TO: Lanham Frazier, Big S
FROM: Drs. Evan Siemann & Meenakshi Bhattacharjee, Rice University
DATE: 3-11-2025
RE: Untargeted metabolomics analyses of EARTI ™ microgreens

We analyzed the metabolites in arugula (Eruca vesicaria) microgreens grown in the EARTI ™ chamber and arugula microgreens bought at local grocery stores. Untargeted metabolomics simultaneously measures all detectable compounds in a sample (hundreds to thousands of compounds), determines the relative concentrations of each compound among the samples via differences in peak areas, and identifies each compound (if possible). However, it does not tell the absolute concentrations of compounds so it is not possible to determine the relative concentrations of different compounds, even in the same sample. But, it gives a comprehensive chemical profile that can be used to compare among samples.

Arugula was grown for seven days post planting in the EARTI ™ chamber. Then the arugula microgreens were cut at the base, ground in liquid nitrogen with a mortar and pestle, and stored at -80°C. Another set of microgreens were harvested two days later and processed the same way. Later, another set of seven day microgreens were grown and processed. Arugula microgreens were bought from HEB and Sprouts and processed the same way as the EARTI ™ samples. All the samples were shipped in a container of dry ice to Creative Proteonomics in Shirley, NY. Details of their chemical analysis methods are at the end of this report.

The data were analyzed at Rice University. First, the untargeted metabolomics data were processed to remove peaks that could not be assigned to a specific compound. Second, Principle Component Analysis (PCA) was used to examine the overall chemical composition of samples and to determine which samples had similar vs. distinct chemical compositions. This was done separately for data generated using positive vs. negative detector modes. Third, each chemical compound was tested to determine whether it differed in concentration between the EARTI ™ vs. commercial samples using ANOVAs. P-values were adjusted using sequential Bonferroni correction (alpha=0.05) to control for false discovery with the large number of tests being performed. Fourth, the ratios of concentrations of each compound in EARTI ™ vs. commercial samples were examined to identify compounds that occurred in much higher concentrations in EARTI ™ vs. commercial samples. Finally, a literature search was conducted to determine effects on human health for compounds that were present in significantly higher concentrations in EARTI ™ vs. commercial samples.

Key findings were: 1) Arugula microgreens grown in EARTI ™ had chemical compositions that were distinct from those of arugula microgreens available at local grocery stores in Houston, TX. 2) A shorter time to harvest in EARTI ™ produced arugula microgreens that had consistent chemical composition. 3) Seventeen compounds were significantly elevated in EARTI ™ arugula microgreens vs. those bought at local grocery stores and ten were significantly lower in EARTI ™ samples. 4) Another three compounds were not significantly different between EARTI ™ samples vs. those bought in local grocery stores but were present at more than 100 times the concentration in EARTI ™ samples. 5) More chemical compounds were elevated in arugula microgreens grown in EARTI ™ than in those bought at local grocery stores. 6) Dipeptides were commonly elevated in EARTI ™ samples.

PCA: The overall chemical composition of the samples fell into three distinct groups (Figure 1). In these graphs, points closer to each other have more similar chemical compositions and points more distant from each other have more distinct chemical compositions. In these graphs, positive vs. negative on the axes is arbitrary. The key finding was that, the same three groups are found in both positive and negative detector mode data. One group is the commercial samples from grocery stores ("C1" – H2Organics bought from HEB, "C2" – BrightFresh bought from Sprouts). Another was the short time to harvest samples from EARTI ™ ("T1" – planted 10/2/24 and harvested on 10/9/24; "T2" – planted 10/19/24 and harvested on 10/26/24). The longer time to harvest EARTI ™ sample was separate from the others ("T3" – planted 10/2/24 and harvested on 10/11/24). At harvest, the T3 sample seemed to have some microbial contamination based on appearance and odor. It is important to note that it is possible that different arugula varieties were grown in EARTI ™ vs. those for commercial samples which could contribute to the differences in their chemical compositions.

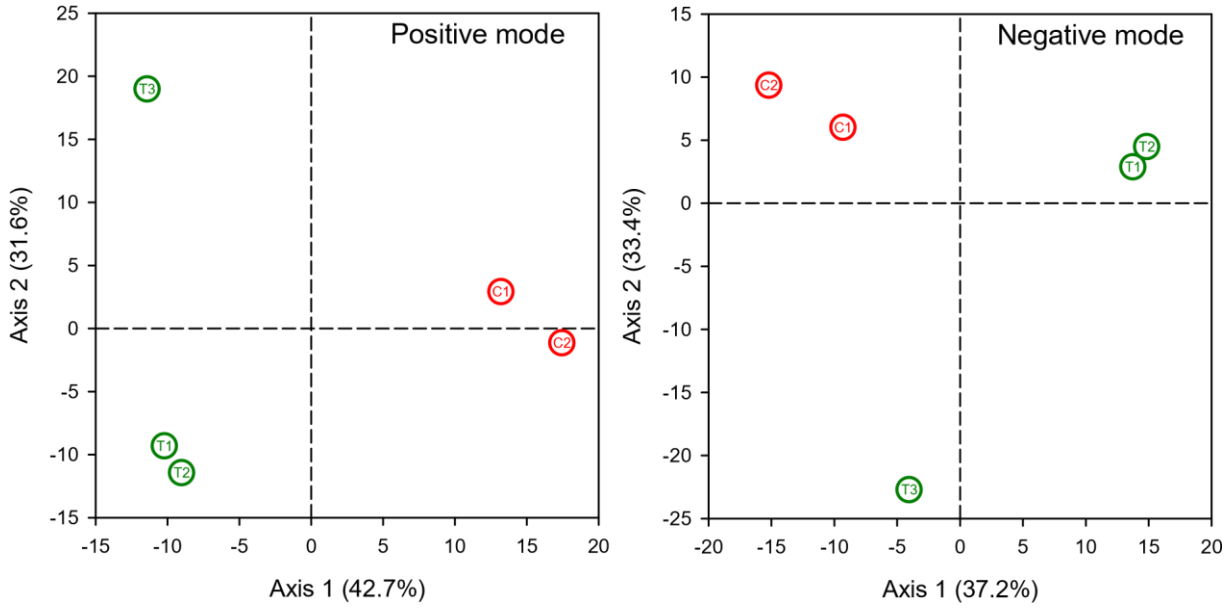


Figure 1 Chemical composition of arugula microgreen samples grown in EARTI ™ for 7 days (T1, T2) or 9 days (T3) along with commercial arugula microgreen samples (C1, C2). Numbers indicate the percent of variation explained by each axis.

ANOVAs: For examining which compounds contribute to these distinct chemical compositions, we restricted statistical analyses to only the shortterm EARTI ™ vs. commercial samples. After correcting for examining a large number of compounds (~1000 in total) with a sequential Bonferroni procedure, there were 27 compounds that differed significantly between EARTI ™ vs. commercial samples (Table 1). If T:C >1 (i.e., log[T/C]>0) then there is a higher concentration in EARTI ™ samples vs. commercial samples [rows in green] while T:C<1 means greater concentrations in commercial samples [rows in red].

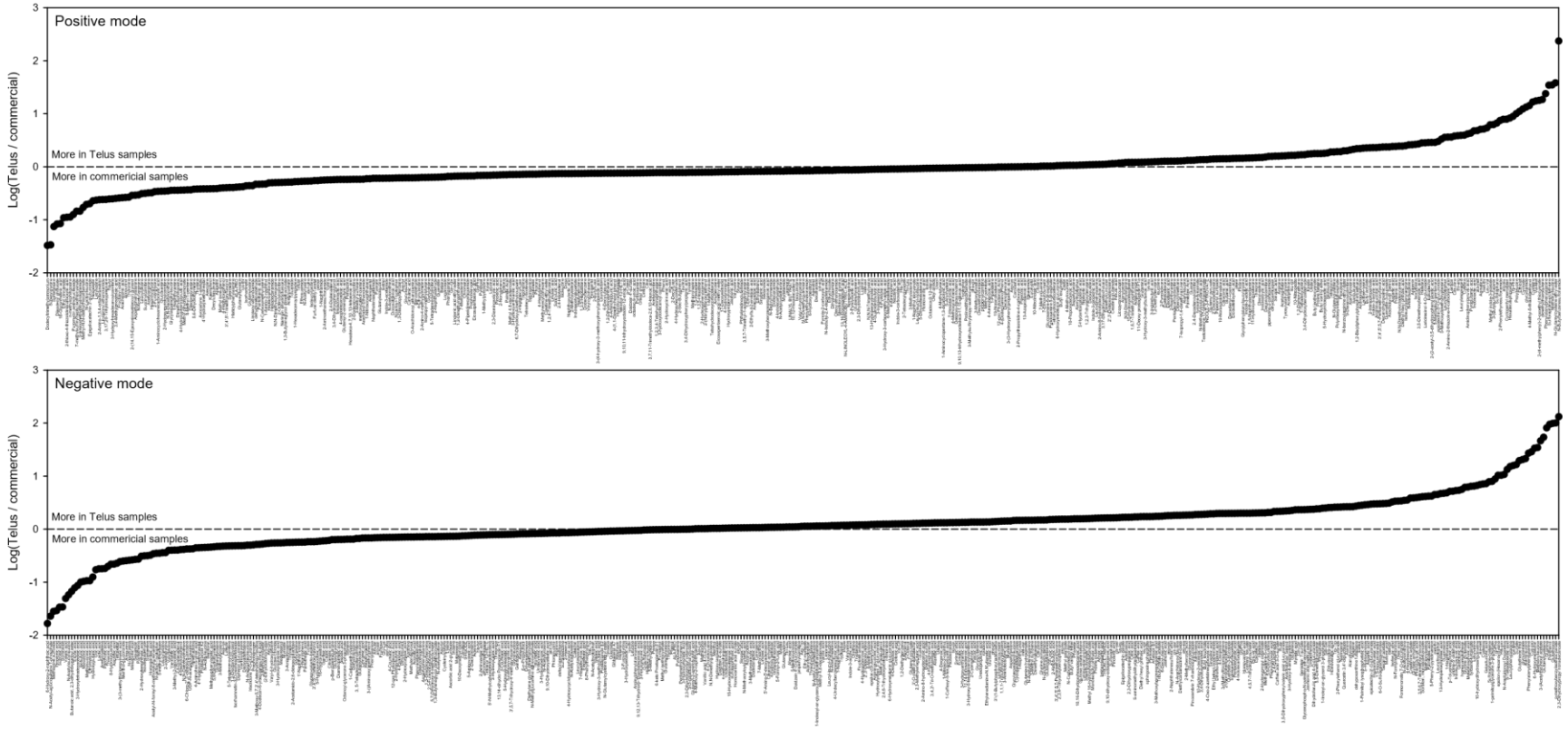
Table 1. The relative concentrations of samples that differed significantly between arugula microgreens grown in EARTI ™ vs. those bought at local grocery stores. Mode = detector mode. CODE indicates the peak number. RT = retention time in minutes. MW = molecular weight. T:C is the ratio of the compound's concentration in EARTI ™ vs. commercial samples.

Mode	CODE	RT [min]	MW	Name	Formula	Chemical class	T1	T2	C1	C2	T:C	log(T/C)	P-value
Pos	C4607	5.781	482.10372	Epigallocatechin 3'-glucuronide	C21 H22 O13	flavonoid metabolite (glucuronidated catechin derivative)	2.93	3.21	15.63	15.01	0.20	-0.70	0.0008
Pos	C2300	4.027	302.0798	Hesperetin	C16 H14 O6	flavanone (Flavonoid compound)	40.28	52.08	195.99	209.74	0.23	-0.64	0.0033
Neg	C2315	5.799	316.18906	Menthol-glucoronide	C16 H28 O6	glucuronidated monoterpenoid (menthol metabolite)	35.30	36.88	143.65	144.66	0.25	-0.60	<.0001
Pos	C1197	1.371	216.12212	N-Acetyl-arginine	C8 H16 N4 O3	acetylated amino acid derivative	8.31	9.10	29.21	30.16	0.29	-0.53	0.0009
Neg	C0762	5.705	212.06876	Vanillactic acid	C10 H12 O5	phenolic acid derivative (vanillin metabolite)	11.80	10.37	27.99	27.64	0.40	-0.40	0.0019
Neg	C0243	1.496	143.05835	Trimethadione	C6 H9 N O3	oxazolidinedione (anticonvulsant drug)	211.74	237.03	534.53	521.89	0.42	-0.37	0.0022
Pos	C0953	7.343	190.13572	Heptanophenone	C13 H18 O	aromatic ketone	24.25	26.56	51.90	50.55	0.50	-0.30	0.0027
Neg	C0452	5.208	173.10551	N-Acetylalloisoleucine	C8 H15 N O3	acetylated amino acid	79.84	83.04	164.46	162.99	0.50	-0.30	0.0005
Pos	C2222	1.805	296.1007	Aspartyl-Tyrosine	C13 H16 N2 O6	dipeptide	220.97	219.63	357.96	359.49	0.61	-0.21	<.0001
Neg	C3451	11.798	367.27253	3, 5-Tetradecadiencarnitine	C21 H37 N O4	acylcarnitine	18.51	18.39	27.84	27.71	0.66	-0.18	<.0001
Neg	C3674	12.394	377.29326	Arachidonoyl Serinol	C23 H39 N O3	N-acyl ethanolamine-related lipid	48.90	50.82	22.48	22.82	2.20	0.34	0.0013
Pos	C0543	0.992	155.06946	Histidine	C6 H9 N3 O2	amino acid	5543.75	5371.65	2100.90	1778.52	2.81	0.45	0.0027
Pos	C0228	75	118.04519	5-Mercapto-2-pentanone	C5 H10 O S	thiol-containing ketone (organosulfur compound)	437.92	405.21	144.53	148.87	2.87	0.46	0.0036
Pos	C1075	2.88	205.07383	Indolelactic acid	C11 H11 N O3	indole derivative (tryptophan metabolite)	195.31	197.99	36.67	46.08	4.75	0.68	0.0010
Pos	C0970	3.078	193.07731	3-(Butylsulfinyl)alanin	C7 H15 N O3 S	sulfoxide-containing amino acid derivative	33.35	31.80	6.74	3.70	6.24	0.80	0.0039
Neg	C0850	1.613	220.08815	Alanine-Methionine	C8 H16 N2 O3 S	dipeptide	369.15	356.18	37.93	66.98	6.91	0.84	0.0026
Pos	C3151	6.016	351.15815	Tryptophan-Phenylalanine	C20 H21 N3 O3	dipeptide	99.38	102.04	12.92	9.70	8.91	0.95	0.0005
Neg	C1092	4.931	244.17847	epsilon-(Hexanoyl)lysine	C12 H24 N2 O3	lipidated amino acid (N-acyl lysine derivative)	3172.16	3370.09	360.59	271.79	10.35	1	0.0013
Neg	C1158	2.408	248.11932	Valine-Methionine	C10 H20 N2 O3 S	dipeptide	414.68	452.32	26.23	28.58	15.82	1.20	0.0021
Neg	C0051	82	97.96737	Sulfate	H2 O4 S	inorganic anion	50321.93	54319.42	2520.49	3845.39	16.44	1.22	0.0018

Neg	C1884	4.865	296.11932	glycyclamide	C14 H20 N2 O3 S	peptidomimetic (amide derivative of glycine)	66.79	73	5.70	3	20.48	1.31	0.0023
Neg	C0682	1.613	202.13176	Alanylleucine	C9 H18 N2 O3	dipeptide	1463.15	1504.32	69.38	69.84	21.31	1.33	0.0002
Neg	C2210	5.769	312.14787	Phenylalanylphenylalanine	C18 H20 N2 O3	dipeptide	135.68	137.11	6.81	3.27	27.05	1.43	0.0002
Neg	C1157	2.886	248.11931	6-Hydroxymelatonin	C13 H16 N2 O3	melatonin metabolite (indole derivative)	131.54	122.38	4.66	2.91	33.55	1.53	0.0014
Pos	C4617	3.873	484.1117	Sulforaphane-glutathione	C16 H28 N4 O7 S3	organosulfur compound (glutathione conjugate of sulforaphane)	53.07	56.96	1.94	1.24	34.53	1.54	0.0014
Neg	C1323	3.956	262.13489	Methionylleucine	C11 H22 N2 O3 S	dipeptide	295.33	322.37	10.05	7.83	34.54	1.54	0.0020
Pos	C1948	1.482	278.09354	N-Glutamylcysteine ethyl ester	C10 H18 N2 O5 S	glutathione precursor (modified dipeptide)	66.13	61.76	1.71	1.63	38.25	1.58	0.0012

Relative concentrations by sample: Although they are not significantly different between EARTI ™ and commercial samples, three compounds were present at more than 100 times the concentration in EARTI ™ vs. commercial samples. They were N-Butylbenzenesulfonamide (sulfonamide) which was ~200 times higher in EARTI ™ samples along with p-methoxybenzylisothiocyanate (isothiocyanate) and 2,3-Dihydroxypropyl nonadecanoate (fatty acid ester) which were ~100 times higher in EARTI ™ samples.

Figure 2. The relative concentrations compounds (log transformed) in arugula microgreens grown in EARTI ™ [7 days] vs. those bought at local grocery stores.


Positive mode
Log(Telus / commercial)
3
2
1
0
-1
-2
More in Telus samples
More in commericial samples
Negative mode
Log(Telus / commercial)
3
2
1
0
-1
-2
More in Telus samples
More in commericial samples

Compound functions: Potential effects on human health for compounds that were present at significantly higher concentrations or at more than 100 times the concentration in arugula microgreens grown in EARTI ™ vs. those bought at local grocery stores (Table 2). It is important to note that untargeted metabolomics analysis does not tell the absolute concentrations of chemical compounds. Rather it tells the relative amounts of a particular compound in different samples. Therefore, a chemical compound may be present at very different concentrations in different samples but the concentrations may be too low to have any effects on human health. In addition, a chemical compound may be present at very different concentrations in different samples but those concentrations may have similar effects on human health. In order to link relative concentrations to human health, it would be necessary to conduct additional, quantitative, targeted chemical analyses. Nevertheless, most dipeptides that differed in concentration between EARTI ™ and commercial samples were higher in EARTI ™ samples. Higher dipeptide content in plants is considered beneficial from a health perspective as they are readily absorbed by the body compared to proteins and possess various health promoting properties.

Table 2: Potential effects of compounds elevated in EARTI ™ arugula microgreen samples vs. those bought in local grocery stores.

Name	Potential effects on human health
Arachidonoyl Serinol	Neuroprotective, Improves motor function, Vasodilation of abdominal aorta.
Histidine	Improves cognitive function, growth& repair of damaged tissue, precursor of carnosine for human muscle and parts of brain, proton buffering, and histaminergic reactions
5-Mercapto-2-pentanone	Potentially hazardous material causes irritation of skin and respiratory tract.
Indole lactic acid	Anti-inflammatory, maintains healthy gut, protects intestinal inflammation cancer prevention
3-(Butyl sulfinyl)alanine	Strong Antioxidant Properties
Alanine-Methionine	Improves protein synthesis, strong antioxidants, modifies DNA, energy production, improves exercise performance, strengthens the immune system.
Tryptophan-Phenylalanine	Mood regulation, improves cognitive function, improves sleep quality, reduces stress.
Epsilon-(Hexanoyl)lysine	Very new oxidative stress marker
Valine-Methionine	Helps muscle growth, Tissue repair, Over all metabolic function, energy production, emotional stability, liver protection.
Sulfate	Liver protection, helps in digestion, building proteins, detoxification of body.
Glycyclamide	Strong antihyperglycemic activity
Alanyl leucine	Promotes muscle growth and repair, ,blood sugar regulation and Cognitive function.
Phenylalanyl phenylalanine	Helps with mood, pain and skin conditions, protein synthesis, Risk: Phenylketonuria
6-Hydroxymelatonin	Potential antioxidant properties, anti-inflammatory and neuroprotective properties, sleep regulator.
Sulforaphane-glutathione	Precursor of glutathione, cancer prevention, anti-inflammatory, detoxification, helps with hormonal imbalance.
Methionyl leucine	Helps with tissue growth, muscle repair, improves liver damage, mineral absorption, immune system boosts up and wound healing.
N-Glutamyl cysteine ethyl ester	Precursor for glutathione, crucial antioxidant, supports liver health, improves brain function, neuroprotective.
2,3dihydroxypropyl nonadeconate	Treating obesity related diseases, diabetes and inflammation.
N-butylbenzenesulphonamide	Bacteriostatic antimicrobials
p-methoxybenzyl isothiocyanate	Anti-inflammatory, antioxidant anticancer properties, generally found in cruciferous plants.

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Analysis Report

Project Name	Untargeted Metabolomics Analysis
Sample Description	Arugula (Eruca vesicaria)
Sample Quantity	5
Order Number	CPMS09302401
Client	Evan Siemann
Project Date	2025-2
Remark	

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1. Sample Information

5 plant samples for untargeted metabolomics analysis in the collected samples by UPLC-MS.

2. Materials and Methods

2.1 Instruments and reagents

Vanquish Flex UPLC combined with Q Exactive plus MS (Thermo)
ACQUITY UPLC HSS T3 (100×2.1 mm×1.8 μm)
Temp functional Centrifugation (Eppendorf)
Acetonitrile (Merck)
Methanol (Merck)
Formic acid (Merck)

2.2 Sample preparation

All samples were thawed and lyophilized, weigh about 100 mg of each sample and 800 μL 80% methanol, two 5-mm metal balls into tube. All samples were ground 180 s at 65 Hz, twice, followed by sonication for 30 min, 4°C. Then each sample was kept at -20°C for 1 h, vortexed for 30 s, and centrifuge 10 min at 12,000 rpm, 4°C. Finally, transfer 200 μL of supernatant and add 5 μL of 0.14 mg/mL DL-o-Chlorophenylalanine into vial, filtered through a 0.22 μm filter for LC-MS analysis.

3. LC-MS Methods

Separation is performed by Vanquish Flex UPLC combined with Q Exactive plus (Thermo) which is equipped with a heated ESI source. A waters T3 column (100×2.1 mm×1.8 μm) was used for LC separation and the mobile phase is composed of solvent A (0.05% formic acid water) and solvent B (acetonitrile) with a gradient elution (0-1 min, 5% B; 1-12.5 min, 5%-95% B; 12.5-13.5 min, 95% B; 13.5-13.6 min, 95%-5% B; 13.6-16 min, 5% B). The flow rate of the mobile phase is 0.3 mL/min. The column temperature is maintained at 40°C, and the sample manager temperature is set at 4°C.

Mass spectrometry parameters in ESI+ and ESI- mode are listed as follows:

ESI+: Heater Temp 300°C; Sheath Gas Flow rate, 45 arb; Aux Gas Flow Rate, 15 arb; Sweep Gas Flow Rate, 1 arb; spray voltage, 3.0 KV; Capillary Temp, 350°C; S-Lens RF Level, 30%.

ESI-: Heater Temp 300°C, Sheath Gas Flow rate, 45 arb; Aux Gas Flow Rate, 15 arb; Sweep Gas Flow Rate, 1 arb; spray voltage, 3.2 KV; Capillary Temp,350°C; S-Lens RF Level,60%.

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4. Analytical Results

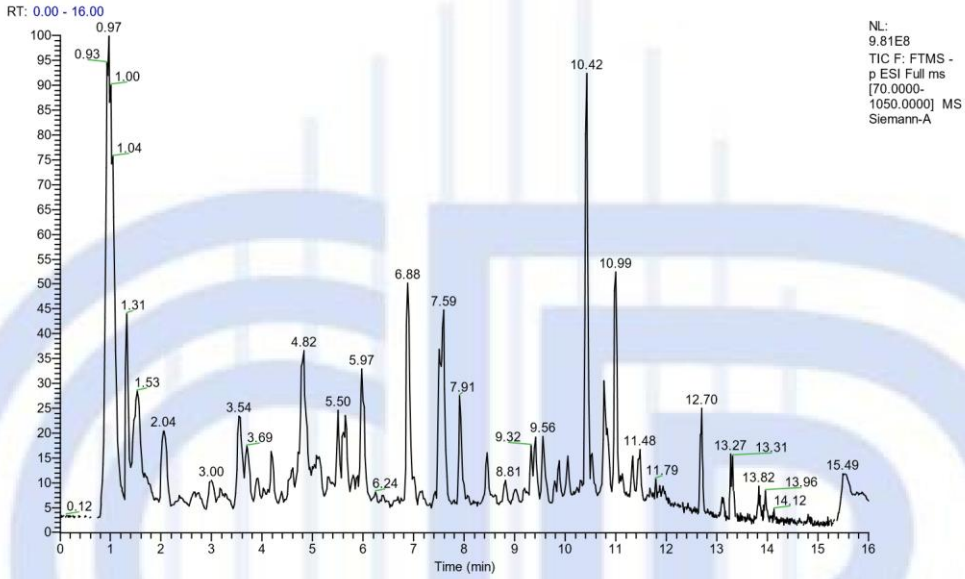

Figure.1 TIC obtained from sample in ESI- mode.

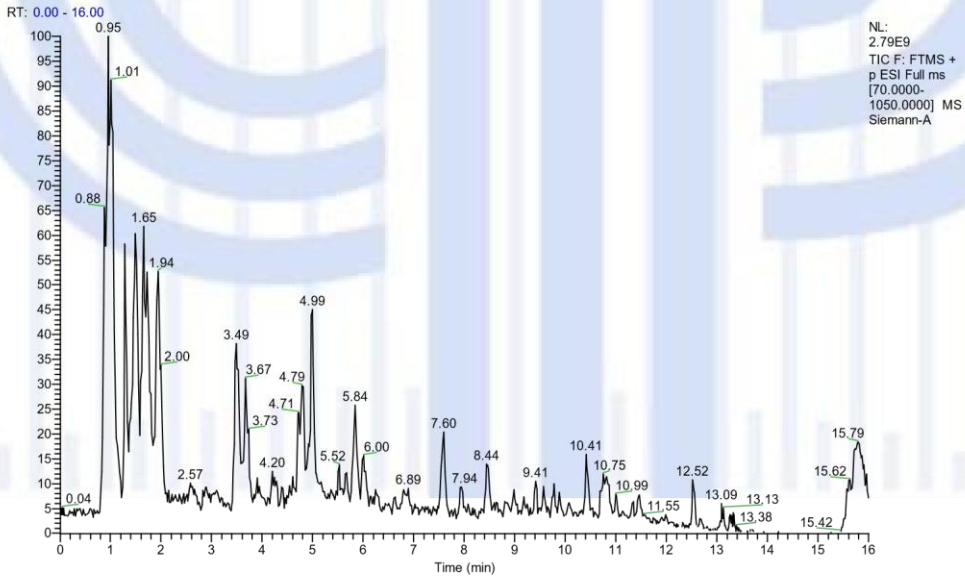

Figure.2 TIC obtained from sample in ESI+ mode.

The results are shown in the excel sheet.