Gas Chromatography–mass Spectrometry Evidences for New Chemical Insights of *Momordica charantia*

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Momordica charantia is known to contain high diversity of biomolecules with antibacterial, antiviral, antihelmintic, abortifacient, antidiabetic and antitumoral properties. Chemical characterization of these molecules and identification of new compounds in M. Charantia sprouts in both aqueous and ethanolic extract was performed in the present study by gas chromatography tandem mass spectrometry (GC-MS). The GC–MS analyses of the alcoholic extract revealed that the main components are phytosterols, methyl esters of saturated straight-chain, branched-chain fatty acids, monoterpenes and chemopreventive agents. Major components such as aliphatic alcohols, 17-pentatriacontene, estranal, diterpene, anticopalic acid, hexanedioic acid, mono(2-ethylhexyl) ester, limonen, lupenone and palmitic acid esters were identified in aqueous extract. Fatty acids and vitamin E were detected only in hydrolized samples. In the case of hydrolized aqueous extract, sugar alcohols and esters of hydroxy carboxylic acids were detected. Two disaccharidic compounds, melibiose and turanose, were identified as trimethylsylil derivatives. The extended characterization of M. Charantia chemical components as well as their comparative analysis gives new information about the aspect of sample treatment in plants. The study shows that sprouts of M. charantia are an excelent source of phytosterols, essential fatty acids, and posses important compounds with antioxidant activity.

Keywords: M. Charantia sprouts, alcoholic extract, aqueous extract, GC-MS analysis, bioactive compounds, acid hydrolysis

Generally speaking, plants are an important source of biomolecules, and many researchers have been studying functional components in that purpose. Essential oils and fatty acids composition of lipids that posses beneficial efect on human health present interest in evaluation of plants extracts[1,2]. Achieved reports showed that various approaches allow exploiting the entire bioactive potential of *M. charantia* constituents [3-8]. Multiple pathways leading to the correlation of different phytochemicals presence and biological activities have been approached [6-9]. Its medicinal potency was also tested in diabetis [10,11] and it was used for his antibacterial, antiviral, anthelmintic, abortifacient and antitumoral properties [12-14]. Due to the fact that plants posess large variations in component amounts, chemical structures and functionalities, elucidating the identity and the relative amounts of these biocompounds is of great importance. The entrance door in different fields especialy medicine is given by the significance of plants that are used as crude extract for their active principles [15,16]. Components with important biological activity in general are extended characterised by high performance analytical techniques such as liquid chromatography and gas chromatography tandem mass spectrometry (MS) [1,2,17].

M. charantia sprouts are a source of polyphenols and fatty acids, representing the substances that cover a wide range of functions and that can be separated by using gas chromatography (GC). Due to its high sensitivity, GC coupled with MS, represents a powerful analytical technique that ensures the identification of volatile compounds. Numerous reports on the identification of *M. charantia* phytochemicals are available [5,18-21]. However, no report for the identification of chemical constituents in sprouts of *M. charantia* is available so far.

To improve the knowledge in the field, in the present study we evaluate the chemical composition of *Momordica charantia* sprouts in both aqueous and ethanolic extract by gas chromatography tandem mass spectrometry. In order to convert conjugated phytosterols into free form needed for GC analysis the goal of sample preparation was to isolate the sterol fraction. Identification of saturated and unsaturated fatty acids and other important nutrients made our purpose.

Experimental part

Materials

Methanol and Ethanol, both GC purity were purchased from Sigma – Aldrich (Darmstadt, Germany). Ultrapure water (Type I in house made) was used for the extraction procedure. Derivatization agent, Bis (trimethylsilyl) trifluoroacetamide (BSTFA) with the addition of 1% trimethylchlorosilane (TMCS) was obtained from Cerriliant (Wesel, Germany). Hydrochloric acid (HCl) 37%, sodium sulphate (Na_2SO_4), analytical grade and hexane (GC purity) were purchased from Sigma – Aldrich.

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Plant material

M. charantia plant sample was collected at the beginning of September from a field belonging to the Banat University Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" and it was identified by qualified personnel. Raw material was cleaned and dried at room temperature protected from light. Sprouts were reduced to a proper degree of fineness and maintained at room temperature, in dark until required for the extraction procedure.

Extraction procedure and preparation prior to GC - MS analysis

Powdered dried sprouts of *M. charantia* (10 g) were extracted under magnetic stirring in dark for 24 h using Type I deionized water and 95% ethanol 5% methanol. The two extracts were evaporated to dryness under reduced pressure. All extracted components were stored in amber bottles at 4°C.

Acid hydrolysis

The obtained extract was completly hydrolized to liberate the sterols from the matrix. Samples were treated with 3.5 M HCl to reduce the *p*H (3 – 4) with reflux at 100 °C, for an hour. Following hydrolysis total lipid extraction was performed by using hexane (three times in order to improve recovery). Prior derivatization samples were concentrated under nitrogen stream.

Derivatization

Following extraction, derivatization was performed to increase the volatility and stability of the extracted compounds. BSTFA was used as trimethylsilyl (TMS) donor for the silylation of hydroxy and carboxy groups. Reaction was catalyzed by the addition of 1 % TMCS. In order to achieve optimal results, both extracts and solvents were dried using rotary evaporator and anhydrous sodium sulphate. The TMS derivatives were obtained after incubation at 70°C for 60 min.

Gas chromatography

The volatile components were separated on 450 gas chromatograph from Varian (Middelburg, Netherlands) using helium as carrier gas at a constant flow rate of 1.2 mL min^{-1}, and a linear velocity of 39.8 cm s^{-1}. For separation a low polarity column Factor Four VF-5ms (30m x 0.25mmID, 0.25μm film), (Varian), was used. Temperature program was set as following: 50°C, 2 min, raised to 200 °C with 10 °C min^{-1}, held for 2 min, raised to 250 °C, with 5 °C min^{-1}, 2 min, and raised again at the final temperature of 300°C, held for 10 min. The temperature of the injection port was set at 280°C. Samples were injected at a volume of 1μL using split mode ratio for 3 min, followed by splitless mode for the rest of the analysis. Using the chromatographic conditions described above, the compounds eluted in less than 60 min.

Mass spectrometry

Mass spectrometry was performed using a 240 MS Ion Trap (Varian, Middelburg, Netherlands). The ionization parameters were set as follows: ion trap temperature at 170°C and transfer line temperature at 240°C. Ionization was performed in EI mode at 70eV. Nominal mass was used for recording full spectra and total ion current (TIC, mass range 50 – 450 amu) technique was applied for the identification of the eluate. For the confirmation of the identity of the separated compounds, the obtained MS spectra was integrated in the spectral database: NIST (National Institute for Standard and Technology), Wiley and PMW (Pfleger, Maurer and Weber).

Results and discussions

GC-MS of M. charantia alcoholic extract

Based on distribution coefficient between the eluent and the efluent, the low polarity column, Factor Four VF-5ms has allowed the separation of the mix composition for both extracts. After separation by gas chromatography, the eluate was introduced into the mass selective detector for the identification of chemical constituents found in both ethanolic and aqueous extract of *M. charantia* sprouts. The relative percentage area of each constituent was calculated by comparing its average peak area to the total area.

The GC – MS analysis of the alcoholic extract is presented in figure 1a. The chromatogram expressed a successful separation for the majority of the compounds (table 1) and revealed that the main components are phytosterols such as Δ5 –avenasterol (19.58%) followed by 25,26-dihydroelasterol (10.44%). Methyl esters of saturated straight-chain fatty acids such as palmitic acid (1.02%) and heptadecanoic acid (0.93%), methyl esters of saturated branched-chain fatty acids such as 14-methyl-pentadecanoate (1.81%), 14-methyl-heptadecanoate (0.78%),

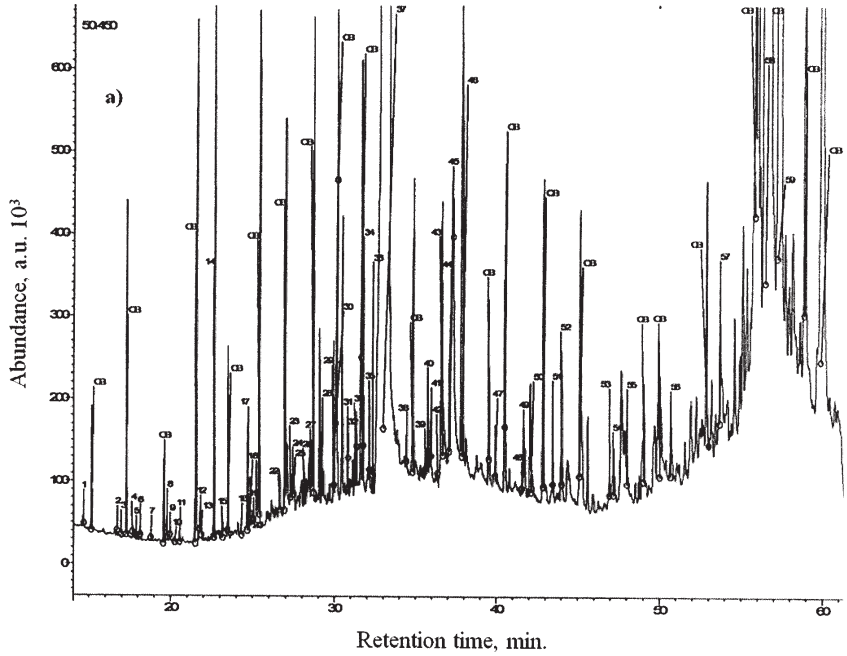

Fig. 1. a) TIC chromatogram of M. *charantia* sprouts alcoholic extract;

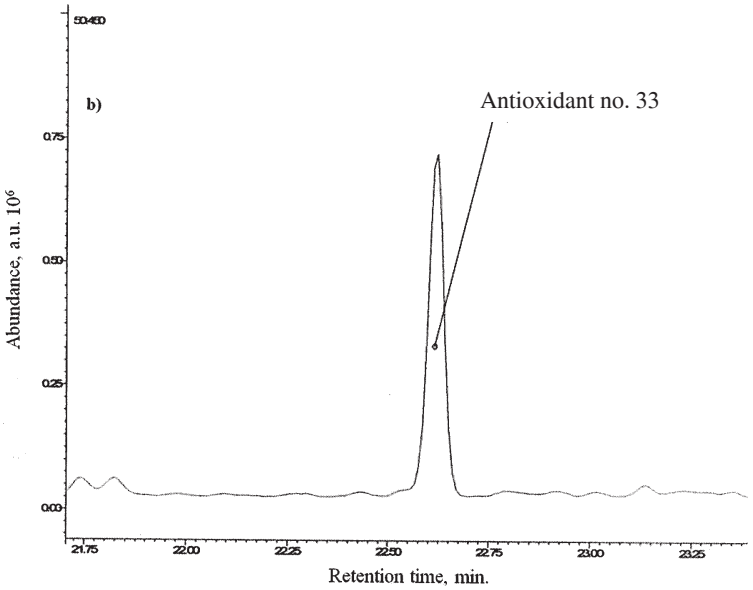

Fig. 1. b) Zoomed area at 21.75-23.25 min retention time show the antioxidant no 33. Carrier gas: helium; flow rate: 1.2 mL/min; velocity: linear, 39.8 cm/sec; CB –column bleed

Table 1
VOLATILE COMPOUNDS IDENTIFICATION IN ETHANOLIC EXTRACT

Compound	Retention time, min	Area, %
tert-butylquinone	14.599	0.37
trans-p-menth-2-ene	16.684	0.27
isobornyl acetate	16.98	0.27
2-methyloctahydro-1-benzofuran	17.637	0.25
2,4-dodecadienol	17.84	0.22
2-menthene	18.09	0.28
1,1,6-trimenthyl-1,2-dihydronaphtalene	18.8	0.23
1-tridecane	19.726	0.38
O-decylhydroxyamine	19.934	0.25
lauryl aldehyde	20.197	0.21
indan-1,3-diol monoacetate	20.533	0.26
1-(2,8,8-trimethyl-5,6,7,8-tetrahydro-4H-cyclohepta[b]furan-5-yl)ethanone	21.743	0.28
n-tridecan-1-ol	21.823	0.28
antioxidant No 33	22.613	4.48
lylial	23.135	0.35
(2,3,6-trimethylphenyl)-2-butanone	24.267	0.32
1-hexadecanol	24.633	1.11
2-hexyl-1-octanol	24.809	0.69
2-(1-hydroxylbut-2-enylidene)cyclohexanone	24.918	0.38
4-phenyl-3-penten-2-one	25.037	0.42
2-ethyl-1-dodecanol	25.853	0.41
n-hexyl salicylate	26.617	0.74
spiro-1-(cyclohex-2-ene)-2'-(5'-oxabicyclo[2.1.0]pentane)-1',4',2,6,6-pentamethyl	27.279	0.70
1,13-tridecanediol diacetate	27.423	0.72
benzestrol	27.49	0.73
estranal	28.135	0.78
miristyc acid	28.422	0.84
2-hexyl-1-decanal	29.201	1.04
3-eicosyne	29.91	0.93
hexahydrofarnesyl acetone	30.032	1.04
(2E)-3,7,11,15-tetramethyl-2-hexadecan-1-ol	30.807	1.16
2-hexyl-1-decanol	31.253	1.06
7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	31.347	1.21
14-methyl-pentadecanoate	31.751	1.81
isophytol	32.13	1.39
palmitic anhydride	32.822	5.31
1,8-dihydroxy-3-methoxy-6-methylanthra-9,10-quinone	33.303	10.44
trimethylsilyl palmitate	34.43	1.02
ethyl heptadecanoate	35.606	0.93
2-hexyl-1-decanol	35.746	1.40
methyl-2-hydroxy-hexadecanoate	35.934	1.25
14-heptadecanoate methylester	36.349	0.78
ethyl 9 cis 11-trans-octadecadienoate	37.077	1.74
ethyl linolenate	37.216	3.02
stearic acid 2-(2-hydroxyethoxy)ethylester	37.29	2.88
ethyl 15-methyl-heptadecanoate	37.819	4.45
1-hexadecanol-2-methyl-2-methylhexadecan-1-ol	39.917	1.10
mono-2-ethyhexyl adipate	41.548	0.65
19-methyl-eicosanoate	41.662	1.09
2,4-bis(1-phenylethyl)phenol	41.784	0.77
2-hexyl-1-decanol	43.46	0.87
[14Z]-14-tricosenyl formate	44.042	1.04
androst-4,9(11) 16-trien-3-one	46.933	0.67
17-pentatriacontene	47.171	0.92
fumaric acid dimyrtenyl ester	48.002	0.87
1-hexacosene	50.663	1.17
gama-linolenic acid tertbutyldimethylsilyl ester	53.646	1.75
Δ5 -avenasterol	56.344	19.58
25,26-dihydroelasterol	57.183	10.44

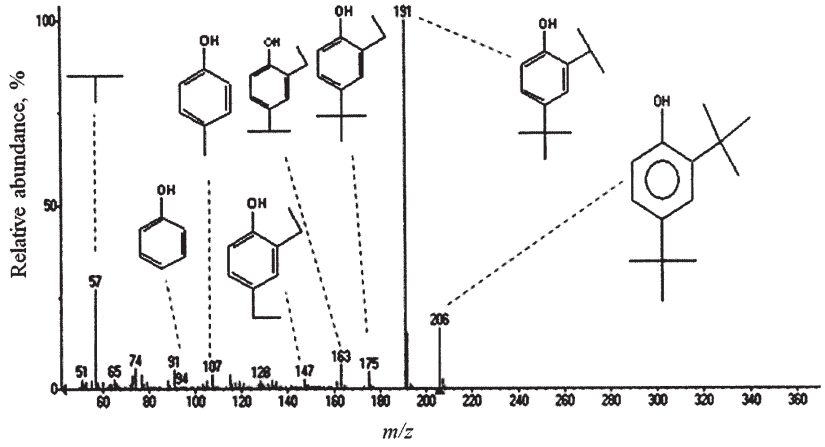

Fig. 2. MS spectrum of Antioxidant No 33 and their corresponding fragments identified in alcoholic extract. Inset: structure of proposed species. EI voltage: 70 eV; ion trap temperature: 170 °C

19-methyl-eicosanoate (1.09%), and ethyl esters of dienoic fatty acids such as 9 cis, 11-trans-octadecadienoate (1.74%) were also observed. Considerable percentage area was registered following retention time in order: antioxidant no 33 (4.48%), palmitic anhydride (5.31%), ethyl linolenate (3.02%), stearic acid 2-(2-hydroxyethoxy)ethyl ester (2.88%) and gama-linolenic acid tert-butyldimethyl-silyl ester (1.75%). Monoterpenes such as trans-p-menth-2-ene and 2-menthene were also found in alcoholic extract.

Because the formation of free radicals is associated with lipid peroxidation, one of the most important roles of antioxidants is to protect against oxidative stress and therefore against injuries from lipid oxidation.[22] Antioxidant no 33 found in the extract (fig. 1b) is part of the antioxidants used to control the free radical attack to the human system. Capillary GC analysis shows baseline separation and gaussian shape of the peak, followed by mass spectrum confirmation using the most abundente mass to charge ratio at *m/z* 191.2.

MS spectrum reveals the fragmentation of molecule with the sequencial cleavage of CH_3-group(s) (fig. 2), which is specific for such molecules. For structural confirmation, the match factor between the target compound is compared against the database library. The biological activity profile of antioxidant no 33 is given by the phenoxyl radical on the donation of hydrogen by interrupting the chain reaction of lipid peroxidation [22]. Various bioactive constituents including saturated fatty acids such as stearic, palmitic, heptadecanoic eicosanoic acids required for energy storage, membrane proliferation and generation of signaling molecules were identified. Chemopreventive agents as trans-p-menth-2-ene and 2-menthene, used for alleviating the proliferation and progression of cancer related – tumors [23] were observed in alcoholic extract.

GC-MS of M. charantia aqueous extract

The chromatogram (fig. 3) of aqueous extract of *M. charantia* showed a satisfactory separation of the compounds eluted from the column. Major components identified in aqueous extract (table 2), were as following their area percentage: aliphatic alcohols, 2-hexyl-1-octanol (6.54%), 2-hexyl-1-decanol (5.44%), 2,4-bis(1-phenyl-ethyl)phenol (4.92%), 17-pentatriacontene (4.87%), estranal (4.83%), a labdane diterpene, anticopalic acid (4.03%), hexanedioic acid, mono(2-ethylhexyl) ester (3.88%), from terpenoids, limonen (3.07%), and lupenone (2.81%). Volatile alkenes, sesquiterpene hydrocarbons such as ä-candinene and nutrients were also found in derivatized aqueous extract. An important synthetic antioxidant, butylated hydroxytoluene, wich posses nutritional and therapeutic value by retarding the peroxidation process in food systems was identified in aqueous extract. The presence of volatile smaller terpenoids, monoterpene hydrocarbons, limonene, oxygenated monoterpenes, bornyl acetate, sesquiterpene hydrocarbons, ä-candinene, with chemopreventive, chemotherapeutic and anti-microbial activities [24,25] should not be neglected. It is believed that minor components work in synergism in order to potentate the activity.

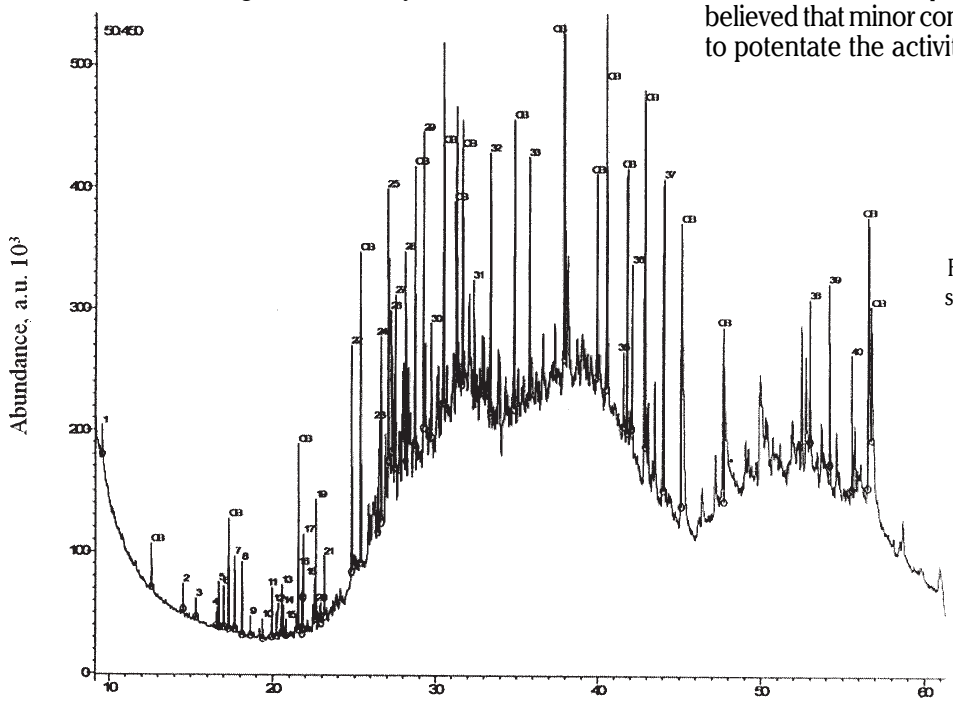

Fig. 3. TIC chromatogram of M. *charantia* sprouts aqueous extract. Conditions as in figure 1. CB –column bleed

Compound	Retention time, min	Area, %
Limonen	9.567	3.07
4-methyl-6-(tetrahydropyron-2-yloxy)hex-4-enol	14.472	0.84
1,2-dimethyl-1-cyclooectene	15.29	0.85
n-tridecan-1-ol	16.552	0.78
2-menthene	16.671	0.86
isobornyl acetate	16.963	0.81
p-menth-3-ene	17.666	1.36
menthyl acetate	18.122	0.96
6-methyltetralin	18.607	0.58
cubenol	19.354	0.50
2,3-dimethyldecane	19.939	0.93
cyclododecanol	20.218	0.74
indan-1,3-diol monoacetate	20.537	1.12
i-propyl-decanoate	20.637	0.62
2,5-di-tert-butylphenol	20.823	0.52
2,2a,4a,7a-tetramethyl-2a,3,4,4a,6,7,7a,7b-octahydro-5H-cyclopenta [cd]inden-5-one	21.754	1.09
isomethyl-alpha-ionone	21.804	1.23
butylated hydroxytoluene	22.562	0.97
Antioxidant No33	22.649	1.06
δ-candinene	22.952	0.81
lilyal	23.114	1.05
methyl-1-undecene	24.824	3.04
2-methylhexadecan -1-ol	26.239	2.27
n-hexyl-salicylate	26.634	3.61
p-tert-octylphenol	27.093	4.88
spiro-1-(cyclohex-2-ena)-2'-(5'-oxobicyclo[2.1.0]pentone), 1',4',2,6-pentamethyl	27.279	3.97
2-methyl-4-hydroxy benzoxazole	27.89	3.31
estranal	28.135	4.83
2-hexyl-1-decanol	29.195	5.44
i-propyl-tetradecanoate	29.704	3.93
anticopalic acid	32.403	4.03
2-hexyl-1-octanol	33.355	6.54
17-pentatriacontene	35.724	4.87
methyl dehydroabietate	40.595	4.44
6-[(2-ethylhexyl)oxy]-6-oxohexanoic acid	41.548	3.88
2,4-bis(1-phenylethyl)phenol	42.075	4.92
phthalic acid octyl-2-pentyl ester	43.946	4.60
5(7a-isopropenyl-4,5-dimethyl-octohydroinden-4-yl)-3-methyl-pent-2-en-1-ol	52.931	3.93
9-n-dodecylperhydrophenanthrene	54.159	3.94
lupenone	55.504	2.81

Table 2
VOLATILE COMPONENTS IDENTIFICATION IN AQUEOUS EXTRACT

Comparative analysis of M. charantia alcoholic vs aqueous extracts

Difference in polarity of the molecules was evaluated using two solvent systems: ethanol with 5 % methanol and deionized type I water. The obtained results showed a promising bioactive potential of extracted compounds for both systems. Lipids were extracted only in alcoholic phase. When using the most polar solvent, terpenoids were the major components extracted. Table 3 contains the common components that were identified in both alcoholic and aqueous extract of *M. charantia* sprouts after derivatization procedure. Retention times are quite similar but percentage area is increased in aqueous phase for 2-menthene, isobornyl acetate, indan-1,3-diol monoacetate n-hexyl, lilyal, salicylate spiro-1-(cyclohex-2-ena)-2'-(5'-oxobicyclo[2.1.0]pentone), 1',4',2,6-pentamethyl, estranal and 2-hexyl-1-decanol and only for antioxidant no 33 in alcoholic phase.

GC-MS analysis of M. charantia hydrolysis products

Plant acids, phenolics and flavonoids identification in raw material is dependent on the polarity of the molecules, the dissolving ability of the extraction solvent and also of the form in wich the compound is found in crude material. Due to the fact that glycosides are the major constituents, acid hydrolysis was the choosen technique to release the aglycone form of the compound. The MS output for the derivatives obtained from both extracts was compared with the respons obtained after hydrolysis and derivatization procedure (table 4 and 5). For the ethanolic extract it was observed that instead of miristyc acid the hydroxy form of the fatty acid, β-hydroxymiristyc acid. Palmitic acid can be seen in both forms (ethyl ester and trimethylsilyl ester). Stearic, pentadecanoic and oleic acid apeared only after hydrolysis procedure in the form of methyl esters of the saturated straight-chain fatty acids. Methyl esters of trienoic acids such as α-linolenic acid and methyl esters of saturated branched-chain fatty acids, such as 13-methyl-penta-decanoate were detected only in hydrolized samples. An important antioxidant, vitamin E, was observed only after acid hydrolysis.

In the case of hydrolized aqueous extract, sugar alcohols such as xylitol in the form of trimethylsylil derivatives, esters of hydroxy carboxylic acids such as lactone and carbo-

Compound	Alcoholic extract		Aqueous extract	
	Retention time, min	Area, %	Retention time, min	Area, %
2-menthene	18.09	0.28	16.671	0.86
isobornyl acetate	16.98	0.27	16.963	0.81
indan-1,3-diol monoacetate	20.533	0.26	20.537	1.12
antioxidant No 33	22.613	4.48	22.649	1.06
lilyal	23.135	0.35	23.114	1.05
n-hexyl salicylate	26.617	0.74	26.634	3.61
spiro-1-(cyclohex-2-ena)-2'-(5'-oxobicyclo[2.1.0]pentone), 1',4',2,6-pentamethyl	27.279	0.70	27.279	3.97
estranal	28.135	0.78	28.135	4.83
2-hexyl-1-decanol	35.746	1.40	33.355	6.54

Table 3
COMMON COMPONENTS FOUND IN BOTH EXTRACTS OF M. *CHARANTIA* SPROUTS

Compound	Retention time, min	Area, %
β-hydroxymiristic acid	12.659	1.70
2,4-ditert-butylphenyl -5-hydroxy-pentanoate	14.191	32.96
1,4-dihydrophenacetic acid 3,5-di-t-butylethyl ester	14.476	3.09
2,6-ditertbutylhydroquinone	16.68	2.14
13-methyl-pentadecanoate	19.348	1.38
trimehtylsiyl pentadecanoate	19.638	1.09
palmitic acid ethyl ester	20.538	1.88
trimehtylsilyl palmitate	21.287	3.67
n-eicosanol	22.047	2.23
ethyl linoleate	23.292	1.55
ethyl-15-methyl-hepta decanoate	23.852	2.07
α-linolenic acid trimethylsilyl ester	24.176	1.18
trimethylsilyl stearate	24.615	1.19
1,2-dipalmitin-glycerol, 1,2-dipalmitate	28.997	1.29
1,3-dipalmitin trimethylsilyl ether	29.792	1.92
1-monopalmitin trimehtylsilyl ether	30.174	1.27
vitamin E	39.116	3.21
3β- stigmasta-5,24(28)-dien-3-ol	41.453	16.53
Δ 5-avenosterol	42.297	13.07
oleic acid	47.766	6.59

Table 4
IDENTIFICATION OF HYDROLYSIS COMPOUNDS IN ETHANOLIC EXTRACT

Compound	Retention time, min	Area, %
2-methoxy cinnamic acid tert-butyldimethylsilyl ester	7.477	67.47
2,6-di-tert-butylquinone	13.667	0.82
2,4-ditert-butylphenyl-5-hydroxy-pentanoate	14.203	7.81
1,2,3,4,5-pentakis-O-(trimethylsilyl)-pentital trimehtylsilyl ether of xylitol	16.184	1.41
1,2,3,4,5-pentakis-O-(trimethylsilyl)-pentital	16.510	0.43
L-gluconic acid, 2,3,5,6-tetrakis-O-(trimethlsilyl) lactone	18.527	0.49
1,2,3,5,6-pentakis-O-(trimethylsilyl) hexafuranose	19.777	5.02
2,3,4,5,6-pentakis-[(trimethylsilyl)-oxy]cyclohexanol	20.165	7.62
1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-myo-inositol	20.435	2.88
1,2,3,4,5,6-hexakis-O-(trimehtylsilyl)-scyllo-inositol	21.842	5.49
octakis-(trimehtylsilyl)-melibiose	22.860	0.38
heptakis-(trimethylsilyl)-D-turanose	30.838	0.17

Table 5
IDENTIFICATION OF HYDROLYSIS COMPOUNDS IN AQUEOUS EXTRACT

hydrates such as furanose and inositol (as *myo* and *schyllo* - naturally occurring stereoisomers) were detected. Two disaccharidic compounds, melibiose and turanose, were identified as trimethylsylil derivatives.

Conclusions

The GC-MS technique provided a complete characterization of complex mixtures extracted from biological material and a high eficiency in identification of chemical composition as well as new molecules. In this work, for the first time, was reported a detailed investigation of *M. charantia* sprouts extract using ethanol and water as extraction solvents, applying hydrolysis and derivatization technique prior identification by gas chromatography tandem mass spectrometry.

In summary, all compounds found in volatile extracts of *M. charantia* sprouts, were baseline resolved and succesfully identified by ion trap mass spectrometer. As shown in the reported results, the identity of the individual components can vary depending on sample preparation prior to injection. Acid hydrolysis used to release the aglycones form of plant acids, flavonoids derived from aromatic amino acids and lipids was eficient for

identification of new compound which could not evidenced in pure extracts. Comparison of the results of the derivatized and hydrolized - derivatized samples gives new information about the aspect of sample treatment in plants.

Functional components profile plays an important role in establishing the specific properties of the part of plant, therefore this is useful knowledge for further researches needed for production of pharmaceutical compounds. On the other side, the study also shows that sprouts of *M. charantia* are an excelent source of phytosterols such as Ä5 –avenasterol and 25,26-dihydroelasterol, essential fatty acids such as palmitic, stearic, oleic, linolenic and myristic acid. It also posses important compounds with antioxidant activity such as antioxidant no 33, vitamine E and butylated hydroxytoluene.

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References

1. LIU, Y., LIU,Z., WANG,C., ZHA, Q., LU,C., SONG, Z. NING,Z., ZHAO,S., LU, X., LU, A.,Chem. Centr. J. **8** (2014) 22.
2. DULF,F.V., PAMFIL.D., BACIU, A.D., PINTEA, A.,Chem. Centr. J. **7** (2013) 8.
3. DANIEL,P., SUPE, U., ROYMON, M.G., Int. J. Adv. Pharm. Biol. Chem. **3** (2014) 214.
4. ORAGWA,N.L., OLAJIDE, O.O., EFIOM,O.O.,. OKWUTE,K.S., Afr. J. Pure Appl. Chem. **7** (2013) 375.
5. ZULBADLI, N.,H. ALWI, K.H. KU HAMID, Int. J. Eng. Tech. **11** (2011) 79.
6. A. DAS, P. KARMAKAR, MD.G. KIBRIA, P.C. DENNATH, S.M. ISLAM, M.M. SATTAR, BRITH, J. PHARM. RES. **4** (2014) 739.
7. R.SINGH, A.KUMAR, D.D. GIRI, K. BHUVANESHWARI, K.D. PANDEY, J. Rec. Adv. Agri. **1** (2012) 122.
8. R.I. BAKARE, A.O. MAGBAGBEOLA, A.I. AKINWANDE, O.W. OKUNOWO, J. Med. Pl. Res. **4** (2010) 2189.
9. L.N. ORAGWA, O.O. OLAJIDE, O.O. EFIOM, K.S. OKWUTE, Afr. J. Pure Appl. Chem. **7** (2013) 405.
10. B. JOSEPH, D. JINI, Asian Pac. J. Trop. Dis. **3** (2013) 93.
11. A. TONGIA, S.K. TONGIA, M. DAVE, Indian J. Physiol. Pharmacol. **48** (2004) 241.
12. K.D. MWAMBETE, Afr. Health Sci. **9** (2009) 34.
13. P.O. LIMTRAKUL, O. KHANTAMAT, K. PINTHA, Cancer Chemother. Pharmacol. **54** (2004) 525.
14. M. Li, Y. CHEN, Z. LIU, F. SHEN, X. BIAN, Y. MENG, Acta Biochim. Biophys. Sin. **41** (2009) 792.
15. L. MAAMRIA, H. HABA, C. LAVAUD, D. HARAKAT, M. BENKHALED, J. Serb. Chem. Soc. **80** (2015) 137.
16. M. DODEVSKA, S. ŠOBAJIĆ, B. DJORDJEVIĆ, J. Serb. Chem. Soc. **80** (2015) 21.
17. T. Yi, Q. Chen. X. He, S. So, Y. Lo, L. Fan, J. Xu, Y. Tang, J. Zhang, Z. Zhao, H. Chen, Chem. Centr. J. **7** (2013) 115.
18. D.O. MORONKOLA, I.A. OGUNWANDE, I.O. OYEWOLE, K.H. CAN BAŞER, T. OZEK,; G. OZEK, J. Ess. Oil. Res. **21** (2009) 393.
19. A. BRACA, T. SICILIANO, M. D'ARRIGO, M.P. GERMAN , Fitoter. 79 (2008) 123.
20. M.E. ABALAKA, H.I. INABO, J.A. ONAOLAPO, O.S. OLONITOLA, Ferm. Tech. Bioeng. **1** (2011) 1.
21. M.E. ABALAKA, H.I. INABO, J.A. ONAOLAPO, O.S. OLONITOLA, Afr. J. Med. Phy. Biomed. Eng. Sc. **2** (2010) 14.
22. M.G. REPETTO, S.F. LLESUY, Braz. J. Med. Biol. Res. **5** (2002) 523.
23. G.J. KELOFF, J.A. CROWELL, C.W. BOONE, J. Cell. Biochem. Supp. **56** (1994) 55-62.
24. J.A. MILLER, J.E. LANG, M. LEY, R. NAGLE, C.H. HSU, P.A. THOMPSON, C. CORDOVA, A. WAER, H.H. CHOW, Cancer Prev. Res. (Phila.) **6** (2013) 577.
25. S. TAVASSOLI, S.M. MOUSAVI, Z. EMAM-DJOMEH, S.H. RAZAVI, Middle-East J. Scien. Res. **9** (2011) 46

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