Tripeptides Observed in Hydro-distillation Analysis of *Ocimum Tenuiflorum L.* by LC-MS/MS

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doi: 10.30731/ijcps.7.2.2018.46-51

Abstract

Tripeptides are molecules of great interest today due to their antioxidant property shown by them at very low concentration. Hydro-distillation of O. tenuiflorum L. leaves was carried out for the qualitative analysis of minor phytoconstituents by LC-MS/MS. The ten tripeptides were observed along with previously reported eugenol, methyl eugenol, cis-caryophyllene, cinnamyl acetate. The present article described the tripeptides occurred in the O. tenuiflorum L and its spectrometric profiling in LC-MS/MS analysis.

Keywords: Eugenol, Fatty acids, Lipids, *Ocimum sanctum*, XCMS

Introduction

Tripeptides are the small building block having three amino in its structure linked by peptide linkage. Tripeptides are known for their relationship in growth factors involved in the healing process and synthesis of collagen. Few tripeptides also exhibit properties such as antioxidant and protease inhibition. Naturally occurring tripeptides are often isolated from the plant parts. Indeed, every plant has some or other medicinal properties and hence plants are treated as source of drug for health care since human civilization. In Asia, *O. tenuiflorum* synonym: *O. sanctum* (Lamiaceae) an aromatic plant is popularly known as Holy basil. *O. tenuiflorum* has been known for its therapeutic activities such as analgesic, anthelmintic, anti-aflatoxigenic, antimicrobial, anticancer, antidepressant, anti-diabetic activity, anticandidal, anti-metastatic, antioxidant, antiprotozoal, anti-stress, antiviral, anxiety, cardio protective, chemoprotective, cognition improvement, hepatosupression, hyperlipidemia, immunomodulatory activity, noise stress release, radioprotective, also including renal damage recovery and wound healing [1]. The plant is also recognized for its ethnopharmocological activities [2,3,4,5,6]. *O. tenuiflorum* has been used in traditional medicine for centuries of years as an anti-inflammatory agent and expectorant. The most abundant components of *O. tenuiflorum* are phenol, lignans, terpene and fatty acids. Therefore the plant is gaining demand in primary heath care for treating various ailments. Number of minor secondary metabolites from *O. tenuiflorum* still remains unrevealed and hence its metabolite profiling has foreseeable future. Due to advancement in analytical instruments it is now possible to detect and identify the phytochemicals exhibiting significant pharmacological activities and which were below limit of detection in previous analytical techniques. The biological activities of the plant are due to the phytochemicals present in different parts of it. The volatile oil of *O. tenuiflorum* was previously studied by analytical methods [7] such as GC, GC-MS. Quantitative estimation of rosmarinic and ursolic acid [8]

of leaves of *O. sanctum* by LC-MS is recently reported. The numbers of phytochemicals are reported for the *O. tenuiflorum* the amount of which in various part of the plant vary on basic of climate, soil quality, place and other such environmental factor. Advanced hyphenated system LC-MS/MS with Q-TOF is found as most appropriate techniques for phytochemical profiling of leaves extract of *O. tenuiflorum*.

Material and Methods

Plant Material and Chemicals

Fresh, healthy leaves of *O. tenuiflorum* were collected from Avasari ghat, Narayangaon, Pune, India. The herbarium of the sample was authenticated by Botanical Survey of India, Pune. Leaves thoroughly washed with double distilled water to remove adsorbed material on it. HPLC grade ethyl acetate, chloroform, methanol, was purchased from Merck (Germany). Water was purified using quartz double distillation system. (Infusil, India).

Hydro-distillation

50 grams of fresh leaves of *O. tenuiflorum* were crushed and subjected to hydro-distillation. Approximately 500 ml aliquot was obtained. The distilled aliquot was then extracted with (250 ml x 2) each of chloroform, diethyl ether and ethyl acetate respectively. The solvents were mixed and evaporated on rotary evaporator under vacuum. 1.254 gm of pale yellow oil was obtained. This oil was diluted with methanol (~1 ppm) and run on LC-MS/MS.

LC-MS/MS Analysis

Mass analyzer (Model: Agilent G6540B MS Q-TOF) was used in full scan mode scanning a range from m/z 40–2000 at a scan time of 3 spectra/s. Dual Electrospray Ionization (ESI) fragmentor was used at 170.0V with positive mode (Reference Mass: 149.02332). The different metabolites are separated on C18 (G1316A) using gradient from 95% (0.1% formic acid/water) and 5% acetonitrile to 5% (0.1% formic acid/water) and 95% acetonitrile within 25 min with flow rate 0.3 ml/min. The gradient was restored to 95% (0.1% formic acid/water) and 5% acetonitrile for last five minute. Injection volume of the hydro-distillation sample is 1.0 µL. The method parameter for Agilent G6540B MS Q-TOF, gradient, solvent composition and column component in detail is given in **Table 1**.

Result and Discussion

The **Figure 1** represents chromatogram for hydro-distillation of *O. tenuiflorum* by LC-MS/MS. These peaks correspond to a range of secondary metabolites such as sugar, amino acids, terpenes and other carbon compounds. Obtained peaks were sorted on the basis of database score and its difference in ppm. The peaks having database score greater than 95 and difference in ppm less than 5 were chosen for further analysis.

The LC-MS/MS Q-TOF data were first analyzed on Agilent Mass Hunter workstation. Subsequently, the same data were analyzed by means of freely available XCMS Online platform at https://xcmsonline.scripps.edu/. XCMS means various forms (X) Chromatography Mass Spectrometry. XCMS Online [9] is bioinformatics software available free of cost for the analysis of chromatographic data and creating its global plot [10,11,12]. The software is link to the METLIN and The LIPID MAPS/Lipidomics Gateway, http://www.lipidmaps.org/. The METLIN and LIPID MAPS databases are having information of known metabolites and known lipids respectively and their tandem mass spectrometry data. Present study will shade some light on the phytochemicals contain in *O. tenuiflorum* and the numerous bioactivities shown by the plant.

Table 1: Parameter for Agilent G6540B MS Q-TOF

Parameter	Value		Parameter	Value
Acquisition Method			**Auxillary**	
Component Name	MS Q-TOF		Draw Speed	200 µL/min
Component Model	G6540B		Eject Speed	200 µL/min
Ion Source	Dual AJS ESI		Draw Position Offset	0.0 min
Stop Time(min)	No Limit/As Pump		**Injection**	
Can wait for Temperature	Disable		Injection Mode	
Fast Polarity	N/A		Injection Volume	1.0 µL
MS Absolute Threshold	100		**Needle Wash**	
MS Relative Threshold (%)	0.010		Needle Wash Location	Wash Vial
MS/MS Absolute Threshold	5		Wash location	Vail 91
MS/MS Relative Absolute Threshold (%)	0.010		**Overlapped Injection**	
Tune File	Autotune.tun		Enabled Overlapped Injection	Yes
Acquisition Mode MS1			Overlapped Injection Start Mode	Prefetch Vial
Min Range (m/z)	40		Overlapped Injection Watt Time	0.00 mim
Max Range (m/z)	2000		**Stop Time**	
Scan Rate (Spectra/Sec)	3.00		Stopping Mode	As Pump/No limit
Source Parameter			**Post Time**	
Gas Temperature (°C)	325		Post time mode	Off
Gas Flow (l/min)	5		**Binary Pump**	
Nebulizer (psig)	20		Model	G1312B
Sheath Gas Temperature	400		Flow	0.300 mL/min
Sheath Gas Flow	10		Use Pressure Limit	Yes
Polarity	Positive		Low Pressure Limit	0.00 bar
Scan Source Parameter			High Pressure Limit	500.00 bar
VCap	4000		maximium Flow Gradient	100.000 mL/min^2
Nosal Voltage (V)	0		**Stroke A**	
Fragmentor	170		Automatic Strock Calcuation A	Yes
Skimmer1	45		**Stroke B**	
OctopolRFPeak	750		Automatic Strock Calcuation B	Yes
Reference Mass	Enabled		**Compress A**	
Use Bottle A Ref Nebulizer	FALSE		Compressibility mode A	Value set

Ref Nebulizer (psig)	0		Compressibility A	50 x 10^{-6} /bar
Auto Calibration			**Compress B**	
Average Scan	1		Compressibility mode B	Value set
Detection Window (ppm)	100		Compressibility A	115 x 10^{-6} /bar
Min Height (Counts)	1000		**Stop time**	
Reference Mass: Positive	149.02332000		Stoptime Mode	Time set
Chromatography Type	TIC		Stoptime	30.00 min
Sampler			**Post time**	
Model	G1329B		Posttime Mode	Time set
			Posttime	5.00 min

Gradient

Time	Function	Parameter
5.00 min	Change Solvent Composition	Solvent Composition A: 95.0%
15.00 min	Change Solvent Composition	Solvent Composition A: 60.0%
20.00 min	Change Solvent Composition	Solvent Composition A: 15.0%
25.00 min	Change Solvent Composition	Solvent Composition A: 5.0%
30.00 min	Change Solvent Composition	Solvent Composition A: 95.0%

Solvent Composition

Channel	Solvent 1	Name 1	Used	Percentage
A	Water	0.1% FA	Yes	95.0%
B	Acetonitrile	ACN	Yes	5.0%

Column Component

Model	G1316A
Left Temperature Control	
Temperature Control Mode	Temperature set
Temperature	40.00 °C
Enable Analysis Left Temperature	
Enable Analysis Left Temperature On	Yes
Enable Analysis Left Temperature Value	0.80 °C
Right Temperature Control	
Temperature Control Mode	Combined
Enable Analysis Right Temperature	
Enable Analysis Right Temperature On	Yes
Enable Analysis Right Temperature Value	0.80 °C
Stop Time	
Stoptime Mode	As Pump/Injector
Post Time	
Posttime Mode	Off

Out of ten tripeptides Lys-His-Pro, Arg-Val-Phe, Ile-Pro-Arg were identified on negative polarity in LC-MS/MS analysis. All the tripeptides observed in the study with less than 5 ppm error in their difference of mass expected were short listed for further studies. Leupeptin is well known naturally occurring tripeptides was also observed in the **Figure 1**.

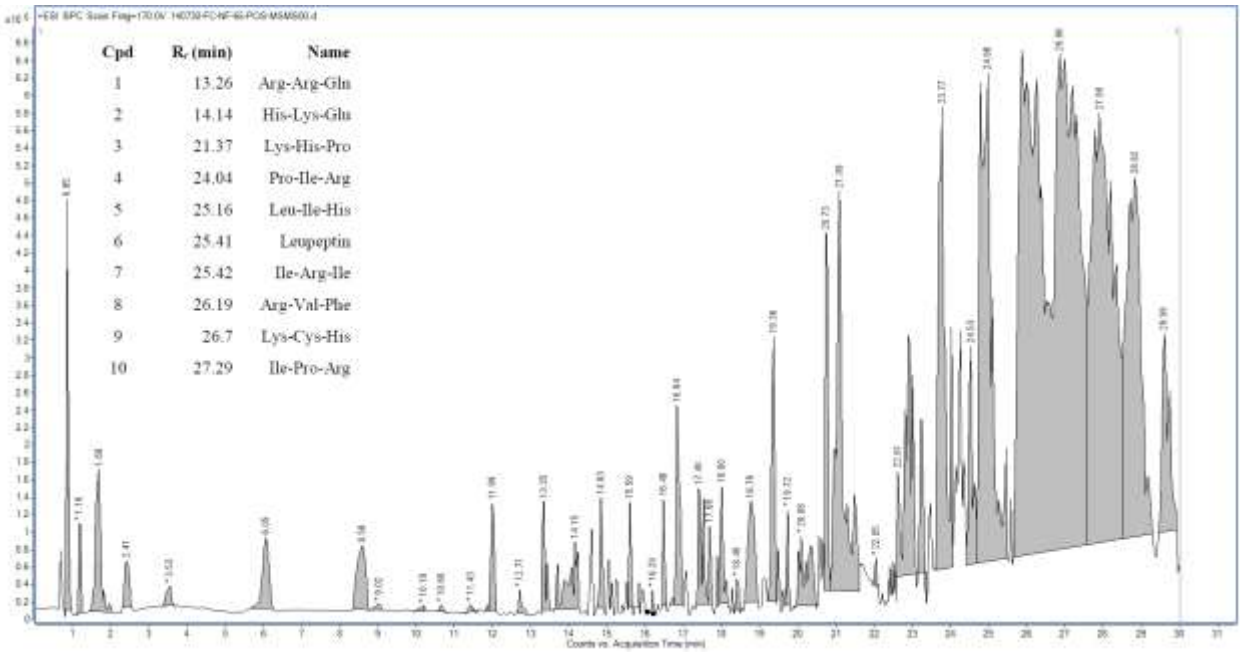
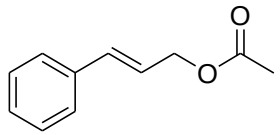
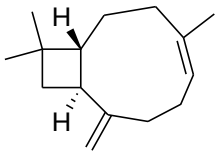
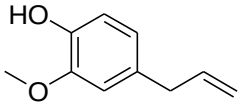
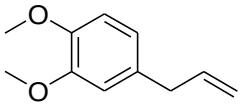


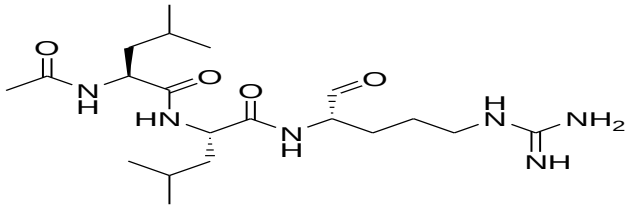
Fig. 1: Chromatogram for hydro-distillation of *O. tenuiflorum* by LC-MS/MS.

Table 2: Tentative assignment of the compounds identified in LC-MS Q-TOF analysis from the leaves extract of *O. tenuiflorum*

Cpd	R_t (min)	MF	m/z (Precursor Peak)	Exact Mass		Error (ppm)	Polarity	Class/sub class	Name
				Observed	Calculated				
1	13.26	$C_{17}H_{34}N_{10}O_5$	476.3049	458.2709	458.2714	1.08	Positive	Peptide	Arg-Arg-Gln
2	14.14	$C_{17}H_{28}N_6O_6$	430.2423	412.2086	412.207	-3.77	Positive	Peptide	His-Lys-Glu
3	21.37	$C_{17}H_{28}N_6O_4$	361.1999	380.218	380.2172	-2.03	Negative	Peptide	Lys-His-Pro
4	24.04	$C_{17}H_{32}N_6O_4$	367.244	384.2475	384.2485	2.72	Positive	Peptide	Pro-Ile-Arg
5	25.16	$C_{18}H_{31}N_5O_4$	381.2598	381.2365	381.2376	2.91	Positive	Peptide	Leu-Ile-His
6	25.41	$C_{20}H_{38}N_6O_4$	444.3306	426.2967	426.2955	-2.88	Positive	Peptide	**Leupeptin**
7	25.42	$C_{18}H_{36}N_6O_4$	405.2593	400.2808	400.2798	-2.52	Positive	Peptide	Ile-Arg-Ile
8	26.19	$C_{20}H_{32}N_6O_4$	401.2316	420.2495	420.2485	-2.37	Negative	Peptide	Arg-Val-Phe
9	26.7	$C_{15}H_{26}N_6O_4S$	369.1696	386.1731	386.1736	1.35	Positive	Peptide	Lys-Cys His
10	27.29	$C_{17}H_{32}N_6O_4$	365.2312	366.2387	384.2485	1.75	Negative	Peptide	Ile-Pro-Arg

			
Cinnamyl acetate	Cis-Caryophyllene	Eugenol	Methyl Eugenol

Eugenol, cis-caryophyllene, cinnamyl acetate, methyl eugenol were appeared in **Figure 1** with retention time 19.07, 24.55, 29.63 29.64 min respectively.



N-acetyl-L-leucyl-L-leucyl-L-argininal
Leupeptin **6**

Leupeptin was appeared in **Figure 1** with retention time 25.41 min. Leupeptin is naturally occurring protease inhibitor also known as *N*-acetyl-L-leucyl-L-leucyl-L-argininal having ability to inhibit cystein, serine and threonine peptidases.

Conclusions

The present study described the observation made in the analysis of *O. tenuiflorum* leaves. The hydro-distillation of *O. tenuiflorum* reveals the qualitative presence of the ten bioactive tripeptides. All the tripeptides observed in the study with less than 3 ppm error in their difference of mass expected.

References

[1] S.S.Pingale, N.P.Firke, A.G.Markandeya, *Journal of Pharmacy Research*, 2012, 5(4), 2215-2220.
[2] P.Prakash, N.Gupta, *Indian Journal of Physiology Pharmacology*, 2005, 49(2), 125-131.
[3] M.Mukherjee, A.K.Datta, *Plant Archives*, 2007, 7(2), 473-483.
[4] S.Singh, M.Taneja, D.K.Majumdar, *Indian Journal of Experimental Biology*, 2007, 45(5), 403-412.
[5] P.Pattanayak, P.Behera, D.Das, S.K.Panda, *Pharmacognosy Review*, 2010, 4(7), 95-105.
[6] B.Joseph, V.N.Nair, *British Journal of Pharmaceutical Research*, 2013, 3(2), 273-292.
[7] P.K.Awasthi, S.C.Dixit, *Journal of Essential Oil Bearing Plants*, 2007, 10(4), 292-296.
[8] R.S.Sundaram, L.Gowtham, M.Ramanathan, V.Venugopal, D.Kamalkannan, *Asian Journal of Biomedical and Pharmaceutical Sciences* 2011, 1(3), 22-28.
[9] R.Tautenhahn, C.Böttcher, S.Neumann, *BMC Bioinformatics* 2008, 9, 504.
[10] C.A.Smith, E.J.Want, G.O'Malille, R.Abaghyan, G.Siuzdak, *Analytical Chemistry*, 2006, 78, 779-787.
[11] G.J.Patti, R.Tautenhahn, G.Siuzdak, *Nature Protocol*, 2012, 7(3), 508-516.
[12] Z.J.Zhu, A.W.Schultz, J.Wang, C.H.Johnson, S.M.Yannone, G.J.Patti, S.Gary, *Nature Protocol*, 2013, 8(3), 451-460.