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Investigation Of Bioactive Compounds In Momordica Dioica Root By Gc-Ms

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Abstract

Momordica dioica, commonly known as "spiny gourd," is a member of the Cucurbitaceae family and holds significant traditional medicinal value. In this study, we conducted a comprehensive evaluation encompassing pharmacognostic parameters, phytochemical screening, and biological activity assessment of Momordica dioica. Macroscopic and microscopic analyses were employed to establish pharmacognostic characteristics. Phytochemical screening was performed to identify various classes of secondary metabolites present in the plant extract. Furthermore, the biological activities including antioxidant, antimicrobial, anti-inflammatory, and cytotoxic properties were evaluated using appropriate experimental assays. Results revealed distinctive pharmacognostic features of Momordica dioica, aiding in its identification and authentication. Phytochemical analysis indicated the presence of diverse bioactive compounds such as alkaloids, flavonoids, terpenoids, and phenolic compounds. The plant extract exhibited notable antioxidant potential, as evidenced by radical scavenging assays. Additionally, significant antimicrobial activity against a panel of pathogens was observed, suggesting its potential in combating microbial infections. Furthermore, the anti-inflammatory activity was demonstrated through inhibition of pro-inflammatory mediators. Moreover, cytotoxicity assessment revealed promising results, indicating the potential of Momordica dioica as a source of anticancer agents. This study provides valuable insights into the pharmacognostic, phytochemical, and biological properties of Momordica dioica, highlighting its potential for further pharmacological exploration and therapeutic applications.

Keywords: Gas Chromatography–Mass spectroscopy, hepoto-protective, phytochemical, secondary metaboilts, economics.

Introduction

The five great elements includes a vast array of medicinal and herbal plants, among them species from the Cucurbitaceae (gourd) family. Momordica dioica Roxb., native to Asia, represents about 965 species within 95 genera of both annual and perennial plants. These plants thrive in warmer tropical regions such as the Amazon, East Africa, the Caribbean, and South America. In India, prominent species include M. charantia L., M. balsamina L., M. dioica Roxb., M. sahyadrica, M. cochinchinensis (Lour.), M. subangulata, and Blume subsprenigera [Foye WO et al., 2008]. Traditional and Ayurvedic

texts document the use of various plant parts, such as flowers, roots, fruits, seeds, stems, bark, roots, root bark, aerial roots, rhizomes, and creepers. In Sri Lanka, Momordica dioica Roxb. fruits have been examined for their potential in diabetes treatment [Ediriweera et al., 2005].

The medicinal properties of these plants are linked to phytochemical components, which have specific physiological effects on the human body. Understanding these chemical constituents is important for synthesizing complex substances [Gordon et al., 2001]. Momordica dioica fruit is rich in nutrients, fiber, protein, minerals, vitamins, and fatty acids [Salvi et al., 2015]. The root extract has been studied for its ability to restore liver enzyme levels and its anti-inflammatory, antioxidant, and hepatoprotective activities [Shreedhara et al., 2006]. The roots of Momordica dioica exhibit bioactivities such as anti-tumor, hypoglycemic, antidiarrheal, antioxidant, and hepatoprotective effects [Nagarani et al., 2014].

Extraction techniques like maceration and Soxhlet are utilized. The roots and fruits of Momordica dioica are extracted using the Soxhlet method with ethanol as the solvent and characterized by HPTLC [Jain et al., 2007; Joshi et al., 2018]. The leaf extract of Momordica dioica is effective against hypertension, seasonal infections, skin issues, liver diseases, respiratory disorders, peptic ulcers with piles, diabetes, brain disorders, cancer, and kidney dysfunction [D'Souza et al., 2019]. Phytochemical tests of the acetone extract from Momordica dioica roots confirm the presence of alkaloids, flavonoids, terpenoids, phenol, saponins, glycosides, phenol, and phytosterol.

Gas chromatography-mass spectrometry (GC-MS) analysis of the acetone leaf extract identified several phytochemicals, including 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione; hexadecanoic acid, 13-methyl-methyl ester; isophytol; octadecane, 3-ethyl-5-(2-ethylbutyl); octadecanal, 2-bromo; phytol; 4-hydroxyphenyllactic acid, ethyl ester, di-TMS; 1-monolinoleoylglycerol trimethylsilyl ether; dodecane, 5,8-diethyl; eicosane, 7-hexyl; dodecanal; 3,7,11,15-tetramethyl-2-hexadecen-1-ol; ethanol, 2-(9-octadecenyloxy)-(Z); and phytol acetate. This study is the first to report on the phytochemical screening of acetone extract from Momordica dioica roots using GC-MS, highlighting the presence of bioactive compounds with medicinal applications.

Novelty

The investigation and establishment of naturally derived vegetative materials in these applications demonstrate their potential for easy and economical use in healthcare. The resulting processes can be standardized, and formulations can be refined using advanced techniques. The findings of the present study are promising, providing valuable insights for accurate botanical identification, drug authentication, standardization, and the development of a monograph.

Materials And Methods

Plant material

Plant seeds of Momordica dioica were collected from Gujarat, India, in March 2022. Their identity was confirmed by comparing them with a voucher specimen available at the Agharkar Research Institute in Pune, Maharashtra, India.

The Momordica dioica roots used for the study were cultivated in Taluka-Palus, Dist. Sangli, Maharashtra, India. Acetone of HPLC grade (Merck, India) was used for Soxhlet extraction. The GC-MS analysis of the acetone extract from the roots was performed at Poona College of Pharmacy, BVDU, Pune, and the results were analyzed.

Fig. 1. Momordica dioica Plant

Preparation Of Sample

The roots were selected from healthy, growing plants. The samples were washed with distilled water 2 to 3 times to remove dust particles and then air-dried at room temperature for 2 days. The roots were then shadow-dried, pulverized, and stored in an airtight container at 4°C for future use. A 140-gram powdered root sample was extracted with acetone using the Soxhlet technique at 30 to 50°C for 24 hours. The extracted product was concentrated using a rotary evaporator at 40°C and evaporated under reduced pressure. The essential oils from the extracted product were obtained in powdered form for further characterization to detect secondary metabolites. [Ilango,, et al. 2012]

Fig. 2. Root extraction

Phytochemical Screening

The Soxhlet-extracted product obtained from the roots of Momordica dioica was analyzed to detect secondary metabolites. The following preliminary tests were conducted based on visual observation of color changes or precipitate formation after adding specific reagents (Harborne, 1973; Krishnaiah et al., 2009). A small portion of the aqueous and acetonic extract was subjected to phytochemical tests to detect alkaloids, terpenoids, saponins, flavonoids, phenols, glycosides, and phytosterols.[Joshi et al. 2018]

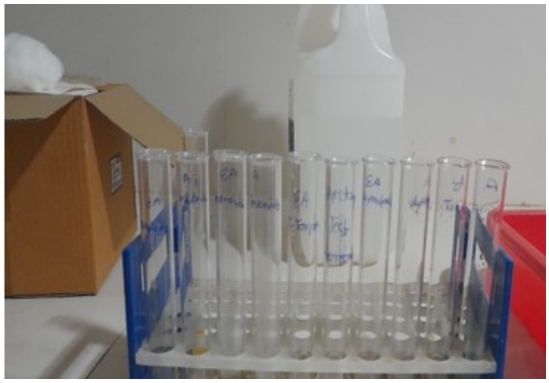

Fig. 3. Phytochemical Test

Gc-Ms (Gas Chromatography- Mass Spectroscopy.)

The Acetone extract of Momordica dioicawas analyzed for the presence of various bioactive compounds by the technique of Gas chromatography- Mass spectroscopy. GC-MS analysis wascarried out at Poona college of Pharmacy, BVDU, Pune, Maharashtra, India.

Gc-Ms Analysis – Sample Preparation

180 grams of the roots extract of Momordica dioica was soaked in ethyl alcohol for 48 hours and then extracted. The roots extract was re-extracted using chloroform to obtain chloroform soluble extract. It was centrifuged at 10,000 rpm for 25 minutes and the clear supernatant oil was injected to GC-MS analysis.[Gong, F., et al. 2001.]

Gc-Ms Experimental Procedures

GC-MS analysis was conducted using GCMS-MS (Triple Quard), Agilent -7890A (G7000B). It is equipped with Column- DB-SMS (30 Meter). The oven temperature was set to 70°C.Helium and Nitrogen gas was used as carrier gas. Solvent used as Ethyl acetate. 1ml of sample was injected by using syringe size 104µm.

Result And Discussion

Analysis of essential oils is carried out with GC-MS. GC-MS investigations of essential oils may be contributed to confirm chemical profile of sample. Relative proportion of bioactive organic compounds with concentration has been detected by GC-MS analysis. Standardization and identification of essential oils extraction easily studied with GC-MS analysis.[Singh, R., et al. 2012. , Ali Hussein Al-Marzoqi, et al. 2016., V. K. Meenakshi, et al. 2012.,R. Madhumitha, et al.2015., Elufioye, , et al. 2014,Hunda, J. M., et al. 2015. Chouni and et al.2021,Barath, and et al. 2016,. M. R. L.,and et al.2019., Faridha Begum, et al. 2016, Junjum Liu, et al.2016].

The GC-MS spectrum of Momordica dioica is shown in fig 3-16 below with 14 absorption peaks, the interpreted data is contained in Table 1 below. Peak 1 was identified as 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dionewith retention time of 9.627, its molecular weight is 276g and a molecular formula of $C_{17}H_{24}O_3$.[Monika M. and et al.2022]Fig 2. Similarly, Peak 2 was also identified asHexadecanoic acid,13-methyl –methyl ester with retention time of 9.713, its molecular weight is 270g and molecular formula $C_{17}H_{34}O_2$.[M. R. L., RossoniJúnior, et al. 2019]Peak 3 was identified as Isophytol with retention time 9.906 of , its molecular weight is 296g and a molecular formula of $C_{20}H_{40}O$.[Faridha Begum, et al. 2016.] in Fig 3. Peak 4 was also identified as

Octadecane,3-ethyl -5-(2-ethylbutyl) with retention time of 9.953, its molecular weight is366g and molecular formula $C_{26}H_{54}O$.[ChaitanyGopu, et al. 2021]Peak 5 was identified as Octadecanal,2-bromowith retention time 10.570of , its molecular weight is346g anda molecular formula of $C_{18}H_{35}BrO$[ChaitanyGopu, et al. 2021].. Peak 6 was identified as Phytol with retention time11.315of, its molecular weight is 296g and a molecular formula of $C_{20}H_{40}O$[34-35,38]. Peak 7 was 4-Hydroxyphenyllactic acid ,ethyl ester,di-TMS with retention time 13.228of , its molecular weight is 354g and a molecular formula of$C_{17}H_{30}O_4Si_2$[Chaitany Gopu, et al. 2021].Peak 8 was identified as1-Monolinoleoylglycerol trimethylsiyl ether with retention time 12.345of, its molecularweight is 498g and a molecular formula of $C_{27}H_{54}O_4Si_2$[Ilango,, et al. 2012].Peak 9 was identified as Dodecane, 5-8-diethylwith retention time6.496of , its molecular weight is226g and a molecular formula of $C_{16}H_{34}$[Faridha Begum, et al. 2016.].Peak 10 was identifiedasEicosane, 7- hexyl with retention time 7.898of , its molecular weight is 366g and a molecular formula of$C_{26}H_{54}$.[Zona Octarga, et al. 2021]Peak 11 was identified as Dodecanal with retention time 6.091of , its molecular weight is 184g and a molecular formula of $C_{12}H_{24}O$.[Singh, R., et al. 2012.]Peak 12 was identified as 3, 7, 11, 15-Tetramethyl-2- hexadecen-1-olwith retention time 8.972of , itsmolecular weight is 296g and a molecular formula of $C_{20}H_{40}O$.[Elufioye, et al. 2014]Peak 13was identified as Ethanol, 2-(9-octadecenyloxy)-(z)with retention time 9.176of , its molecular weight is 312g and a molecular formula of $C_{20}H_{40}O_2$.[Hunda, J. M., et al. 2015.] Peak14was identified as Phytol, acetate with retention time 9.335of, its molecular weight is 338g and amolecular formula of $C_{20}H_{40}O_2$[PrabhannaBanakar ,et al. 2010.]

Conclusions

The hyphenated GC-MS technique is utilized to identify the authentic chemical database of bioactive compounds. The applications of GC-MS include:

a) The capillary column's remarkable separation ability allows for the generation of high-quality chemical profiles akin to fingerprints.

b) The associated mass provides both qualitative and relatively quantitative insights into spectral studies, leveraging a pertinent mass spectral database. This enables the verification of chemical databases of herbal plants.

GC-MS represents a pioneering method that enables the visualization of individual bioactive constituents within herbal plants. Its significance underscores the need for further investigation into pharmacological studies.

This research delves into the traditional medicinal application of the plant in treating various conditions such as hypertension, seasonal infections, skin issues, liver ailments, respiratory disorders, peptic ulcers with piles, diabetes, neurological disorders, cancer, and kidney dysfunction, among others.

Table 1. GC-MS analysis - Bioactive Compounds of acetone rootsextractofMomordica dioica

Sr No.	Ret. Time	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area%	Nature of compound

1	9.627	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	72.3	Ketone
2	9.713	Hexadecanoic acid,13-methyl – methyl ester	$C_{17}H_{34}O_2$	270	15.8	Fatty acid ester
3	9.906	Isophytol	$C_{20}H_{40}O$	296	16.8	Terpenoid
4	9.953	Octadecane,3-ethyl -5-(2-ethylbutyl)	$C_{26}H_{54}O$	366	16.9	Alkane
5	10.570	Octadecanal,2-bromo	$C_{18}H_{35}BrO$	346	8.67	Long chain aldehyde
6	11.315	Phytol	$C_{20}H_{40}O$	296	75.0	Acylicditerpenoids alcohol
7	13.228	4-Hydroxyphenyllactic acid ,ethyl ester,di-TMS	$C_{17}H_{30}O_4Si_2$	354	11.8	Phenylic ester
8	12.345	1-Monolinoleoylglycerol trimethylsiyl ether	$C_{27}H_{54}O_4Si_2$	498	10	Ether
9	6.496	Dodecane, 5-8-diethyl	$C_{16}H_{34}$	226	5.57	Alkane
10	7.898	Eicosane, 7- hexyl	$C_{26}H_{54}$	366	10.8	Alkane
11	6.091	Dodecanal	$C_{12}H_{24}O$	184	15.7	Aldehyde
12	8.972	3, 7, 11, 15- Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	26.3	Alcohol
13	9.176	Ethanol, 2-(9-octadecenyloxy)-(z)	$C_{20}H_{40}O_2$	312	8.63	Diterpenoid
14	9.335	Phytol, acetate	$C_{20}H_{42}O_2$	338	9.63	Acylicditerpenoids

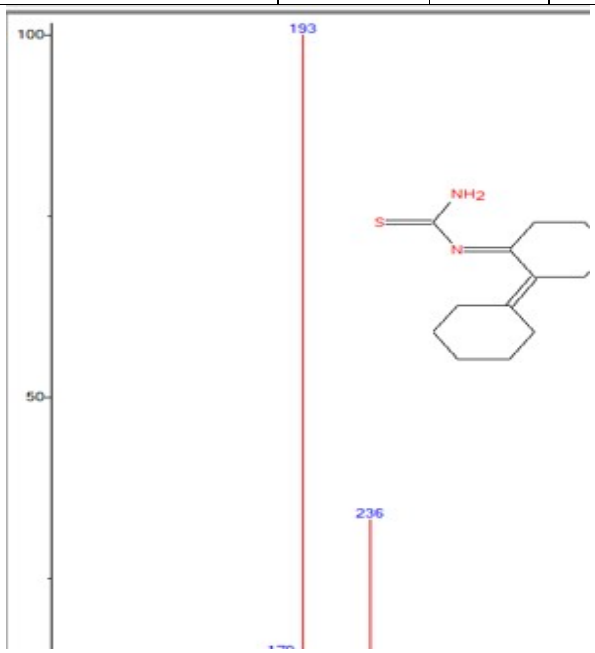

Fig. 3. GC-MS graphs of bioactive compounds from roots extract of Momordica dioica

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