

Comprehensive Tools of Alkaloid/Volatile Compounds–Metabolomics and DNA Profiles: Bioassay-Role-Guided Differentiation Process of Six *Annona* sp. Grown in Egypt as Anticancer Therapy

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Table S1. Number of total bands, monomorphic bands and polymorphic bands and percentage of polymorphism revealed by the twelve 10-mer primers in the studied samples by SCoT and ISSR.

SCoT	Primer Name	Total Band	Monomorphic Band	Polymorphic band	Unique Band	Polymorphic %
	SCoT 1	8	4	4	-	50%
	SCoT 2	3	3	-	-	-
	SCoT 3	5	4	1	1	20%
	SCoT 4	7	3	4	4	57.14%
	SCoT 6	3	2	1	1	33.33%
	SCoT 8	5	1	4	3	80%
	Total	31	17	14	9	45.16%
ISSR	14A	4	3	1	-	25%
	44A	5	4	1	1	20%
	HB-8	5	3	2	-	40%
	HB-10	6	2	4	1	66.66%
	HB-12	8	5	3	3	37.5%
	HB-13	6	5	1	-	16.16%
	Total	34	22	12	5	35.29%
Combination Between SCoT and ISSR	ISSR	34	22	12	5	35.29%
	SCoT	31	17	14	9	45.16%
	Total	65	39	26	14	40.0%

Table S2. Similarity Index Using SCoT and ISSR analysis for six *Annona* sp.

species	1	2	3	4	5
1	1.0				
2	0.166	1.0			
3	0.233	0.538	1.0		
4	0.114	0.132	0.682	1.0	
5	0.589	0.238	0.875	0.530	1.0
6	0.308	0.283	0.845	0.413	0.378

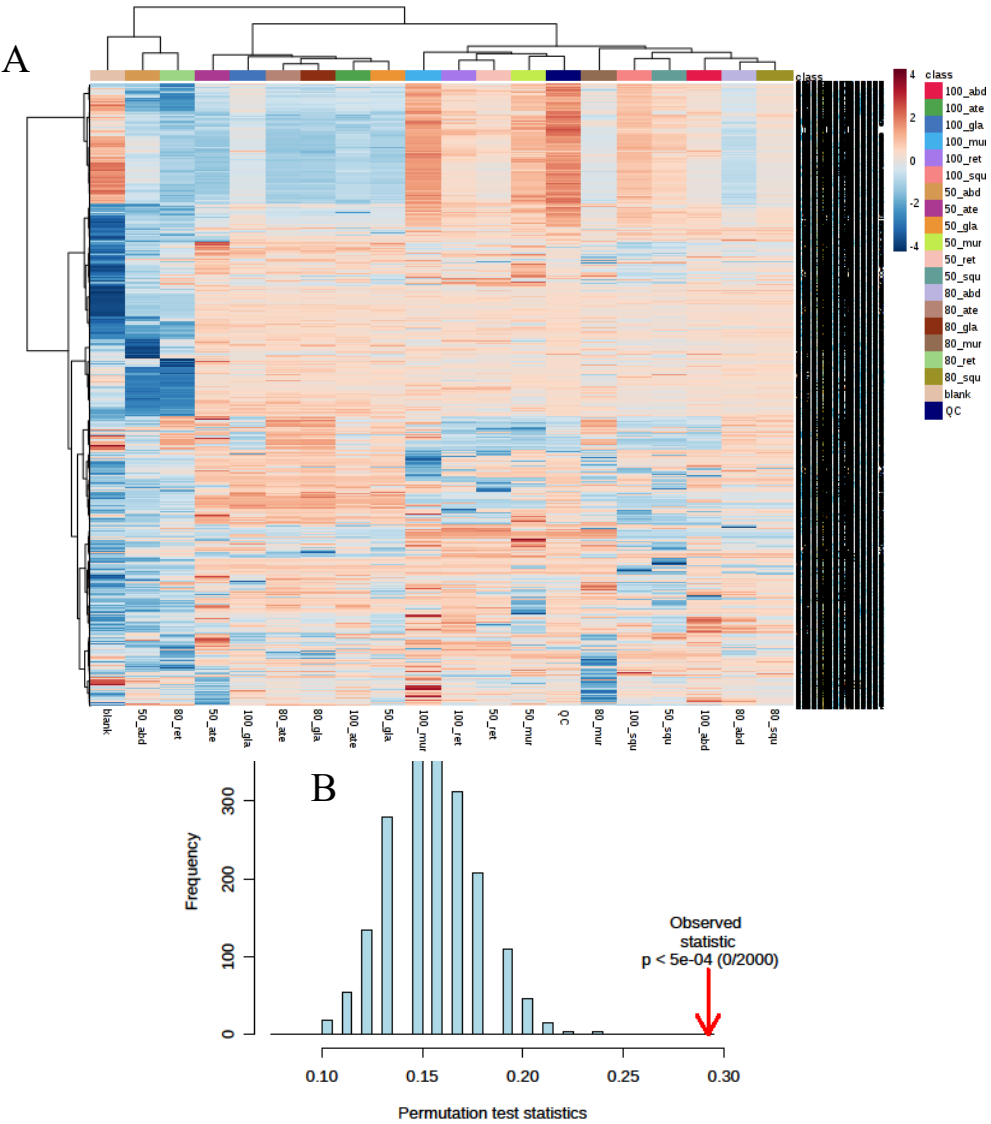
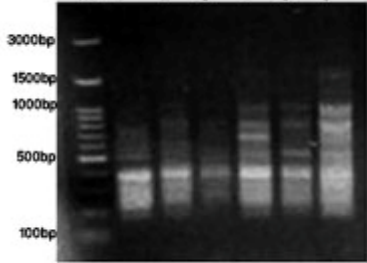


Figure S1. Bioinformatic pre-processing of LC/MS data resulted in detection of 67933 total signals in both modes in (A) the pearson correlation of heatmap of metabolic profiling of positive mode it appear different classification of compound showed in (sPLS-DA and PCA loading plot of different extract species. Hierarchical clustering of all signals from different *Annona* species showed in clusters positive mode. (B) metabolomics data positive 18 samples with three replicates Empirical p value.

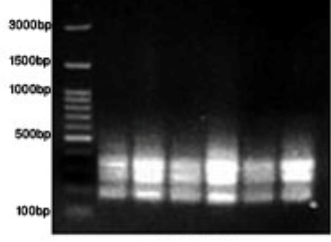
2. Taxonomic - DNA finger-printing for six *Annona* species grown in Egypt.

A. SCoT analysis

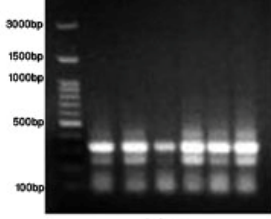
SCoT 1

 <p style="text-align: center;">SCoT</p>	Band No	M.W bp						
			1	2	3	4	5	6
	1	1740	0	0	0	1	0	1
	2	1160	0	1	0	1	1	1
	3	920	1	1	1	1	1	1
	4	700	1	0	0	1	0	0
	5	540	1	1	1	1	1	1
	6	380	1	1	1	1	1	1
	7	265	1	1	1	1	1	1
	8	235	1	0	0	1	1	1
Total			6	5	4	8	6	7

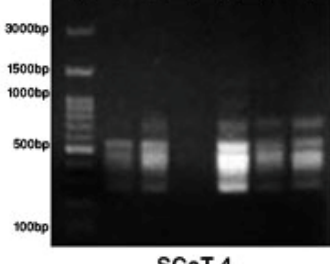
SCoT 2

 <p style="text-align: center;">SCoT 2</p>	Band No	M.W bp						
			1	2	3	4	5	6
	1	365	1	1	1	1	1	1
	2	245	1	1	1	1	1	1
	3	185	1	1	1	1	1	1
Total			3	3	3	3	3	3

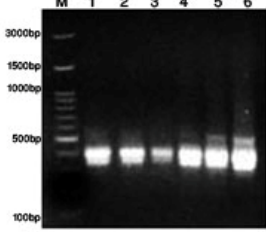
SCoT 3

 <p style="text-align: center;">SCoT</p>	Band No	M.W bp						
			1	2	3	4	5	6
	1	400	1	1	0	1	1	1
	2	330	1	1	1	1	1	1
	3	245	1	1	1	1	1	1
	4	175	1	1	1	1	1	1
	5	130	1	1	1	1	1	1
Total			5	5	4	5	5	5

SCoT 4

 <p style="text-align: center;">SCoT 4</p>	Band No	M.W bp						
			1	2	3	4	5	6
	1	1185	0	0	0	1	0	0
	2	840	0	0	0	1	0	0
	3	715	1	1	0	1	1	1
	4	550	1	1	1	1	1	1
	5	470	1	1	1	1	1	1
	6	380	1	1	1	1	1	1
	7	260	1	1	0	1	1	1
Total			5	5	3	7	5	5

SCoT 6

 <p>SCoT</p>	Band No	M.W bp						
			1	2	3	4	5	6
	1	565	1	1	0	1	1	1
	2	400	1	1	1	1	1	1
	3	345	1	1	1	1	1	1
	Total		3	3	2	3	3	3

SCoT 8

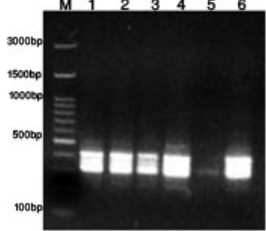
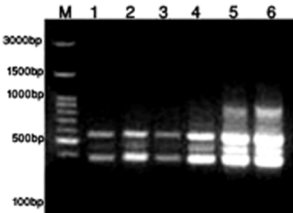
 <p>SCoT</p>	Band No	M.W bp						
			1	2	3	4	5	6
	1	500	1	1	0	1	0	1
	2	430	1	1	1	1	0	1
	3	370	1	1	1	1	0	1
	4	315	1	1	1	1	1	1
	5	245	1	1	1	1	0	1
	Total		5	5	4	5	1	5

Figure S2. DNA of primers in the studied 6 *Annona* samples by SCoT. As 1 = *A. atemoya*, 2 = *glabra*, 3 = *abdel razek*, 4 = *reticulata*, 5 = *squamosa*, then 6 = *muricata*.

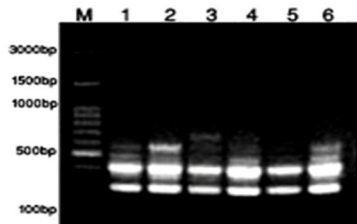
B. ISSR analysis

The ISSR banding profiles produced by the six 10-mer primers in the six samples of *Annona* species are illustrated in Fig.(5) and Tables 5 for primers 14A, 44B, HB-08, HB-10, HB-12 and HB-14.

14A

 <p>14A</p>	Band No	M.W bp	Samples					
			1	2	3	4	5	6
	1	835	0	0	0	0	1	1
	2	580	1	1	1	1	1	1
	3	475	1	1	1	1	1	1
	4	390	1	1	1	1	1	1
	Total		3	3	3	3	4	4

44A

 <p>44B</p>	Band No	M.W bp	Samples					
			1	2	3	4	5	6
	1	700	0	0	1	0	0	0
	2	580	1	1	1	1	1	1
	3	500	1	1	1	1	1	1
	4	400	1	1	1	1	1	1
	5	270	1	1	1	1	1	1
	Total		4	4	5	4	4	4

HB-8

M	1	2	3	4	5	6	Band No	M.W bp	Samples					
									1	2	3	4	5	6
3000bp							1	845	0	1	0	1	1	0
1500bp							2	715	0	1	0	1	1	0
1000bp							3	580	1	1	1	1	1	1
500bp							4	485	1	1	1	1	1	1
100bp							5	400	1	1	1	1	1	1
HB-8							Total		3	5	3	5	5	3

HB-10

M	1	2	3	4	5	6	Band No	M.W bp	Samples					
									1	2	3	4	5	6
3000bp							1	650	0	1	0	0	1	1
1500bp							2	570	0	1	0	0	0	0
1000bp							3	480	0	1	0	0	1	1
500bp							4	430	0	0	0	0	1	0
100bp							5	300	1	1	1	1	1	1
HB-10							6	265	1	1	1	1	1	1
							Total		2	5	2	2	5	4

HB-12

M	1	2	3	4	5	6	Band No	M.W bp	Samples					
									1	2	3	4	5	6
3000bp							1	1040	0	0	0	0	0	1
1500bp							2	900	0	0	0	0	0	1
1000bp							3	680	0	0	0	0	0	1
500bp							4	510	1	1	1	1	1	1
100bp							5	425	1	1	1	1	1	1
HB-12							6	365	1	1	1	1	1	1
							7	270	1	1	1	1	1	1
							8	230	1	1	1	1	1	1
							Total		6	6	6	6	6	6

HB-13

M	1	2	3	4	5	6	Band No	M.W bp	Samples					
									1	2	3	4	5	6
3000bp							1	910	1	1	1	1	1	1
1500bp							2	600	1	1	1	1	1	1
1000bp							3	480	1	1	1	1	0	0
500bp							4	400	1	1	1	1	1	1
100bp							5	290	1	1	1	1	1	1
HB-13							6	180	1	1	1	1	1	1
							Total		6	6	6	6	5	5

Figure S3. DNA of primers in the studied 6 Annona samples by ISSR. As 1 = *A. atemoya*, 2 = *glabra*, 3 = *abdel razek*, 4 = *reticulata*, 5 = *squamosa*, then 6 = *muricata*.

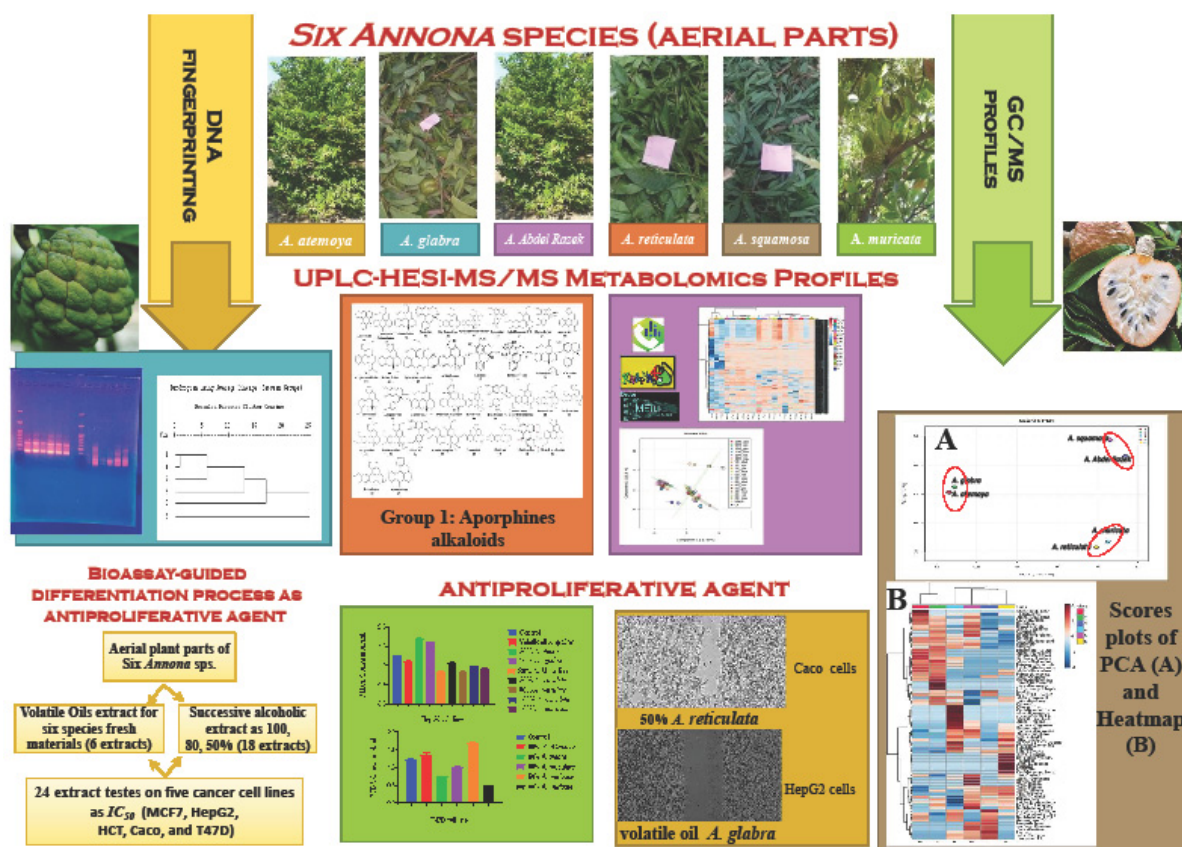


Figure S4. Role-bioassay-guided differentiation process of Six *Annona* cultivated in Egypt on anti-cancer therapy.