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Phytochemical Screening and GC-MS Analysis in the Wild and Cultivated Varieties of *M.charantia* L. (Cucurbitaceae)

Suhara Beevy S[1], Haseena Bai N[2], Soumya N M[3]
[1,2,3]*Department of Botany, University of Kerala, Thiruvananthapuram, India*

Abstract- ***Momordica charantia*** **L., an important medicinal plant and a common vegetable belonging to the family Cucurbitaceae is grown throughout the tropics. The species consists of the wild and cultivated varieties viz, *M. charantia* var. *muricata* (wild) and *M. charantia* var. *charantia* (cultivar) that exhibits variations at intra specific levels. The present investigation analysed the chemical constituents in the leaves of wild and cultivated varieties of *M.charantia* by preliminary phytochemical and physicochemical screening. Two accessions of the wild variety representing two morphotypes collected from the state of Kerala, and the cultivar, Preethy, procured from Kerala Agricultural College, Vellayani, Thiruvananthapuram, Kerala were the materials for the study. GCMS profiling of leaf extracts of the two morphotypes of the wild variety, *M. charantia* var. *muricata*, was also carried out to develop the fingerprint of the plant and to detect the major chemical constituents of the wild varieties. Qualitative phytochemical screening by methanol, chloroform and petroleum ether extracts showed the presence of medicinally active constituents like alkaloids, flavanoids, tannins, phlobatannins, saponins, steroids and cardiac glycosides in both the wild and cultivated varieties. Superiority of the wild variety over the cultivar was noticed by the presence of coumarins and anthraquinones, suggesting the significance of wild variety in breeding programme. Quantitative phytochemical and physicochemical screening summarizes that the cultivar was pharmaceutically more important than the wild variety. Despite this, GC-MS analysis highlighted the presence of many medicinally important secondary metabolites like Diosgenin, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Vitamin E, 3,4-Dihydro-3,5,8-Trimethyl-3-(4,8,12-Trimethyl Tridecyl)-(2H)1-Benzopyran-6-Acetate and Aspidospermidine in the wild accessions, which justifies the antioxidant, antimicrobial, antidiabetic, antitumor properties of the wild variety which needs further evaluation. Intra varietal variations were noted in the wild variety suggesting the use of it separately.**

Keywords- ***M. charantia*** **var.*charantia*, *M.charantia* var.*muricata*, Intra specific variation, Phytochemical screening, GC MS.**

I. INTRODUCTION

Medicinal plants have been used by human beings since ages in traditional medicine due to their therapeutic potential.

The therapeutic efficiency of these medicines has mainly been attributed to the presence of various bioactive constituents of plants called phytochemicals which include vitamins, terpenoids, phenolic acids, lignin, stilbene, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other secondary metabolites. Studies have demonstrated that many of these phytochemicals can act as anti oxidants, anti inflammatory, anti atherosclerotic, anti tumorous, anti mutagenic, anti carcinogenic, anti bacterial, and anti viral agents (Sala *et al*., 2002). The genus *Momordica* includes species having immense neutraceutical values and have much significance in indigenous medical systems and in various countries like Asia and Africa (Joseph and Antony, 2010). Of these, *M. charantia* , known commonly as bitter gourd, balsam pear, bitter melon, bitter cucumber and African cucumber (Dasgupta *et al*., 2009) consists of the both wild and cultivated varieties viz, *M. charantia* var. *muricata* and *M. charantia* var. *charantia* (Chakravarthy 1990). Diversity analysis of the wild variety *M. charantia* var *muricata* pointed out the occurrence of two morphotypes, one with fruits having markedly sculptured seeds and other with fruits having feebly sculptured seeds (Beevy and Bai, 2013). *M. charantia* has been reported to have many pharmacological activities like antioxidant, adipogenesis-reducing, antilipolytic, hypoglycemic, antidiabetic, anticancer, antifertility, anthelmintic, antimicrobial, antiviral and hepatoprotective activity (Upadhayay *et al*., 2015). Grover and Yadav (2004) reported that these medical activities are attributed to an array of potent bioactive plant chemicals, including triterpenes, pisteins and steroids. New source of therapeutically and industrially valuable compound from plants can be identified from phytochemical evaluation of these active compounds. Knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies (Milne, 1993).

Phytochemical analysis including preliminary phytochemical evaluation and GC-MS analysis in the cultivated varieties of *M.charantia* was carried out by researchers like Yuwai *et al*. (1991), Bakare *et al.* (2010), Santhi *et al.* (2011), Ullah *et al*. (2011) and Moronkola *et al*.(2009) and identified its immense value as medicine.

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But phytochemical evaluation of the wild variety *M.charantia* var. *muricata*, which has been widely used as health foods and folk medicine, is not readily available except a few reports (Wu and Ng, 2008 and Ullah *et al*. 2011). However the chemical constituents of the wild fruits remain unknown. Edible and medicinal wild plants can provide healthy alternatives to highly processed foods and pharmaceuticals, bringing greater health into our lives. In the present investigation preliminary qualitative and quantitative phytochemical evaluation of the species was carried out giving special emphasis to the wild varieties. The study also focussed to find out the phytochemical variations and GC-MS analysis of the two morphotypes of the wild accessions.

II. Materials And Methods

Two accessions of the wild variety *M. charantia* var. *muricata* (morphotype1and 2) collected from the state of Kerala, and the cultivar, Preethi, procured from Kerala Agricultural College, Vellayani, Thiruvananthapuram, Kerala were used in the present study. Details of the plants and the characteristics of fruits and seeds of the accessions are presented in Table1(Fig 1, Fig 2A&B). Fresh leaves collected from the plants were washed properly with tap water and with double distilled water to remove impurities. They were then shade dried at room temperature for two weeks and ground to powder using an electric blender. The powder was allowed to pass through a 0.5 mm metallic mesh. The resultant crude fine powder was stored in a sample tube and kept in a refrigerator for further analysis. Plant extraction was carried out using three solvent systems viz, methanol, chloroform and petroleum ether. The dried powder sample (20gm) was extracted with 100ml solvents for 12hours and the filtered extracts were subjected to preliminary phytochemical, physico-chemical and GC-MS analysis.

Qualitative phytochemical analysis as carried out according to the methods described by Harborne (1984) and Trease and Evans (1989). The presence of Alkaloids (Dragendroff's test), Flavanoids (Ammonia test), Tannin (Braemer's test), Phlobatannin (Hydrochloric acid test), Saponin (Frothing test), Terpenoids (Salkowski's test), Coumarins (Alkaline test), Steroid (Liebermann Burchard test), Anthraquinones (Borntrager's test) and Cardiac glycosides (Keller Kiliani test) were estimated following the standard methods. Quantitative phytochemical analyses were carried out following the methods described by Obadoni *et al*.(2001), Boham *et al*.(1994) and Van-Burden & Robinson (1981). Total alkaloid, total phenolic (Folin –Ciocalteu method), total flavonoid, total saponin and total tannin content (Folin Denis method) were also estimated.

The procedure recommended by (WHO, 2002), Indian Pharmacopoeia (1996), Ahmad and Sharma *et al*.(2001), Gupta *et al*.(2003) and Indrayan *et al*. (2005) was followed for the determination of total ash, acid soluble/insoluble ash, water soluble/insoluble loss on drying at 105°C, alcohol soluble extractive, swelling index and foaming index.

For GC-MS analysis crude cold methanol leaf extract of the two morphotypes of the wild variety *muricata* were analysed following the method of Hema *et al*.(2010). Analysis was performed by using a GC, Varian CP 3800 and MS Saturn 2200 (VF 5ms 30 X 0.25 system) equipped with Elite-1, fused silica capillary column composed of 5% phenyl-arylene-95% dimethyl poly siloxane. The system comprised of COMBIPAL autosampler. The following conditions were maintained: helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 1µl EI (split ratio of 1:10). Injector temperature was 250°C and the oven temperature was programmed from 100-270^{0}C at the rate of 5^{0}C; total GC running time was 63 minutes. Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns and the name, molecular weight and molecular formula, retension time and peak area percent of the components of the test materials were ascertained.

III. Results

Qualitative phytochemical analysis using methanol, chloroform and petroleum ether extracts of the leaves of the *M.charantia*, revealed the presence of medicinally active constituents like alkaloids, flavanoids, tannins, phlobatannins, saponins, coumarins, terpenoids, steroids, anthraquinone and cardiac glycosides. The results are shown in Table 2. The cultivated variety, *M.charantia* var. *charantia* contained compounds like alkaloids, flavanoids, tannins, phlobatannins, saponins, terpenoids, steroids and cardiac glycosides whereas in the morphotype (1) of the wild variety *M. charantia* var. *muricata* had anthraqiunones in addition the alkaloids, flavanoids, tannins, phlobatannins, saponins, terpenoids, steroids and cardiac glycosides. But all the phytochemicals except terpenoids were detected in the morphotype (2).

Quantity of total phenol, flavanoid, tannin and saponin are shown in Figure-3. The total phenol, flavanoid and tannin content were higher in *M.charantia* var.*charantia*. Within the wild morphotypes, the morphotype (2) possessed higher amount total phenol and flavanoid while higher quantity of tannin was present in the wild morphotype (1). Total saponin content in these species were too low ie, below the detectable level.

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Determination of different ash values like total ash, acid insoluble and soluble ash and water soluble and insoluble ash revealed that these values were higher in the cultivar. The cultivar also showed higher amount of swelling index and loss of drying at 105°C. The alcohol soluble extractive value was higher in the wild morphotype (2).The physico-chemical parameters of the wild and cultivar are given in the Figure-4.

GC-MS analysis of methanolic extract of the two wild accessions identified the presence of phytochemicals like diosgenin, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Vitamin E, Methyl (Z)-5,11,14,17-eicosatetraenoate, Pentadecanoic acid,14-methyl-methyl ester, Propane,2-fluoro-2-meth, Palmitic anhydride, 3,4-Dihydro-3,5,8-Trimethyl-3-(4,8,12-TrimethylTridecyl)-(2H)1-Benzopyran-6-Acetate, Phthalic acid and Aspidospermidine-3-carboxylic acid,2,3-didehydro-,methyl ester,hydrochloride. The list of compounds, name, molecular weight, molecular formula, retension time and percent of peak area and amount of each component of the two morphotypes are given in Table 3 and 4 respectively. GC-MS chromatogram of the accessions (Figs.5 & 6) revealed the presence of 67 phytochemicals in morphotype (1). Of these, 25 were characterized and identified, 18 peaks were unknown and 14 compounds were present in less amounts thus it cannot be represented as peaks. In morphotype (2), 78 peaks were obtained, out of these 35 were identified, 18 unidentified and the rest of them were missing peaks. Various compounds having medicinal activities have been identified and listed in Table 6.

IV. Discussion

Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance and to make the best and judicious use of available natural wealth (Grover and Patni 2013). Presence of various phytochemicals like alkaloids, flavanoids, tannins, phlobatannins, saponins, steroids and cardiac glycosides in the wild and cultivated varieties of *M. charantia* may be attributed to its immense medicinal value. Grover and Yadav (2004) opined that medicinal activities of *M. charantia* are due to an array of biologically active plant chemicals, including triterpenes, pisteins and steroids. Presence of alkaloids, flavanoids, tannins, phlobatannins, saponins, terpenoids, steroids and cardiac glycosides and absence of coumarins and free anthraquinones were reported in *M.charantia* var. *charantia* by Raman and Lau (1996) and Ullah *et al.* (2011). Presence of coumarins and anthraquinones in the wild variety *M.charantia* var. *muricata* compared to the cultivar indicated the superiority of the wild variety in terms of phyto constituents.

Present findings were in contrast to the report of Ullah *et al*. (2011) who indicated that both cultivar and the wild varieties contained similar types of phytochemical constituents. The two morphotypes of the wild variety *M.charantia* var. *muricata* differ in terms of presence phytochemical constituents which is also in conformity with the previous report on nutritional and pomological variation of (Beevy and Bai, 2015). Intraspecific and intravarietal diversity observed in *M.charantia* in terms of the phytochemicals suggests high therapeutical value of the species. The present study also point out the significance of wild varieties especially var. *muricata* morphotype(2) that contained both coumarins and anthraquinones in plant breeding programme to develop a medicinally improved variety. Kole *et al*. (2010 a,b) reported high content of phytomedicines in a variety of *muricata*, CBM12 with inferior fruit quality.

Quantitative phytochemical analysis revealed both intra specific and intra varietal variations in the amount of total phenol, flavanoid and tannin. The present investigation revealed that the cultivated variety possessed higher concentration of these secondary metabolites (Figure 3). Flavonoids and phenolic compounds are the main antioxidative compounds in fruits and vegetables (Huang *et al*., 1998). The highest amount of flavanoids in the cultivar (16.14µg/ml) justifies its use as an immunomodulator that may improve immune function of cells. The study noticed comparatively higher quantity of total phenol in the cultivar (0.994µg/ml). The polyphenols possessed a wide range of physiological properties such as antimutagenic, anti- carcinogenic, cardio-protective as well as the ability to modify gene expression (Nakamura *et al*., 2003). Sharma (2006) reported that the poly phenols possess anti-spasmodic, anti-neoplasmic and anti-viral activities. Veerapur *et al*., (2009) and Nagalingam *et al*.,(2012) reported the linear correlation between consumption of dietary supplements rich in natural phyto chemicals that reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing. The presence of these phytochemical constituents in the wild and cultivated varieties revealed that both can be used as basic medicinal agent for analgesic, antispasmodic, antibacterial, anti viral, anti cancer, anti inflammatory and anti oxidant properties. The antiviral and antibacterial properties of leaf extract of *M. charantia* was reported by Au *et al.* (2000) and Omoregbe *et al.* (1996) respectively. The researchers like Jilka *et al.*(1983), Battelli *et al.*(1996) and Sun *et al.*(2001) reported the anti-tumor and anti-cancerous activity of crude extract of *M. charantia.*

Anti-nutrient analysis revealed moderate amount of tannin and absence of saponin in *charantia*. Total tannin content found in *M.charantia* var. *charantia* (0.23µl) and *M.charantia* var. *muricata* (var.2), was very low.

Thompson *et al*., (1993) opined that saponins in food have traditionally been considered as 'anti-factors' and in some cases limited their use due to their bitter taste (Ridout *et al*., 1991).Tannins are polyhydric phenols that form insoluble complexes with proteins, carbohydrates and lipids leading to reduction in digestibility of nutrients (Fisher, 1996). The concentrations of saponin and tannins in *Momordica charantia* was however not higher amount when compared to their concentrations in other food stuffs (Chakraborty and Eka, 1978).

Evaluation of physico-chemical parameter is an indispensable part in raw drug standardisation for maintaining the consistency and quality of herbal formulations. The ash value was determined from measures like total ash, acid-insoluble ash, water soluble ash and sulphated ash, and it usually represents the inorganic part of the plant (Tambe and Kadam, 2012). The high total ash value (21.02%) in *M. charantia* var. *charantia* suggests that the cultivar is a rich source of minerals. The total ash value can also be used to detect foreign organic matter and adulteration with sand and earth, therefore, reflecting the kind of care that must be taken in preparing the plant for drug. It was observed that the cultivated variety of *Momordica* possessed higher amount of acid insoluble ash and water soluble ash. Percentage of weight loss on drying an important factor in determining the probable rate at which a drug can undergo deterioration due to fungal attack or enzyme activities was almost similar for both the wild and the cultivar of *M. charantia*. Alcohol extractive value an index of purity of crude extracts is a valuable test to check the quality of drug and to determine adulteration of the drug. Comparatively high alcohol extractive value found in the Morphotype-2., of the wild variety suggests its significance to determine the purity of the drug. The quantitative and physico-chemical analysis revealed that the cultivar was pharmaceutically more important than the wild variety.

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractions of the methanolic extract of the two morphotypes of the wild variety *M. charantia* var.*muricata* which have medicinal properties. The chromatograms revealed that, the prevailing compound in the wild morphotype-1 was Diosgenin followed by 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Vitamin E and Methyl (Z)-5,11,14,17-eicosatetraenoate. Diosgenin is the precursor for the semi synthesis of progesterone which in turn formed an ingredient in oral contraceptive pills (Djerassi, 1992). The unmodified steroid has estrogenic activity (Liu *et al*., 2005) and it can reduce the level of serum cholesterol (Cayen and Dvornik, 1979). Raju and Mehta (2009) reported the chemopreventive or therapeutic nature of Diosgenin against several cancer types.

Vitamin E has many biological functions, the most important functions are the antioxidant property, enzymatic activities, geneexpressio n, neurological function and cell signalling (Zingg and Azzi 2004). In morphotype-2, the major compound was 3,4-Dihydro-3,5,8-Trimethyl-3-(4,8,12-Trimethyl Tridecyl)-(2H)1-Benzopyran-6-Acetate followed by Phthalic acid, Aspidospermidine-3-carboxylic acid,2,3-didehydro-,methyl ester,hydrochloride, Palmitic anhydride and Vitamin E. Presence of pentacyclic triterpenoid 3,4-Dihydro-3,5,8-Trimethyl-3-(4,8,12-Trimethyl Tridecyl)-(2H)1-Benzopyran-6-Acetate the fungicidal and insecticidal property of the wild variety (Morphotype-2).

There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity (Fernie *et al*., 2004). L-(+)-Ascorbic acid 2,6-dihexadecanoate has been reported to have antioxidant, antiinflammatory and anti-nociceptive properties (Okwu and Emenike 2006; Akinmoladun *et al*., 2007). 9, 12, 15-Octadecatrienoic acid having anti inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, antiarthritic and anticoronary properties (Sermakkani and Thangapandian, 2012). Tetradeacanoic acid having antioxidant, anti cancerous, hypercholesterolemic, nematicide activity and is a component in cosmetics. Ha *et al*. (1990) suggested conjugated linoleic acid like 9,12-Octadecadienoic acid (Z,Z) have antioxidant activity. The n-hexane extract of seeds of Momordica has been reported to contain conjugated octadecatrienoic fatty acids and ά-eleostearic acid. These acids have been studied for their anti-oxidant activities and are proven to be successful in an in vitro study. Thus it may help to reduce the risk of coronary heart diseases in non-diabetic as well as diabetic patients.(Sharma et al., 2011). Oh et al., (2012) reported the acaricidal toxicity of 2-Hydroxy-4-methylacetophenone and its derivatives. Other compounds and its bioactivity have been mentioned in Table 6.

The present study identified various bioactive compounds in the wild varieties which justifies the use of the wild variety for various ailments by traditional practitioners. Traditionally the leaves and fruits of wild bitter gourd has been drunken as preventative or treatment of stomachache, toothache, liver diseases, diabetes, hypertension and cancer (Chiu & Chang, 1995) and its leaves are crushed to obtain the juice for applying on the skin for treating insect bites, bee stings, burns, contact rashes, and wounds (Wu and Ng, 2008). Therefore a detailed study of the various compounds present in the wild variety of *M. charantia* and their pharmaceutical importance requires to be carried out such that a drug with multiple effects can be made available in near future.

The present investigation also points out that the two morphotypes of the wild variety *muricata* should be used separately since intra varietal variation was observed in the presence of phytochemicals. However the two morphotypes had 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 9,12,15-Octadecatrienoic acid and Vitamin E, but in different percentages. Difference in the presence of phytochemicals between two species of *Marchantia* was identified by Krishnan and Murugan (2014). According to Nazlina *et al*. (2011) the wild and cultivar of *M. charantia* had the common chemicals viz, Hexadecanoic acid, methyl ester (20.683), Pentadecanoic acid14-methyl-, methyl ester and Vitamin E. Moronkola et al (2009) reported (*Z*)-3-hexenol (34.7%), (E)-2-hexenol (10.1%), and phytol (8.3%) as the major compounds. In addition to these Nazlina *et al*. (2011) reported linoleic acid methyl ester along with the non fatty acid compounds viz, stigmasterol, β-sitosterol, cucurbitacin B dihydro and gentisic acid. The presence of phytochemicals like gentisic acid, 1- pentadecyne, cucurbitacin B dihydro, cis-9-hexadecenal-sitoserol, stigmasterol, oleic acid, stigmastan-3-ol, ethyl-4,5-dimethyl-phenol and linoleic acid were also noticed in the cultivar (Ritu *et al.*2012).

V. Conclusion

The investigation is pivotal in opening up a new dimension regarding the medicinal value of the wild varieties of *Momordica charantia.* The phytochemical evaluation of the species indicates high nutritive and pharmacological values. The study stresses the importance of wild varieties of *M.charantia* for the phytochemicals coumarins and anthraquinones, which were absent in the cultivar, indicating its utility in breeding programmes. The study concludes that the variation in the morphological differences also expressed in the phytochemical profiling of the wild varieties. The preliminary phytochemical screening of different extracts revealed the presence of major secondary metabolites. The phytochemical profile of the species gives an insight into its medicinal value as well as its high nutritious property, as safe for consumption, both as medicine and as natural source for antioxidant and antioxidant promoting activities. GC-MS profiling gave the composition of several chemical constituents in the methanol extract of the plant with immense potential. The present study is the first report on the GC-MS analysis of *M.charantia* var. *muricata*. Intra varietal variations were also noticed in the present study. The study thus showed that the wild variety of *M. charantia* is a reservoir of medicinally useful phytoconstituents which can be utilized beneficially by isolating these compounds using appropriate methods.

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Table 1:
Details of the Accessions of *M.charantia* and the Characteristics of Fruits and Seeds

Plant Material	***M.Charantia* var. *charantia* (Cultivar)**	***M.Charantia* var. *muricata*(Morphotype-1)**	***M.Charantia var. muricata* (Morphotype-2)**
Code	MC1	MC7	MC15
Collection Place	Agricultural university	Neyyardam(Trivandrum)	Thrissur
Fruit Color	Whitish green	Green	Green
Fruit Shape	Elliptical	Globular	Cylindrical
Fruit Bitterness	Very bitter	Low	Medium
Density of Tubercles	Dense	Sparse	Sparse
Nature of Tubercle	Raised & blunt	Soft&flat	Raised & pointed
Seed Sides	Wavy bitten	Wavy bitten	Smooth
Seed Ends	Clearly sub tridentate	Clearly sub tridentate	Feebly sub tridentate
Extent of Seed Sculpturing	Markedly sculptured	Markedly sculptured	Feebly sculptured
Seed Shape	Broad rectangular	Broad rectangular	Narrow rectangular

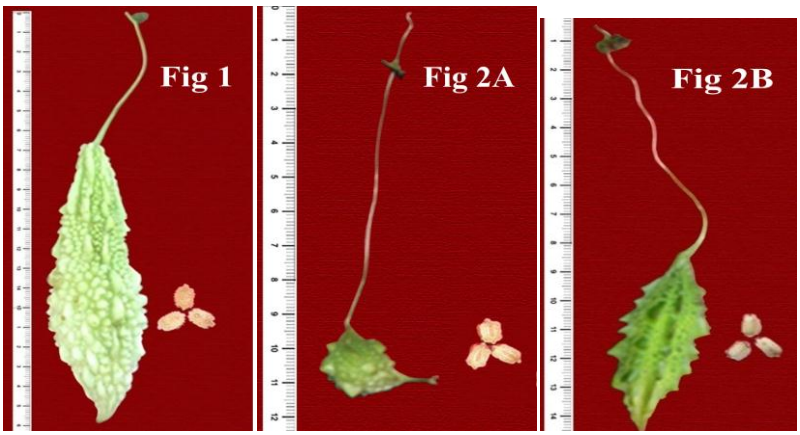

FIG 1: M. charantia var. charantia (Cultivar); Fig 2A&2B M. charantia var. muricata Morphotype-1& Morphotype-2

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Table 2:
Qualitative Phytochemical Analysis In *M. charantia*

Phytochemicals	*M.charantia var.charantia*			*M.charantia var. muricata*					
				Morphotype-1			*Morphotype-2*		
	M	***C***	***P***	***M***	***C***	***P***	***M***	***C***	***P***
Alkaloids	+	-	+	-	+	+	+	-	-
Flavanoids	+	-	-	+	+	-	+	-	-
Tannins	-	+	-	+	-	-	+	-	-
Phlobatannins	-	+	-	-	+	-	-	+	-
Saponins	-	+	-	-	+	-	-	+	-
Coumarins	-	-	-	-	-	-	-	+	-
Terpenoids	-	+	-	-	+	-	-	-	-
Steroids	+	+	+	-	+	+	-	+	-
Anthraquinone	-	-	-	+	-	-	+	-	-
Cardiac glycosides	-	-	+	+	+	-	+	-	-

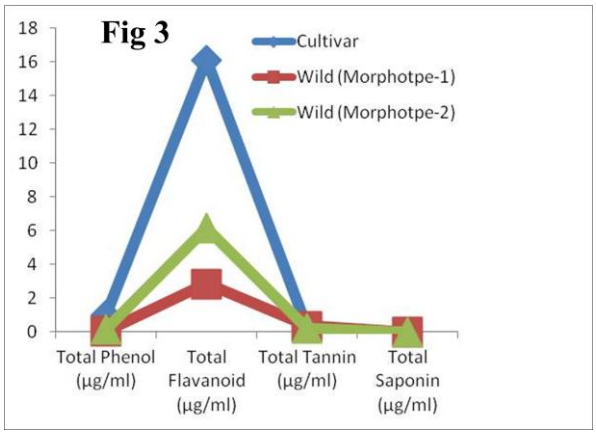

FIG 3. Quantitative Analysis of M. charantia L. ;

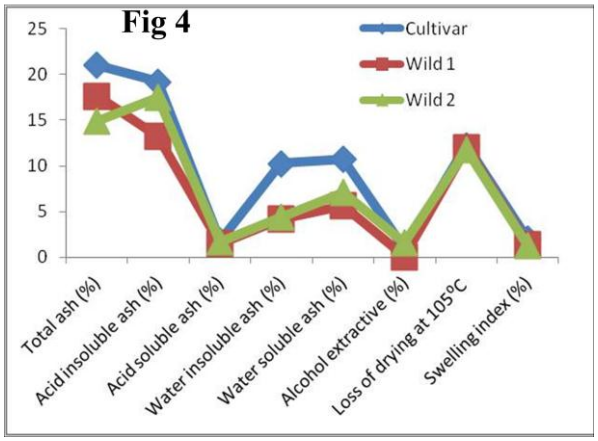

Fig4. Physicochemical Analysis of M. charantia L.

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Table 3.
Phytocomponents Identified in the Methanolic Extract of *M. charantia* var. *muricata* Morphotype-1 (MC-7) using GC-MS.

No.	Name of the compound	Retension Time	Molecular Weight	Molecular Formula	Amount	Peak Area %
1	Propane, 2-fluoro-2-methyl	3.05	385.21	$C_{16}H_{17}BrO_6$	810455	2.56
2	7-Chloro-N-(4-{[1-(dimethylamino)cyclohexyl]methyl}phenyl)-4-quinolinamine	4.07	393.95	$C_{24}H_{28}$	263314	0.83
3	2-Naphthyl-.beta.-D-galactopyranoside	5.39	306.32	$C_{16}H_{18}O6$	228534	0.72
4	2-Methoxy-4-vinylphenol	9.24	150.17	$C_9H_{10}O_2$ ·	156636	0.5
5	Phenol, 3-(1-methylethyl)-, methylcarbam	10.13	193.27	$C_{11}H_{15}NO_2$	106533	0.37
6	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-	22.08	210.31	$C_{13}H_{22}O_2$	312793	0.99
7	1,5,5,8-Tetramethylbicyclo[4.2.1]non-9-yl)acetic acid	24.37	238.37	$C_{15}H_{26}$	179037	0.57
8	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyp	23.37	180.20	$C_{10}H_{12}O_3$	469147	1.48
9	1,3-Benzenediol, 5-pentadecyl-	24.93	320.51	$C_{21}H_{36}O_2$	581060	1.85
10	3-Eicosyne	26.31	278.52	$C_{20}H_3$	1165994	3.68
11	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	26.31	296.53	$C_{20}H_{40}O_8$	2002326	6.32
12	Cyclopropanenonanoic acid	53.71	422.45	$C_{17}H_{22}N_6OS$	190114	0.6
13	Pentadecanoic acid, 14-methyl-, methyl ester	27.74	270.45	$C_{17}H_{34}O_2$	773259	2.44
14	Pentadecanoic acid, 13-methyl-, methyl ester	28.57	270.45	$C_{17}H_{34}O_2$	388512	1.23
15	L-(+)-Ascorbic acid 2,6-dihexadecanoate	29.53	652.94	$C_{38}H_{68}O_8$	1107650	3.5
16	Hexadecanoic acid, ethyl ester	30.11	256.42	$C_{16}H_{32}O_2$	861816	2.72
17	9,12-Octadecadienoic acid (Z,Z)	31.89	280.45	$C_{18}H_{32}O_2$	610354	1.93
18	Methyl eicosa-5,8,11,14,17-pentaenoate	32.30	316.49	$C_{21}H_{32}$	687664	2.17
19	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	32.51	296.53	$C_{20}H_{40}O$	4842786	15.29
20	Methyl (Z)-5,11,14,17-eicosatetraenoate	33.22	318.49	$C_{21}H_{34}O_2$	2989004	9.44
21	9,12,15-Octadecatrienoic acid	33.61	280.45	$C_{18}H_{32}O_2$	1021855	3.23
22	Rhodopsin	44.09	554.89	$C_{40}H_{58}O$	74798	0.24
23	Dihydrosmilagenin 26-tosylate	46.94	572.84	$C_{34}H_{52}O_5S$	1378254	4.35
24	Vitamin E	51.32	430.71	$C_{29}H_{50}O_2$	4635489	14.64
25	Diosgenin	56.46	414.62	$C_{27}H_{42}O_3$	5833356	18.42

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Table 4.
Phytocomponents Identified in the Methanolic extract of M. charantia var. muricata Morphotype-2 (MC-15) using GC-MS.

No.	Name of the Compound	Retension Time	Molecular Weight	Molecular Formula	Amount	Peak Area %
1	Pyrovalerone	4.01	245.36	$C_{16}H_{23}NO$	1320096	2.92
2	Benzofuran, 2-ethenyl-	5.4	144.17	$C_{10}H_8O$	1199559	2.65
3	Benzoic acid	5.57	122.12	$C_7H_6O_2$	1036251	2.29
4	Benzaldehyde, 3-methyl-	6.84	120.15	C_8H_8O	1464930	3.24
5	Indole	8.84	117.15	C_8H_7N	662772	1.47
6	4-Hydroxy-2-methylacetophenone	9.2	150.17	$C_9H_{10}O_2$	1569421	3.47
7	3-Allyl-6-methoxyphenol	10.31	164.20	$C_{10}H_{12}O_2$	508939	1.13
8	Pyrrole-2-carboxylic acid	10.43	111.1	$C_5H_5NO_2$	223174	0.49
9	Pyrrole-2-methanamine,	17.12	196.13	$C_5H_8N_2$	221493	0.49
10	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	15.41	180.24	$C_{11}H_{16}O_2$	285886	0.63
11	Benzeneacetic acid, 4-hydroxy-, ethyl ester	15.53	180.20	$C_{10}H_{12}O_3$	457543	1.01
12	4-Methyl(trimethylene)silyloxyoctane	16.37	214.42	$C_{12}H_{26}OSi$	247242	0.55
13	3-Hydroxy-7,8-dihydro-beta-ionol	18.50	212.33	$C_{13}H_{24}O_2$	94921	0.21
14	2,3,4-Trihydroxybenzaldehyde	19.42	154.12	$C_7H_6O_4$	643828	1.42
15	1-{2-[3-(2-Acetyloxiran-2-yl)-1,1-dimethylpropyl]cycloprop-2-enyl}ethanone	21.62	236	$C_{14}H_{20}O_3$	606010	1.34
16	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-	22.08	210.31	$C_{13}H_{22}O_2$	1371622	3.03
17	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	23.35	180.20	$C_{10}H_{12}O_3$	686118	1.52
18	Tetradecanoic acid	24.20	228.37	$C_{14}H_{28}O_2$	460010	1.02
19	Aspidospermidine-3-carboxylic acid, 2,3-didehydro-, methyl ester, hydrochloride,	24.94	374.90	$C_{21}H_{27}ClN_2O_2$	5082365	11.24
20	Acetamide, N-(4-ethoxy-3-hydroxyphenyl)-	25.74	195.22	$C_{10}H_{13}NO_3$	1335909	2.96
21	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	32.51	296.53	$C_{20}H_{40}O$	372248	0.82
22	Tricyclo[4.3.1.1(3,8)]undecane, 1-methoxy	26.44	180.29	$C_{12}H_{20}O$	129532	0.29
23	Pentadecanoic acid,14-methyl-,methyl ester	28.58	270.45	$C_{17}H_{34}O_2$	352739	0.78
24	Palmitic anhydride	29.57	494.83	$C_{32}H_{62}O_3$	4265432	9.44
25	9,12,15-Octadecatrienoic acid	32.30	280.45	$C_{18}H_{32}O_2$	677707	1.49
26	6,9,12,15-Docosatetraenoic acid methyl ester	33.15	346	$C_{23}H_{38}O_2$	282984	0.63
27	Bis[2-(dimethylamino)ethyl] methylphosphonotrithioate	35.73	270.40	$C_9H_{23}N_2OPS_2$	1298345	2.87

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28	Cedran-diol, 8S,14-	36.51	238.37	$C_{15}H_{26}O_2$	922129	2.04
29	9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol	36.87	536	$C_{28}H_{40}O_{10}$	273827	0.61
30	1-Hexacosene	37.71	364.69	$C_{26}H_{52}$	264571	0.59
31	Phenol, 2-methoxy-6-(3,7,11,15,19,23,27,	38.20	737.19	$C_{52}H_{80}$	299159	0.66
32	Phthalic acid, monocyclohexyl ester	39.86	248.27	$C_{14}H_{16}O_4$	6641678	14.69
33	16-Oxapentacyclo[13.2.2.0(1,13).0(2,10).	42.90	350.53	$C_{22}H_{34}D_2O_3$	434331	0.96
34	3, 4-Dihydro-3, 5, 8-Trimethyl-3-(4, 8, 12-Trimethyl Tridecyl)-(2H)1-Benzopyran-6-Acetate.	49.07	458	$C_{30}H_{50}O_3$	7371778	16.31
35	Vitamin E	51.28	430.71	$C_{29}H_{50}O_2$	2144424	4.74

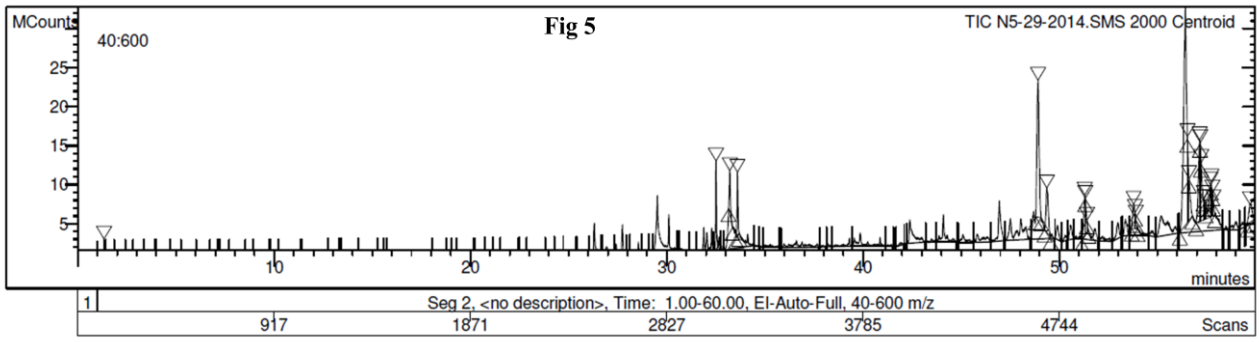

FIG 5: GC-MS Chromatogram of Methanolic extract of M. charantia var. muricata Morphotype-1 (MC-7)

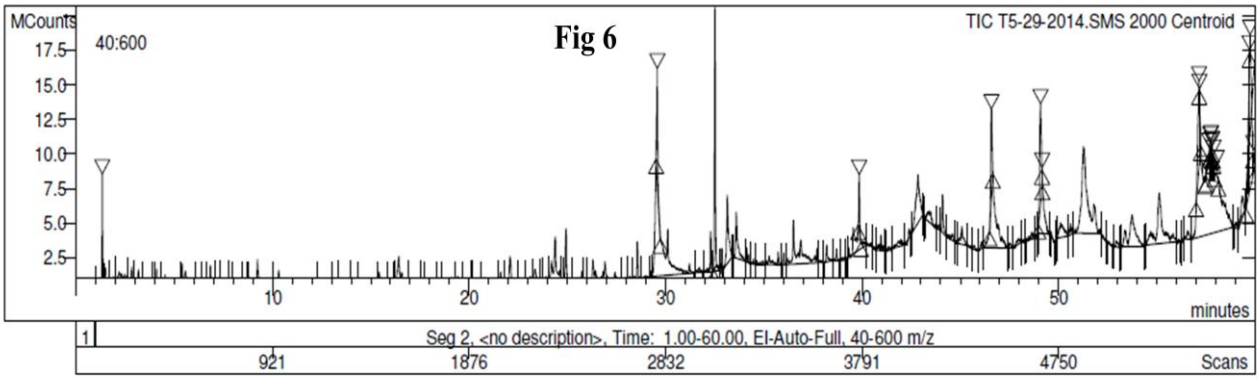

FIG 6: GC-MS Chromatogram of Methanolic extract of *M. charantia* var. *muricata* Morphotype-2 (MC-15)

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Table 5.
Activity of Phyto-Components Identified in the GC-MS Analysis of Methanol Extracts of *M. charantia* var. *muricata*

No	Name of the Compound	Biological Activity
1	Diosgenin	Antitumor
2	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Antimicrobial, Antioxidant, Antiinflammatory, Analgesic, Cancer preventive
3	Vitamin E	Antioxidant, Antiinflammatory
4	L-(+)-Ascorbic acid 2,6-dihexadecanoate	Antioxidant, Antiinflammatory and Anti-nociceptive
6	Hexadecanoic acid, ethyl ester	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Flavor, Lubricant, Antiandrogenic, Hemolytic 5-Alpha reductase inhibitor, Hemolytic, Lubricant, Nematicide, Antialopecic
7	Pentadecanoic acid, 14-methyl-, methyl ester	Antioxidant
8	9,12-Octadecadienoic acid (Z,Z)	Antioxidant
9	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyp	Antimicrobial, Antioxidant, Antiinflammatory, Hypocholesterolemic, Analgesic
10	Cyclopropanenonanoic acid	Antibacterial
11	2-Methoxy-4-vinylphenol	Antiinflammatory, Antimicrobial, Antioxidant, Analgesic
12	3, 4-Dihydro-3, 5, 8-Trimethyl-3-(4, 8, 12-Trimethyl Tridecyl)-(2H)1-Benzopyran-6-Acetate.	Fungicides, Insectiidess
13	2-Hydroxy-4-methylacetophenone	Acaricidal activity
14	Bis[2-(dimethylamino)ethyl] methylphosphonotrithioate	Anticholesterol
15	Benzofuran, 2-ethenyl-	Analgesic, Anti-cancer agents
16	Benzoic acid	Antifungal, Expectorant, Analgesic, Antibacterial
17	Cedran-diol, 8S,14-	Antimicrobial, Antiinflammatory, Anti-cancerous, Fragrance compound
18	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	Antimicrobial
19	9,12,15-Octadecatrienoic acid	Antiinflammatory, Insectifuge, Hypocholesterolemic, Cancer preventive, Nematicide, Hepatoprotective, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary
20	Indole	Fragrance compound
21	2,3,4-Trihydroxybenzaldehyde	COX-2 enzyme inhibitory
22	Tetradecanoic acid	Antimicrobial, Antioxidant, Anti cancerous, Hypercholesterolemic, Nematicide activity
23	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Antimicrobial, Antiinflammatory
24	Pentadecanoic acid,14-methyl-,methyl ester	Antioxidant
25	6,9,12,15-Docosatetraenoic acid methyl ester	Anticholesterol compound
26	9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol	Anti- inflammatory effects
27	1-Hexacosene	Antidiabetic