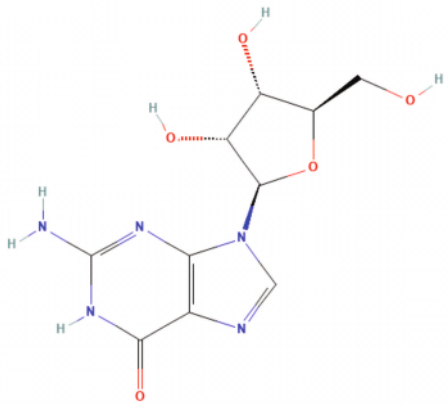

Figure 2(a): Chemical Structure of Kuguacin E (652.50)[34]

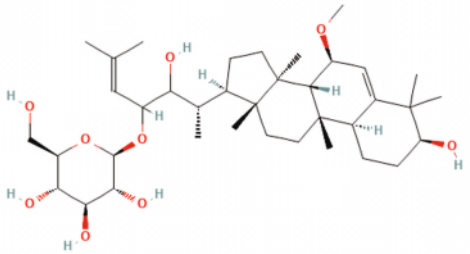

Figure 2(b):): Chemical structure of Karaviloside-IV (437.39)[34]

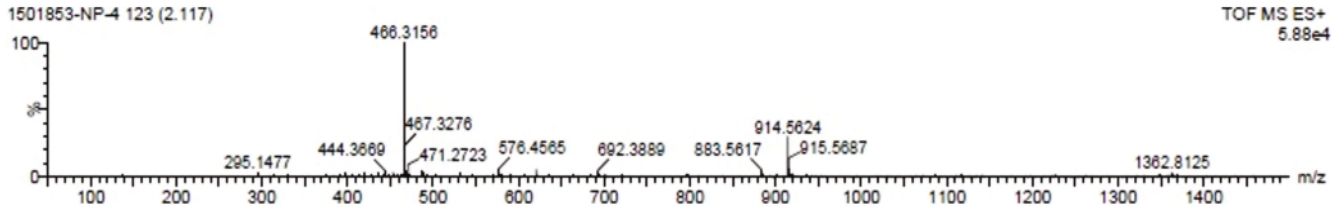

Figure 3: Qualitative Analysis of the Fraction of *M. charantiaby* LC-MS Method

The peak shown in the figure 3 shows the molecular weight 466.31 which corresponds to the molecular weight of Karavilagenin-D (470.68)[34]

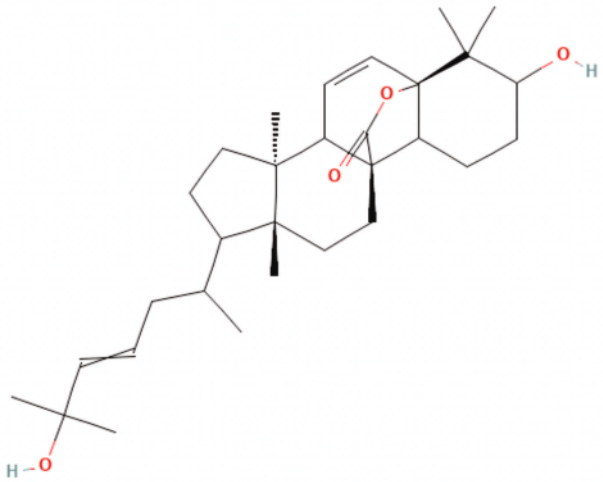

Figure 4: Chemical structure of karavilagenin-D (466.31)[34]

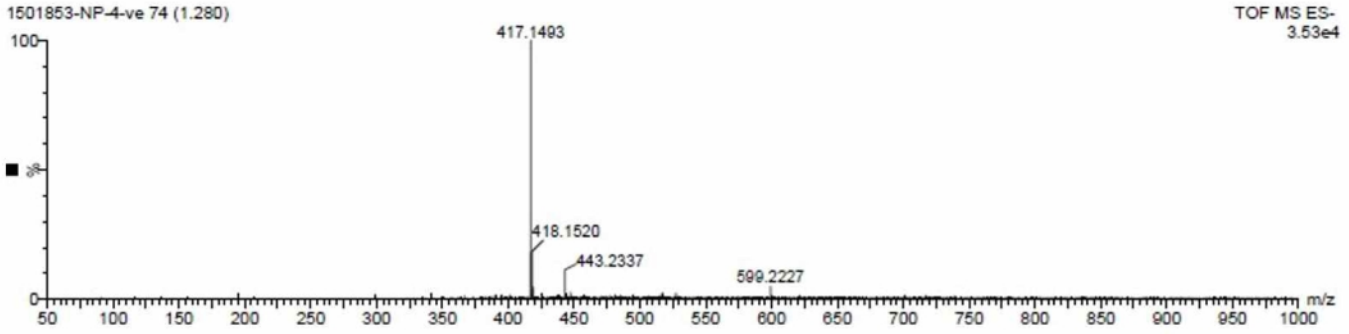

Figure 5: Qualitative Analysis of the Fraction of *M. charantia by* LC-MS Method

The peak shown in the figure 5 shows the molecular weight 417.14 which corresponds to the molecular weight of Cucurbitane (414.74)[34]

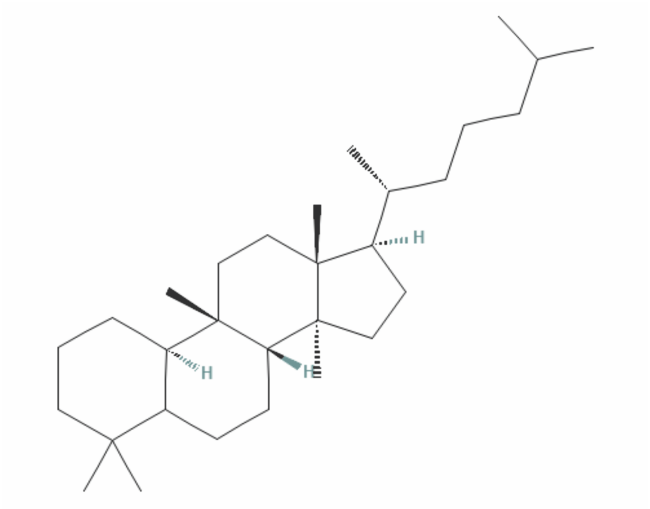

Figure 6: Chemical Structure of Cucurbitane (417.14)[34]

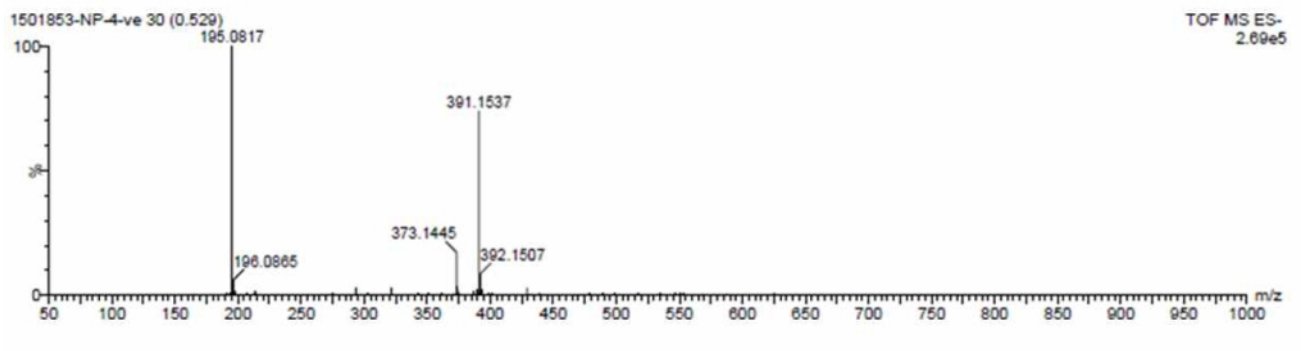

Figure 7: Qualitative Analysis of the Fraction of *M. charantiaby* LC-MS Method

The peak shown in Fig: 7.depicts molecular weight 195.08 which corresponds to the molecular weight of Ferulic acid (194.18)[34]

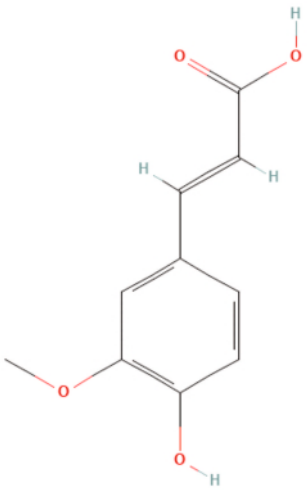

Figure 8: Chemical Structure of Ferulic acid (195.08)[34]

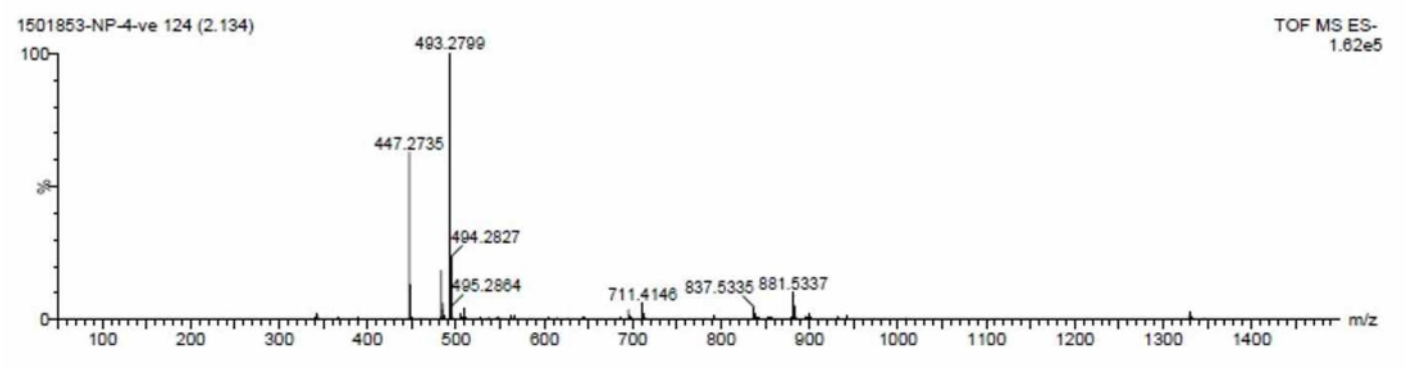

Figure 9: Qualitative Analysis of the Fraction of *M. charantia* by LC-MS Method

The peak shown in Figure: 9. shows molecular weight 493.27 which corresponds to the molecular weight of Karavilagenin-A (486.76)[34]

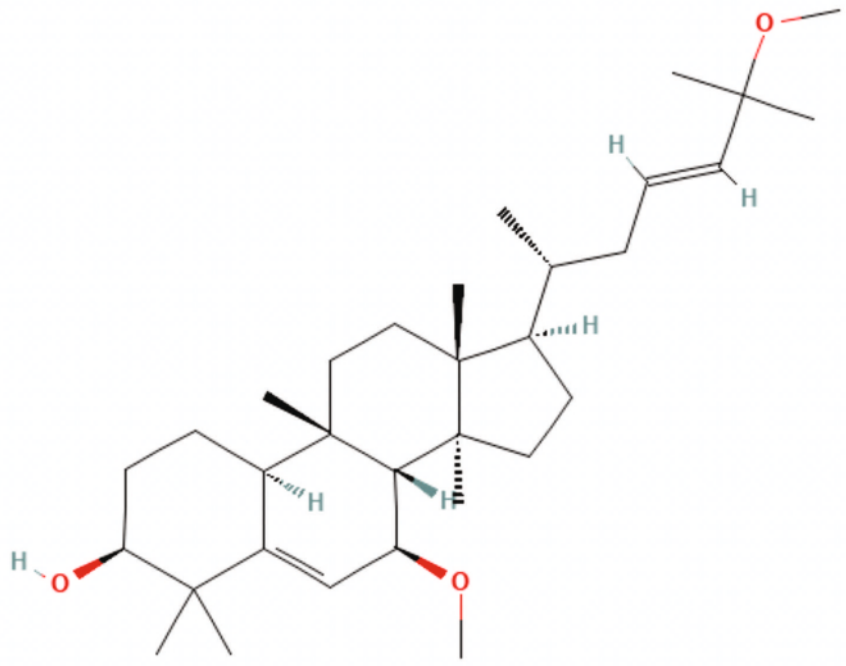

Figure 10: Chemical Structure of Karavilagenin-A (493.27)[34]

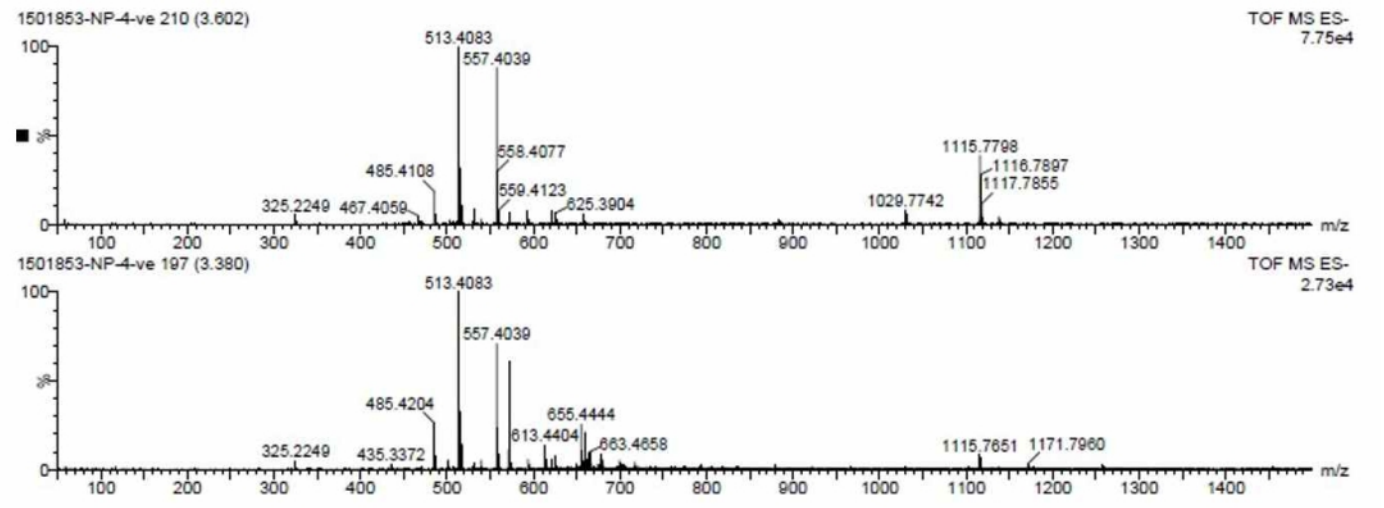

Figure 11: Qualitative Analysis of the Fraction of *M. charantia* by LC MS Method

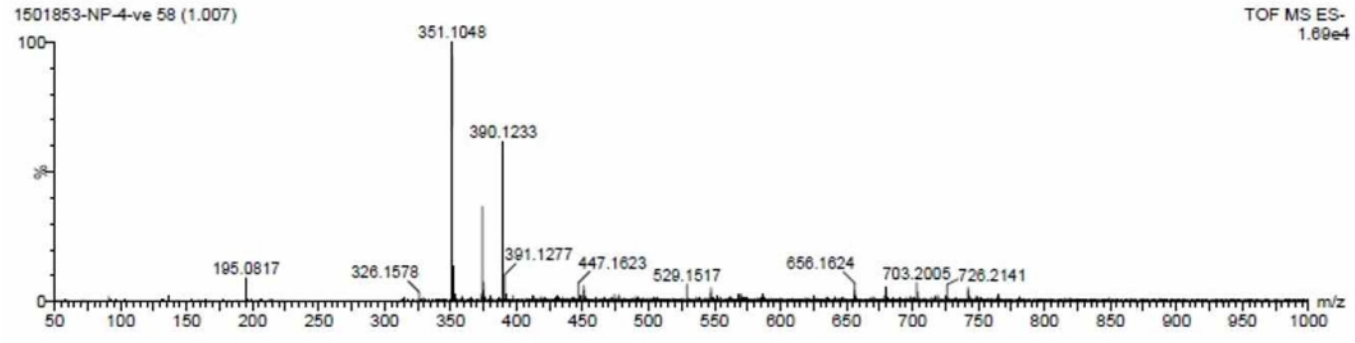

Figure 12: Qualitative Analysis of the Fraction of *M. charantia* by LC-MS Method

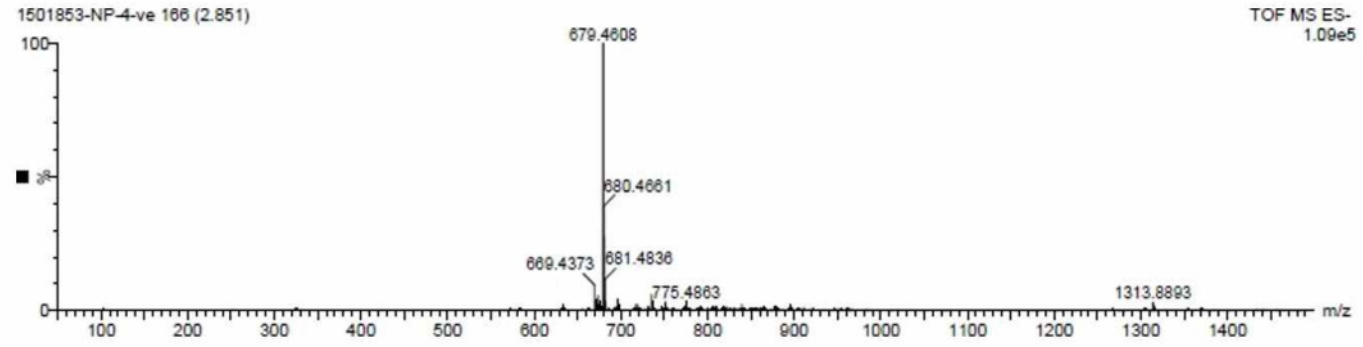

Figure 13: Qualitative Analysis of the Fraction of *M. charantia* by LC-MS Method

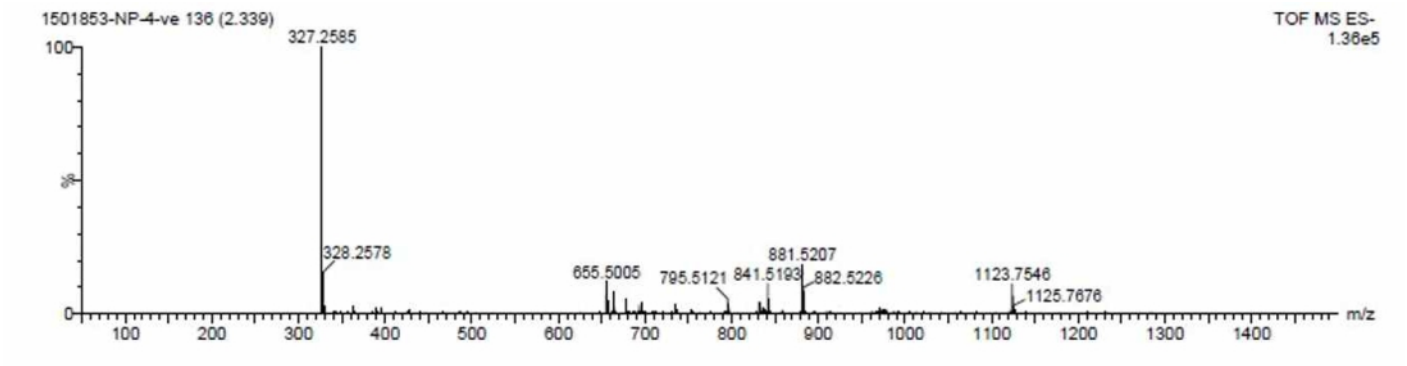

Figure 14: Qualitative Analysis of the Fraction of *M. charantia* by LC-MS Method

The peak shown in Figures: 11, 12, 13, and 14 shows molecular weight 513.40, 351.10, 679 and 327.25 respectively which are novel compounds since the compounds of these molecular weight have not been reported from *M. charantia* by any literature till this date. In summary, a total of 10 compounds were found including four novel compounds by using LC-MS method.

CONCLUSION

A simple, effective and suitable method combining LCMS, TLC and Column chromatography was employed to detect the active compounds present in 50% ethanolic extract of *M. charantia* fruit powder. All the studies carried out on the extract of *M. charantia* so far have shown that the extract of *M. charantia* contains different phenolic compounds. In the present study, the LCMS data obtained from 50% ethanolic extracts of *M. charantia* showed ten major compounds out of which four compounds were predicted to be novel compounds. Much more data are available for biological activities of phenolic acids but very less is reported for their metabolites. Among the compounds identified from *M. charantia* the active compound will be further purified and studied for their therapeutical activity and further studies can be carried out to elucidate the structure of these novel compounds as well as can be used in phytosome technology for treatment of cancer and other diseases.

CONFLICTS OF INTEREST: None

FINANCIAL SUPPORT: None

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