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Metabolite Profiling of Fruit and Seed Extracts of *Garcinia xanthochymus* using Rp-Hplc- Esi-Q-Tof-Ms and Progenesis Qi

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ABSTRACT

Natural products research is the most enormous field of research in terms of the amount of data and importance of information. Natural products discovery and metabolomics deals with a crucial mode of representation of the profile of biologically active metabolites. In this regard, the profiling of the chemical makeup of complex natural plant extracts necessarily requires employing sophisticated and advanced analytical methods like RP-HPLC–ESI-Q-TOF-MS as well as data mining and processing methods. The genus Garcinia (Clusiaceae) contains phenolic, flavonoids, xanthones, triterpenes, and benzophenones which have been reported for their significant biological properties. Due to its high content of secondary metabolites and its large domestic usage, we have developed a simple, rapid and precise method to characterize all the secondary metabolites using Reverse-Phase Ultra Performance Liquid Chromatography coupled to Electrospray Ionization Quadruple Time-of-Flight Mass Spectrometry (RP-HPLC–ESI-Q- TOF-MS) for the hydro-methanolic extract. A total of about 3443 secondary metabolites from the fruit and 3757 secondary metabolites from the seed were identified by the Progenesis-QI data analysis. Among these a total of 74 compounds from fruits and 86 polar compounds from seeds were manually identified using the mass error limit of < ± 5 ppm including the score less than 40. The unexplored bioactives belonging to the class of glycosides, flavones, xanthones, organic acids and other phenolic derivatives. Garcinia xanthochymus was found to contain significant number of diverse phytochemical components. These results indicate the profile of molecules present in G. xanthochymus and will be helpful for industries and researchers involved in isolation of their molecules of interest.

Keywords: *Garcinia xanthochymus*, RP-HPLC –ESI-Q-TOF-MS, Glycosides, Flavones, Xanthones.

INTRODUCTION

Garcinia xanthochymus has attracted the interest of researchers because of its very rich constituents like phenolics, xanthones, antioxidantents, flavonoids and are well known as secondary metabolites [1]. Because of increased consumption and health benefits, the identification of secondary metabolites is equally important. The genus Garcinia (Clusiaceae) is large family of shrubs or trees which is mainly distributed in Asia, Africa, and Polynesia [2]. In India, the genus is represented by about 30 species. Several phenolic, flavonoids, xanthones, triterpenes and benzophenones have been reported from this genus which have significant biological properties such as antibacterial [3-5], antioxidant [6], antiviral [7,8] and anticancer activities [9,10].

G. xanthochymus, commonly well known as gamboges, is a tree endemic to India growing 8-10 m in height [10,11]. The trees have dark green leaves and bear yellow fruits 6-7 cm in diameter with juicy, acidic, yellow pulp containing two seeds [11]. Traditionally, fruits of this plant are used in the treatment of diarrhea and dysentery [12]. Other important uses of this fruit are in the preparation of jams, preserves and vinegar, and also as a yellow fabric dye [13]. Previous phytochemical analysis of the leaves, seeds, and fruits of *G. xanthochymus* revealed the presence of two xanthochymol, benzophenones and isoxanthochymol, biflavonoids including fukugiside, fukugetin and volkensiflavone, the flavonoid vitexin, and a number of triterpenes, xanthones and lipids [11,14,15]. Biological activities, mainly like analgesic, antibacterial, antioxidant, antiviral, antiinflammatory, antiplasmodial and cytotoxic have been reported for previously isolated benzophenones and biflavonoids [14-19].
Characterization of components using an advanced and powerful technique becomes crucial to have an all-inclusive overview on the

metabolite profile. Analytical strategies in this regard which can analyze and profile the metabolites of the natural plant extracts have strong potential [20]. Advances in chromatographic techniques including LC-MS, GC-MS, and LC- NMR as well as data mining and processing methods has been instrumental in the field of natural product research. These tools collectively provide the development of metabolomics [20,21]. The present work focused on the metabolite profiling of *G. xanthochymus* using a newly developed comprehensive and reliable method by using High-Performance Liquid Chromatography coupled to Electrospray Ionization Quadropole-Time-of-Flight Mass Spectrometry (HPLC–ESI-Q-TOF-MS). Furthermore, the obtained results were subjected to Waters Progenesis QI Data analysis software for the identification of metabolites based on the high resolution mass data and isotope distribution of the compounds.

MATERIAL AND METHODS

Plant material and chemicals

G. xanthochymus was collected from different parts of Western Ghats, South-West Karnataka, India. The collected fruits cleansed with distilled water and dried. The seeds were removed from the fresh fruit using knife. The fresh fruit and the seeds were frozen in liquid nitrogen. The frozen fruits and seeds were grounded into powder using pestle and mortar. The powder was collected in a polythene bag and stored at -20°C until the use.

Formic Acid AR grade, HPLC grade Methanol and Acetonitrile (Merck, USA) were used as mobile phase solvent. Deionized water was purified by a reverse osmosis and ion exchange method (Milli-Q system, USA).

Preparation of extracts

The stored powder which served as the starting material was weighed (0.5 g) and subjected for extraction according to the previous methods [22]. Briefly, powder was mixed with 16 ml of 80: 20 (v/v) methanol/H_2O and sonicated for 30 min at room temperature. Then, the mixture was centrifuged for 15 min at 4000 rpm and the supernatant was decanted into a round bottom flask. Thereafter, the solvent was evaporated by using a rotary evaporator under vacuum at 40ºC and the dry residue was resolved in 0.5 ml of 80: 20 (v/v) methanol/H_2O. Finally, the extract was filtered through a 0.2 µm syringe filter and stored at −20ºC till analysis.

Chromatographic system and conditions

Analysis of metabolites of *G. xanthochymus* fruit and seed was done by the Waters ACQUITY UPLC (Waters corp., MS) quaternary pump equipped with column thermostat and the auto sampler. The HPLC column Phenomenex Gemini (100 mm x 4.6 mm, 3 µm, 110 Å USA) was set at 40°C, auto sampler was at 10°C and flow rate was 0.5 ml/min. The mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. The eluting conditions were used as follows: 0 min, 0% B; 10 min, 10% B; 15 min, 30% B; 30 min, 55% B; 40 min, 65% B; 50 min, 90% B; 56 min, 90%; 58 min, 0%; 65 min, 0%.

ESI-Q-TOF-MS analysis conditions

The UPLC system was connected to Quadrupole-Time-of-Flight (Synpat G2, Waters corp., USA) which is an orthogonally accelerated Q-TOF mass spectrometer, furnished with electrospray ionization source (ESI). Parameters for analysis were set using positive mode and the spectral range was set to 100-1500 m/z. The MS was optimized with following parameters: Polarity, ES+ ; Analyzer, High Resolution Mode; Capillary (1.8 kV), Source Temperature (150°C), Sampling Cone 40, Extraction Cone 4.0, Desolvation Temperature (200°C), Desolvation Gas Flow (500 l/H), Trap Collision Energy 4.0 and Nitrogen was used as carrier gas and Nitrogen-Argon were used as collision gas. The MS data was processed using Mass Lynx VMassLynx SCN781 software. The characterization of metabolites was done using Waters Progenesis QI Data analysis software based on the accurate mass and isotope distribution. Principal Component Analysis was performed to assess the interrelationship between the extract of fruit and seed.

RESULTS AND DISCUSSIONS

Selection of the compounds from fruit and seeds

Tables 1 and 2 provides the list of the tentatively selected compounds of fruit and seed extract of *G. xanthochymus.* About 3443 compounds were found from the fruit and about 3757 compounds from the seed. The tentative list was constructed based on the deconvolution of UPLC-ESI-Q-TOF-MS using the positive ionization mode and the isotope distribution. Tentatively, 74 out of 3443 from fruit and 86 out of 3757 from seeds were manually selected based on the least mass error (> ± 5ppm) and p-value (score less than 50). The compounds were summarized along peak number, retention time, observed m/z, empirical formula, mass error, and mSigma score.

Table 1: Proposed secondary metabolites obtained from fruit extract using UPLC-ESI-Q- TOF-MS and Progenesisi-QI

Peak No.	Rt (min)	m/z	Formula	Mass Error (ppm)	mSigma Score	Proposed compound
1	1.69	290.8476	C_4Cl_6	4	22.2	Hexachlorobutadiene
2	1.79	248.1131	$C_{10}H_{17}NO_6$	1	39.6	Linamarin
3	2	184.0731	$C_{10}H_{11}NO$	-0.9	38.4	Tryptophol
4	2.08	385.1341	$C_7H_{12}O^6$	-0.4	39.3	D-(-)-Quinic acid
5	2.13	319.1018	$C_{17}H_{19}ClN_2S$	-0.6	31.7	Chlorpromazine

6	2.15	325.1131	$C_{12}H_{22}O_{11}$	0.6	39.3	Lactose
7	2.18	292.1029	$C_{11}H_{19}NO_9$	0.6	37.1	N-Acetylneuraminic acid
8	2.32	325.1132	$C_{12}H_{22}O_{11}$	0.7	39	beta-Gentiobiose
9	2.37	301.0554	$C_{16}H_{13}ClN_2S$	-1.3	32.5	sulazepam
10	4.14	193.0342	$C_6H_8O_7$	-0.3	39.4	L-threo-Hexo-2,3-diulosonic acid
11	7.58	109.0282	$C_6H_6O_3$	-1.9	38.2	Maltol
12	10.96	163.0382	$C_5H_{11}NO_4P+$	-2.7	38	[(3S)-3-Amino-3-carboxypropyl](hydroxymethyl)oxophosphon ium
13	12.82	323.0367	$C_{12}H_{19}O_2PS_3$	2.8	34.7	Merdafos
14	13.21	147.0435	$C_9H_8O_3$	-3.1	37.3	Cinnamic acid, 2-hydroxy-, cis-
15	14.59	377.1449	$C_{20}H_{24}ClN_2OS$	0.2	33.5	N-(2-((3-(Dimethylamino)propyl)thio)phenyl)-3- phenyl-2-propenamide Monohydrochloride
16	15.02	301.1793	$C_{19}H_{26}O_4$	-1.6	39.3	Ubiquinone 2
17	15.02	347.1849	$C_{20}H_{28}O_6$	-1.1	39.2	(3aR,4S,7aR)-6-(Hydroxymethyl)-5-[(2S)-5- hydroxy-2-pentanyl]-3-methylene-2-oxo-2,3,3a,4,7,7a-hexahydro-1-benzofuran-4-yl (2Z)-2-methyl-2-butenoate
18	15.19	331.1896	$C_{20}H_{28}O_5$	-2.3	38.8	gibberellin A53
19	15.19	285.1842	$C_{19}H_{26}O_3$	-2.4	38.4	Formestane
20	15.93	315.1941	$C_{20}H_{28}O_4$	-4.3	36.7	(1alpha,4aalpha,4bbeta,10beta)-1,4a-Dimethyl-8-methylenegibbane-1,10- dicarboxylic acid
21	16.59	285.184	$C_{19}H_{26}O_3$	-3	36.4	Formestane
22	16.91	437.0701	$C_{13}H_{19}N_4O_{12}P$	-0.6	38.8	Succino-AICAR
23	17.42	363.1789	$C_{16}H_{22}N_6O_4$	2	38.3	Protirelin
24	17.66	719.1552	$C_{36}H_{30}O^1_6$	-2.1	36.4	(+-)-Fukugiside
25	17.68	557.1063	$C_{25}H_{26}O_{12}$	1.4	33	(6aR,11aR)-9-Methoxy-6a,11a-dihydro-6H- [1]benzofuro[3,2-c]chromen-3-yl 6-O- (carboxyacetyl)-beta-D-glucopyranoside
26	18.74	261.0382	$C_{13}H_8O_6$	-4.3	38.4	1,2,6,8-Tetrahydroxyxanthone
27	19.62	285.1839	$C_{19}H_{26}O_3$	-3.4	37.3	Formestane
28	19.91	274.2732	$C_{16}H_{35}NO_2$	-3.1	38.6	(2S,3R)-2-Amino-1,3-hexadecanediol
29	20.96	555.0905	$C_{25}H_{24}O_{12}$	1.1	37.9	Cynarine
30	21.13	423.0692	$C_{14}H_{20}N_6O_5S$	-0.7	38.2	S-Adenosyl-L-homocysteine
31	22.75	539.0947	$C_{30}H_{18}O_{10}$	-4.9	38.3	Amentoflavone
32	22.87	245.0434	$C_{13}H_{10}O_6$	-4.1	38.2	Laguncurin
33	23.2	541.1113	$C_{15}H_{10}O_5$	-3.1	36.2	Emodin
34	24.17	539.0955	$C_{30}H_{18}O_{10}$	-3.3	38.7	amentoflavone
35	25.17	485.2161	$C_{25}H_{33}NaO_8$	3.1	34.2	Corlan

36	26.35	229.0484	$C_{13}H_{10}O_5$	-4.5	38.5	Isopimpinellin
37	27.62	581.2735	$C_{24}H_{40}N_2O_{10}$	-0.6	37.9	Terbutaline sulfate
38	28.89	329.1004	$C_{14}H_{18}N_4O_3$	-2.1	38.8	Trimethoprim
39	29.07	273.0381	$C_{10}H_{13}ClN_2O$	1.5	34.6	Metoxuran
40	29.28	329.1001	$C_{14}H_{18}N_4O_3$	-3.4	38.7	Benomyl
41	29.89	313.1043	$C_{14}H_{12}N_6O_3$	-0.1	37.9	p-[(2-Amino-4-hydroxy-6- pteridylmethyl)amino]benzoic Acid
42	30.28	441.259	$C_{25}H_{38}O_5$	-4.6	37.7	(+)-Simvastatin
43	30.58	585.303	$C_{33}H_{44}O_9$	-4.9	36.8	(6Z)-4,9,11-Triacetoxy-3a-hydroxy- 2,5,8,8,12-pentamethyl-2,3,3a,4,5,8,9,10,11,13a-decahydro-1H-cyclopenta[12]annulen-1-yl benzoate
44	30.82	329.0995	$C_{16}H_{18}O_6$	-0.3	38	(2S)-7-(Hydroxymethyl)-2-(2-hydroxy-2- propanyl)-4-methoxy-2,3-dihydro-5H- furo[3,2-g]chromen-5-one
45	32.63	343.1158	$C_{17}H_{19}NaO_6$	1.8	38.8	Mycophenolate sodium
46	33.63	557.1049	$C_{25}H_{26}O_{12}$	-1.4	38.2	(6aR,11aR)-9-Methoxy-6a,11a-dihydro-6H-[1]benzofuro[3,2-c]chromen-3-yl 6-O- (carboxyacetyl)-beta-D-glucopyranoside
47	38.62	549.2812	$C_{28}H_{40}N_2O_9$	0.9	36.8	3-[(3-Formamido-2-hydroxybenzoyl)amino]- 8-hexyl-2,6-dimethyl-4,9-dioxo-1,5- dioxonan-7-yl 3-methylbutanoate
48	39.68	451.2086	$C_{16}H_{31}N_6O_7P$	4.7	37.5	Valyl-N-[(1S,2Z)-1-carboxy-4-phosphono-2- buten-1-yl]-L-argininamide
49	39.75	551.2962	$C_{27}H_{38}N_2O_8$	-0.2	35.7	NPAB
50	40.44	637.3689	$C_{33}H_{44}N_6O_5$	-3.2	37	N1-{(1S,2R)-1-Benzyl-3-[[(tert-butylamino)carbonyl](isobutyl)amino]-2- hydroxypropyl}-N2-(quinolin-2-ylcarbonyl)- L-aspartamide
51	42.78	467.2398	$C_{26}H_{36}O_6$	-1.3	38.5	Prednival
52	42.99	447.2708	$C_{24}H_{40}O_5$	-3.6	37.1	3a,6a,7a-Trihydroxy-5b-cholan-24-oic acid
53	44.48	411.177	$C_{22}H_{28}O_6$	-2.2	39.1	Surinamensin
54	45.13	639.324	$C_{36}H_{50}O_7$	-4.8	36.1	1-O-[(2E,4E,6E,8E,10E,12E,14E,16E,18E)-2,6,10,15,19,23-Hexamethyl- 2,4,6,8,10,12,14,16,18,22-tetracosadecaenoyl]-beta-D-glucopyranose
55	45.43	619.3585	$C_{32}H_{46}N_2O_8$	-0.8	39	[(1alpha,6beta,14alpha,16beta)-20-Ethyl-7,8- dihydroxy-1,6,14,16-tetramethoxyaconitan-4- yl]methyl aminobenzoate
56	46.31	601.3478	$C_{31}H_{45}NO_8$	-0.9	36.3	(1S,7aR)-Hexahydro-1H-pyrrolizin-1- ylmethyl 4-(beta-D-glucopyranosyloxy)-3,5-bis(3-methyl-2-buten-1-yl)benzoate

57	46.58	604.3663	$C_{38}H_{50}O_6$	-4.8	33.1	Cycloxanthochymol
58	47.06	583.3371	$C_{31}H_{43}NO_7$	-1.3	36.8	(1S,3S,4S,5S,7S,9S,14S,15R,16S,17R)-3,4,7-Trihydroxy-9,11-dimethyl-19-methylene-11-azahexacyclo[12.3.2.0~1,13~.0~4,9~.0~5,12~.0~5,17~]nonadecane-15,16-diyl (2E,2'E)bis(2-methyl-2-butenoate)
59	47.47	409.161	$C_{20}H_{28}O_6$	3.3	38.7	(3aR,6aS,7R,8R,10R,10aR,10bS)-3a,7,8,10a-Tetrahydroxy-5-(hydroxymethyl)-8- isopropenyl-2,10-dimethyl-4,6a,7,8,9,10,10a,10b-octahydrobenzo[e]azulen-3(3aH)-one
60	47.48	465.2233	$C_{17}H_{33}N_6O_7P$	2.6	36	Isoleucyl-N-[(1S,2Z)-1-carboxy-4- phosphono-2-buten-1-yl]-L-argininamide
61	47.68	601.348	$C_{36}H_{50}O_6$	-3.4	37.8	(11beta)-11,17-Dihydroxy-3,20-dioxopregna- 1,4-dien-21-yl (2E,6E)-3,7,11-trimethyl-2,6,10-dodecatrienoate
62	47.96	523.2988	$C_{21}H_{38}N_4O_9$	2.9	38.7	(6R)-5-Acetamido-2,6-anhydro-4- carbamimidamido-3,4,5-trideoxy-6-[(1R,2R)- 2-hydroxy-1-methoxy-3-(octanoyloxy)propyl]-L-threo-hex-2-enonic acid hydrate (1:1)
63	48.24	657.3352	$C_{34}H_{50}C_{l2}N_4O_3$	4.7	29.1	N-(2,6-Dimethylphenyl)-2-(tetrahydro-1H- pyrrolizin-7a(5H)-yl)acetamide hydrochloride hydrate (2:2:1)
64	48.63	625.3447	$C_{31}H_{55}O_9P$	-4.8	36.8	1-O-(Hydroxy{[(6Z,10E,14E)-3,7,11,15,19-pentamethyl-6,10,14,18-icosatetraen-1- yl]oxy}phosphoryl)-beta-D-glucopyranose
65	48.8	641.3333	$C_{18}H_{24}O_5$	2	36.3	b-Zearalenol
66	49.71	991.6204	$C_{55}H_{92}O_{11}P_2$	1.7	37.7	1-O- [{[(2Z,6Z,10Z,14Z,18Z,22Z,26Z,30Z,34E)-3,7,11,15,19,23,27,31,35,39-Decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaen-1-yl]oxy}(hydroxy)phosphoryl]-5-O- phosphono-beta-D-ribofuranose
67	49.99	465.2231	$C_{17}H_{33}N_6O_7P$	2	37.7	Isoleucyl-N-[(1S,2Z)-1-carboxy-4-phosphono-2-buten-1-yl]-L-argininamide
68	51.1	329.1718	$C_{15}H_{26}N_2O_7$	3.2	38.6	(2R,4aS,5aR,6S,7S,8R,9S,9aR,10aS)-4-Methoxy-2-methyl-6,8-bis(methylamino)- 5a,6,7,8,9,9a-hexahydro-2H-pyrano[2,3-b][1,4]benzodioxine-4a,7,9(10aH)-triol
69	51.86	413.2641	$C_{22}H_{39}NaO_4$	0.7	38	Sodium (2E)-4-(octadecyloxy)-4-oxo-2-butenoate
70	52.05	507.3039	$C_{21}H_{38}N_4O_8$	3	38.5	N-[(2S,3R)-3-Amino-2-hydroxy-5-methylhexanoyl]-L-valyl-L-valyl-L-aspartic acid
71	52.71	603.3614	$C_{38}H_{50}O_6$	1.9	16	Guttiferone E/Guttiferone H

72	53.29	603.3506	$C_{38}H_{50}O_6$	4.8	32	Xanthochymol
73	54.55	413.2624	$C_{22}H_{39}NaO_4$	-3.7	38.8	Sodium (2E)-4-(octadecyloxy)-4-oxo-2-butenoate
74	55.51	587.3686	$C_{33}H_{56}O_6$	-4.1	36.9	(3beta)-Cholest-5-en-3-yl beta-D-glucopyranoside

Table 2: Proposed secondary metabolites obtained from seed extract using UPLC-ESI-Q- TOF-MS and Progenesisi-QI

Peak No.	Rt (min)	m/z	Formula	Mass Error (ppm)	mSigma Score	Proposed compounds
1	1.52	151.0305	C_9H_9ClO	-2.5	35.6	Chlorindanol
2	1.79	248.1052	$C_{11}H_{17}ClNO_3$	3.9	34	Pressomin
3	2.08	421.1417	$C_{20}H_{24}N_2O_6S$	-1.7	37.3	10-Oxo-8-azatricyclo[5.3.1.0~3,8~]undec-5-yl 1H-indole-3-carboxylate methanesulfonate (1:1)
4	2.32	343.1129	$C_8H_{19}N_6O_7P$	2.3	38.4	(1S,2R,3S,4S,5R,6S)-2,4-Dicarbamimidamido-3,5,6-trihydroxycyclohexyl dihydrogen phosphate
5	4.06	210.0547	$C_{13}H_9NO_3$	-1.3	36.9	2-Nitro-9H-fluoren-9-ol
6	6.92	208.0538	$C_9H_9N_3OS$	-0.4	38.3	Benzthiazuron
7	9.3	208.0541	$C_9H_9N_3OS$	1	36.8	Benzthiazuron
8	10.39	163.0339	$C_5H_{10}O_3$	-2	38.8	5-Hydroxypentanoic acid
9	13.21	339.0968	$C_{19}H_{18}O_3$	0.2	39.1	2-Butyl-3-(4-hydroxybenzoyl)-benzofuran
10	13.75	163.0338	$C_5H_{10}O_3$	-3.4	38.7	5-Hydroxypentanoic acid
11	14.5	207.0586	$C_{10}H_{10}N_2OS$	-0.2	36.8	N-Hydroxy-2-(1H-indol-3-yl)ethanethioamide
12	14.6	377.1338	$C_{19}H_{24}O_5$	0.7	39.4	(1R,2R,5R,8R,9S,10R,13R)-13-Hydroxy-11-methyl-6-methylene-16-oxo-15-oxapentacyclo[9.3.2.1~5,8~.0~1,10~.0~2,8~]h eptadecane-9-carboxylic acid
13	15.02	147.0394	$C_4H_6N_2O_4$	-4.2	38.5	3-(Carbamoylamino)-3-oxopropanoic acid
14	15.19	331.1799	$C_{22}H_{24}N_2O_2$	-1.7	38.1	Acrivastine
15	15.55	415.1443	$C1_4H_9NO$	0.4	39.2	2H-Dibenzo[b,f]azepin-2-one
16	15.8	331.18	$C_{22}H_{24}N_2O_2$	-1.3	38.1	Acrivastine
17	15.92	233.11	$C_{13}H_{16}N_2S$	0.7	38	Rezatomidine
18	15.95	331.1797	$C_{22}H_{24}N_2O_2$	-2.2	38	Acrivastine
19	16.07	433.0989	$C_{19}H_{20}N_2O_7$	1.7	38.9	Aranidipine
20	16.41	261.0311	$C_{15}H_{10}O_2$	-0.7	36.8	Isoflavone
21	16.69	701.4036	$C_{22}H_{26}N_2O_2$	-3.7	36.7	Vinpocetine
22	16.93	351.2055	$C_{22}H_{28}N_2O_3$	-3.3	37	Hirsutine
23	17.1	529.175	$C_{24}H_{32}O_{11}S$	2.4	36.8	(17-beta)-3-(Sulfooxy)estra-1,3,5(10)-trien-17- yl beta-D-glucopyranosiduronic acid
24	17.64	719.1376	$C_{36}H_{30}O_{16}$	0.9	15.7	(+-)-Fukugiside
25	17.93	535.2357	$C_{20}H_{40}N_4O_{10}$	-3.9	37.4	Geneticin
26	18.15	543.1903	$C_{28}H_{30}N_6OS$	-2.1	36.4	Masitinib

27	18.15	397.1366	$C_{16}H_{22}N_4O_9$	3	38.2	(3R)-3-[(3R,5S)-7-Oxo-4-oxa-1-azabicyclo[3.2.0]hept-3-yl]seryl-(3S)-3- [(3R,5S)-7-oxo-4-oxa-1-azabicyclo[3.2.0]hept-3-yl]serine
28	18.41	397.1001	$C_{18}H_{18}N_2O_7$	-1.5	35.7	(2S)-4-[(E)-2-{[(1S)-1-Carboxy-2-(4-hydroxyphenyl)ethyl]amino}vinyl]-2,3- dihydro-2,6-pyridinedicarboxylic acid
29	18.71	235.0892	$C_{11}H_{10}N_2S$	-3.6	37.5	.alpha.-Naphthylthiourea
30	18.98	533.0761	$C_{14}H_{26}N_4O_{11}P_2$	-4.9	37.4	Citicoline
31	19.1	541.0962	$C_{22}H_{24}ClN_2O_9$	0.4	34.4	Tetran
32	19.52	533.0757	$C_{13}H_{11}ClO_4$	-1.3	34.3	3-[5-(4-Chlorophenyl)-2-furyl]-3-hydroxypropanoic acid
33	19.98	411.1158	$C_{19}H_{20}N_2O_7$	-1.2	37.9	Aranidipine
34	20.35	277.1347	$C_{18}H_{18}N_2O_2$	3.9	37.6	2-[3-Ethyl-5-(4-methoxyphenyl)-1H-pyrazol-4-yl]phenol
35	21.05	557.0904	$C_{24}H_{22}O_{14}$	0.4	38.1	2-(3,4-Dihydroxyphenyl)-5-hydroxy-4-oxo-4H-chromen-7-yl 6-O-(carboxyacetyl)-beta-D- glucopyranoside
36	22.94	543.1115	$C_{23}H_{24}N_6O_7S_2$	0	36.7	sulfamazone
37	24.14	539.0804	$C_{30}H_{18}O_{10}$	0.4	39	Amentoflavone
38	24.61	549.3257	$C_{26}H_{44}O_{10}$	-2.4	37.5	3-O-(alpha-L-olivosyl)oleandolide
39	24.9	549.3255	$C2_6H_{44}O_{10}$	-2.9	37.3	3-O-(alpha-L-olivosyl)oleandolide
40	26.36	527.0325	$C_{12}H_{26}O_{16}P_2$	-0.5	36.7	1-O-[(3-{[(2,3-Dihydroxypropoxy)(hydroxy)phosphoryl]oxy}-2-hydroxypropoxy)(hydroxy)phosphoryl]- alpha-D-glucopyranose
41	27.64	457.24	$C_{25}H_{38}ClN_2O$	4.2	33.8	Bunamidine hydrochloride
42	28.23	417.2489	$C_{22}H_{32}N_4O_4$	-0.6	39.2	Tonapofylline
43	29.64	587.3004	$C_{35}H_{40}N_4O_3$	4.4	33.3	Methyl (2alpha)-14-[(3aR,6R)-2,3,3a,4,5,6-hexahydro-1H-indolo[3,2,1- de][1,5]naphthyridin-6-yl]-13- hydroxyibogamine-18-carboxylate
44	30.3	859.5052	$C_{43}H_{70}O_{15}$	0.3	38.9	(3beta,6alpha,9beta,16beta,20R,24S)-3-[(2-O- Acetyl-beta-D-xylopyranosyl)oxy]-16,25- dihydroxy-20,24-epoxy-9,19-cyclolanostan-6-yl beta-D-glucopyranoside
45	32.02	825.4777	$C_{38}H_{68}N_2O_{13}S$	-0.1	38	(3R,4S,5S,6R,7R,9R,11R,12R,13S,14R)-6-{[(2S,3R,4S,6R)-4-(Dimethylamino)-3- hydroxy-6-methyltetrahydro-2H-pyran-2-yl]oxy}-14-ethyl-7,12,13-trihydroxy-4-{[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6- dimethyltetrahydro-2H-pyran-2-yl]oxy}-3,5,7,9,11,13- hexamethyloxacyclotetradecane-2,10-dione thiocyanate (1:1)
46	32.85	579.0715	$C_{24}H_{22}O_{14}$	-1.2	38.5	2-(3,4-Dihydroxyphenyl)-5-hydroxy-4-oxo-4H-chromen-7-yl 6-O-(carboxyacetyl)-beta-D- glucopyranoside
47	33.08	547.3096	$C_{35}H_{40}N_4O_3$	4.9	32.2	Methyl (2alpha)-14-[(3aR,6R)-2,3,3a,4,5,6-hexahydro-1H-indolo[3,2,1-de][1,5]naphthyridin-6-yl]-13- hydroxyibogamine-18-carboxylate

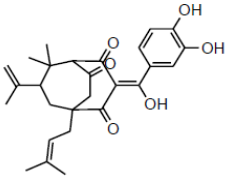
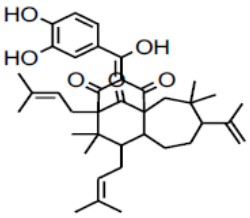
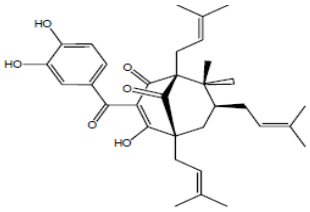
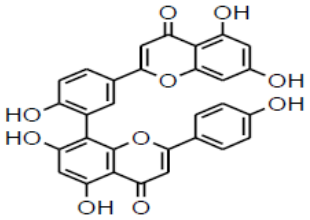
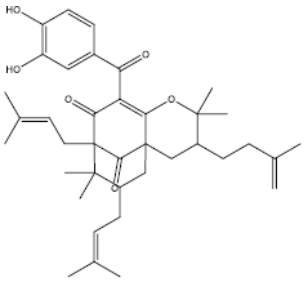
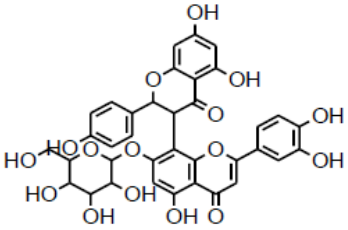
48	35.46	423.2399	$C_{22}H_{27}N_3O_3$	2.2	38.7	tryprostatin A
49	36	553.2955	$C_{33}H_{43}FO_7$	-0.8	33.5	Dexamethasone cipecilate
50	36.96	419.2656	$C_{22}H_{36}N_4O_5$	0.7	38.4	Cipemastat
51	37.11	401.27	$C_{13}H_{16}N_2$	0	38.9	2-[(1S)-1-(2,3-Dimethylphenyl)ethyl]-1H-imidazole
52	37.92	541.2951	$C_{28}H_{40}Cl_2N_2O_2$	-1.4	28.5	Biallylamicoldihydrochlorde
53	38.55	417.2501	$C_{22}H_{34}N_4O_5$	1.1	35.1	(3S)-3-[({(3R)-1-[3-(4-Piperidinyl)propanoyl]- 3-piperidinyl}carbonyl)amino]-3-(3-pyridinyl)propanoic acid hydrate (1:1)
54	39.29	441.2493	$C_{12}H_{16}N_2O_2$	-0.8	37.1	N~2~-Acetyl-N,N-dimethyl-N~2~-phenylglycinamide
55	39.77	433.2446	$C_{20}H_{29}N_3O_5$	0.1	39.5	N-[1-(1-Piperidinyl)-2-propanyl]-N-(2- pyridinyl)propanamide (2E)-2-butenedioate (1:1)
56	39.83	541.0957	$C_{24}H_{22}O_{13}$	0.9	38.4	5-Hydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl 6-O-(carboxyacetyl)-beta-D- glucopyranoside
57	40.72	533.3301	$C_{21}H_{41}N_5O_8$	1.6	38.2	(1R,2S,3S,4R,6S)-4,6-Diamino-3-{[3-deoxy-4-C-methyl-3-(methylamino)-beta-L- arabinopyranosyl]oxy}-2-hydroxycyclohexyl 2-acetamido-6-amino-2,3,4,6-tetradeoxy-alpha-D-erythro-hexopyranoside
58	41.32	431.2652	$C_{23}H_{36}N_4O_5$	-0.3	38.7	N-Acetylleucylleucyltyrosinamide
59	41.71	347.1742	$C_{22}H_{22}N_2O_2$	-1.9	38.9	Cintazone
60	41.71	523.2864	$C_{26}H_{44}O_9$	-1.5	39	Mupirocin
61	42.9	347.1736	$C_{22}H_{24}N_2O_3$	-4.9	38.2	4-(4,4-Dimethyl-3-oxopentyl)-1,2-diphenyl-3,5-pyrazolidinedione
62	42.93	413.2547	$C_{22}H_{36}O_7$	4	37.2	(14R)-3beta,5,6beta,10,16-pentahydroxygrayanotoxan-14-yl acetate
63	43.6	483.2946	$C_{26}H_{42}O_8$	-1.3	39.3	(4R,5R,6R,6aS,9S,9aE,10aR)-5-Hydroxy-9-(hydroxymethyl)-3-isopropyl-6,10a-dimethyl- 1,2,4,5,6,6a,7,8,9,10a-decahydrodicyclopenta[a,d][8]annulen-4-yl alpha-D-glucopyranoside
64	44.32	537.3007	$C_{18}H_{20}O_2$	1.4	37.3	Equilin
65	45.09	453.2461	$C_{27}H_{32}O_6$	3.1	38.8	Gambogenone
66	45.18	524.2886	$C_{24}H_{47}NO_{10}S$	-0.2	35.9	(2S,3R,4E)-2-Amino-3-hydroxy-4-octadecen-1-yl 6-O-sulfo-beta-D-galactopyranoside
67	46.5	539.3157	$C_{27}H_{42}N_2O_5S$	1.6	37.7	Ixabepilone
68	47.92	523.2855	$C_{26}H_{44}O_9$	-4.5	37.6	Mupirocin
69	48.24	417.25	$C_{22}H_{32}N_4O_4$	0.8	33.2	Tonapofylline
70	48.38	401.2555	$C_{23}H_{30}NO_3+$	0	38.2	N-Isopropyl-N-methyl-N-{2-[(9H-xanthen-9-ylcarbonyl)oxy]ethyl}-2-propanaminium
71	48.56	555.3108	$C_{26}H_{43}NO_7S$	1.9	38.3	N-[(3alpha,5beta)-24-Oxo-3-(sulfooxy)cholan-24-yl]glycine

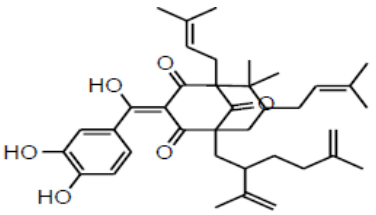
72	48.88	533.2735	$C_{29}H_{40}O_9$	-1.8	36.2	(1R,3aS,3bR,5aS,6aR,7aS,9R,11R,11aS,12aR,13aR,13bS,15aR)-3a,11,11a-Trihydroxy- 9,15a-dimethyl-1-(5-oxo-2,5-dihydro-3-furanyl)icosahydro-7aH,13aH- cyclopenta[7,8]phenanthro[2,3-b]pyrano[3,2- e][1,4]dioxin e-13a-carbaldehyde
73	49.06	535.2357	$C_{33}H_{42}O_6$	-3.9	37.4	Aristophenone A
73	48.92	601.3328	$C_{25}H_{46}N_8O_{10}$	3.9	35.1	(2S)-2-({[(2S)-3-[(4R)-2-Amino-4,5-dihydro-1H-imidazol-4-yl]-1-{[(1S,2S,3R,4S,5S,6S)-4-{[(2R,3S,5S,6R)-3,5-diamino-6-methyltetrahydro-2H-pyran-2-yl]oxy}-2,3,5,6-tetrahydroxycyclohexyl]amino}-1- oxo-2-propanyl]carbamoyl}amino)-4-methylpentanoic acid
74	49.09	603.3629	$C_{31}H_{55}O_9P$	-4.6	37.3	1-O-(Hydroxy{[(6Z,10E,14E)-3,7,11,15,19-pentamethyl-6,10,14,18-icosatetraen-1-yl]oxy}phosphoryl)-beta-D-mannopyranose
75	49.59	277.1329	$C_{18}H_{16}N_2O$	2.3	38.4	Sudan II
76	49.94	359.2098	$C_{19}H_{32}N_2O_2$	1	38.9	Camylofin
77	49.94	415.2707	$C_{22}H_{38}O_7$	4.1	37.4	Quicifal
78	50.37	537.3007	$C_{18}H_{20}O_2$	1.4	30.4	1-Ethyl-2-(4-hydroxyphenyl)-3-methyl-5-indanol
79	51.76	599.375	$C_{40}H_{48}N_4O_2$	0.8	36.4	Methyl (3beta,5alpha,19E)-3-[(2alpha)-ibogamin-13-yl]vobasan-17g-oate
80	52.03	273.1396	$C_{19}H_{16}N_2$	3.6	38.1	sempervirene
81	52.7	603.3614	$C_{38}H_{50}O_6$	0.8	38.5	isoxanthochymol
82	53.18	923.5005	$C_{48}H_{76}O_{18}$	0.7	36.1	Colubrin
83	53.25	345.1947	$C_{23}H_{26}N_2O_2$	-4	37.8	Benzetimide
84	53.79	277.134	$C_{18}H_{16}N_2O$	1.8	38.8	Sudan II

Characterization of the compounds using MS data in comparison with the database

The compounds detected were tentatively characterised by means of MS data, together with the interpretation of the observed m/z spectra in comparison with those found in the literature. The knowledge of previously studied phytochemicals from the same genus or same plant has also been employed in sorting the list. Databases such as Pubchem (https://pubchem.ncbi.nlm.nih.gov/), ChemSpider (http://www.chemspider.com) and SciFinder Scholar (https://www.scifinder.cas.org) were employed during the identification and cross verification of the compounds. The initial molecules in the fruit extract between peak 1-20 appeared on the list were that of glycosides, organic acids, aromatic alcohols and sugar derivatives. Some of these molecules viz., Linamarin, Chlorpromazine, Sulazepam, have been reported for their biological properties [23,24]. The next set of molecules between the peak 21-40 include (+-)-Fukugiside, 1,2,6,8-Tetrahydroxyxanthone, Amentoflavone which have been extensively been studied in the *Garcinia* species [11,25]. The results are helpful for rapid purification of these components using the parameters applied during the UPLC. Other than the previously identified molecules the peaks 21-40 also included glucopyranosides, flavones and some organic acids. Peaks 71 and 72 were proposed to be that of the wellknown signature molecules of *G. xanthochymus* which are Guttiferone E/Guttiferone H and Xanthochymol respectively [11,14]. The peaks of seeds contained unidentified class of molecules of some benzofurans, glucopyranosides, and substituted pentanoic acids; and a huge array of characterized molecules viz., Acrivastine, Aranidipine, Isoflavone, Vinpocetine, Hirsutine, Geneticin, Citicoline having biological activities were proposed to be the constituents based on the results obtained [26,27]. The previously identified molecules of the *Garcinia* species include (+-)-Fukugiside, Amentoflavone, Gambogenone, Aristophenone A, Isoxanthochymol at peaks 24, 37, 65, 73, 81 were found and conferred to be the signature molecules [11,25,28]. Table 3 includes the characterized molecules with specific biological activities from the fruits and seeds of *G. xanthochymus* which have been extensively been studied. Base peak chromatograms (Figures 1 and 2) of the fruit and seed extracts of *G. xanthochymus*, respectively shows the array of metabolites present in the extracts. The biplot generated by principal component analysis (Figures 3 and 4) illustrates the identical nature of the compounds between fruit and seed extracts. The overall triplicate data can be visually assessed by the PCA.

Table 3: Some of the characterized bioactives of *Garcinia xanthochymus*

S. No.	Plant Part	Molecule	Structure	Activities
1	Seed	Gambogenone (PubChem CID:72763153)		Antioxidant [11]
2	Fruit	Guttiferone H (PubChem CID:72783686)		Antioxidant [11] (Baggett et al., 2005)
3	Fruit	Aristophenone A (PubChem CID:10907594)		Anticancer, Antioxidant [7,11]
4	Fruit and seed	Amentoflavone (PubChem CID:5281600)		Anticancer, Antioxidant [11,14]
5	Fruit	Cycloxanthochymol (PubChem CID:23244759)		Antibacterial (Iinuma et al., 1996)
6	Seed	(+-)-Fukugiside (PubChem CID:11968471)		Anti-Helicobacter pylori (Nontakham et al., 2014)

7	Fruit	Xanthochymol (PubChem CID:9938674)		Anticancer (Baggett et al., 2005)

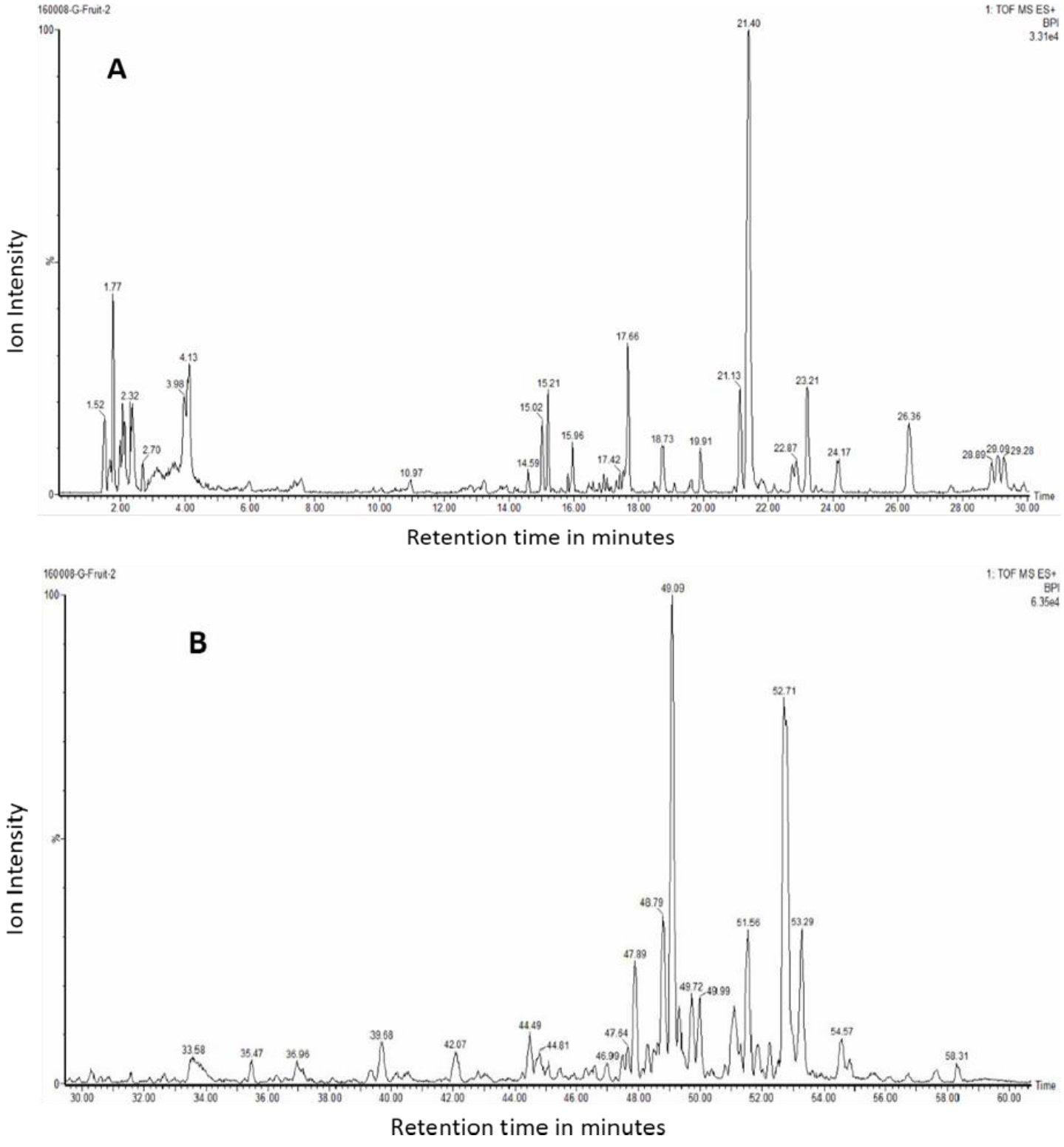


Figure 1: Total Ion Current (TIC) Base peak chromatogram (BPC) of *Garcinia xanthochymus* fruit extract by HPLC-ESI-QTOF-MS, Retention time of 0-30 min (A), Retention time of 30- 60 min (B)

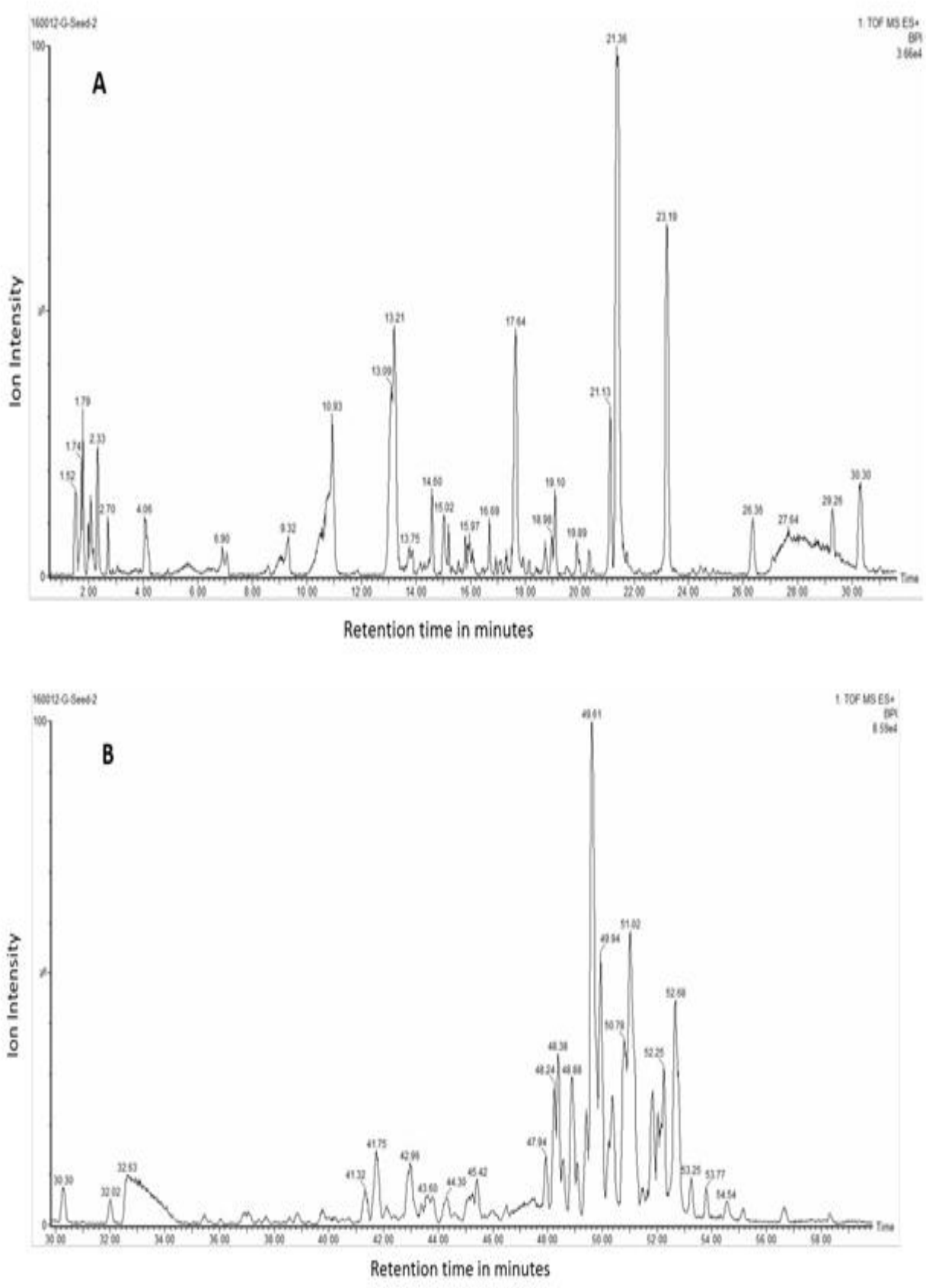


Figure 2: Total Ion Current (TIC) Base peak chromatogram (BPC) of *Garcinia xanthochymus* seed extract by HPLC-ESI-QTOF-MS, Retention time of 0-30 min (A), Retention time of 30- 60 min (B)

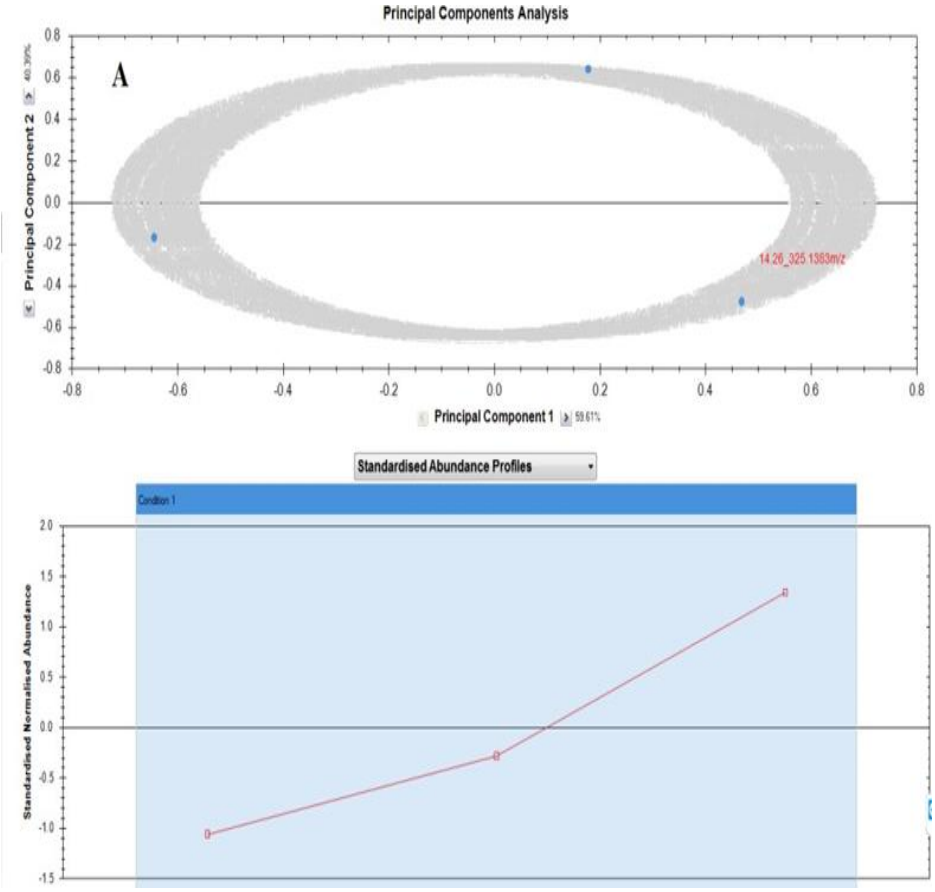


Figure 3: Principal Component Analysis (PCA) of Methanolic extract of *Garcinia xanthochymus* fruit (A) and its seed (B) illustrating the identical nature of the compounds between fruit and seed extracts

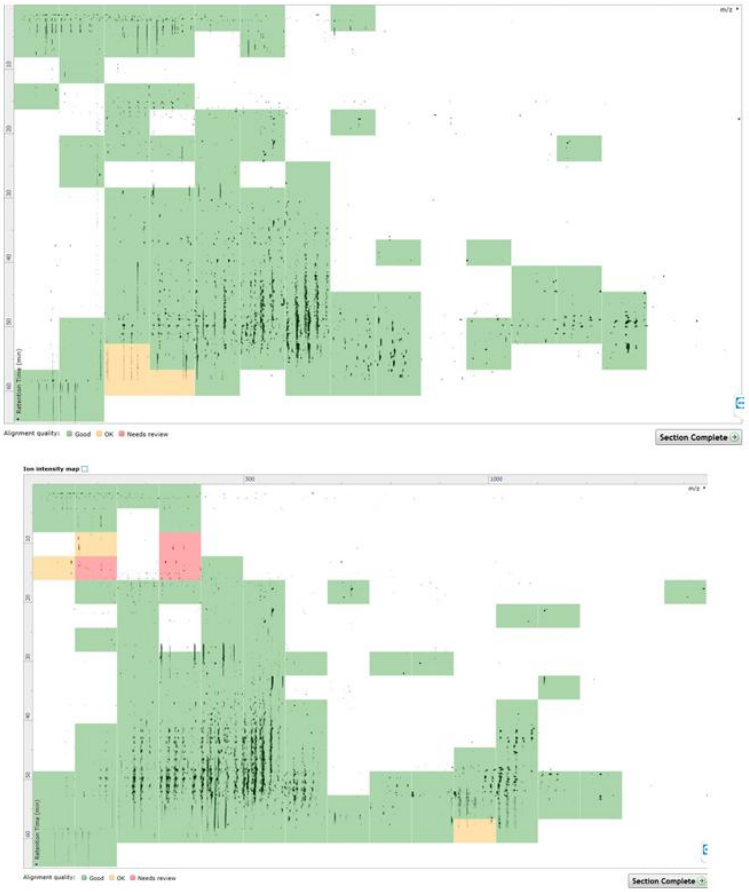


Figure 4: Ion Intensity map of seed and fruit extract of *Garcinia xanthochymus*

CONCLUSION

In the present study, the novel UPLC with ESI–QTOF–MS method adopted has been a useful analytical technique for deconvolution and characterization of phytochemical compounds of G. *xanthochymus* fruits and seeds. Analysis by Waters Progenesis QI Data analysis software based on the accurate mass and isotope distribution resulted in identification of a wide array of phytochemicals in the extracts. The molecules were tentatively identified by the method proposed. Other than the well known molecules of Garcinia species and the native characterized molecules of G. *xanthochymus*, the bioactive molecules proposed were glycosides, organic acids, aromatic alcohols, sugar derivatives, benzofurans, glucopyranosides, and substituted pentanoic acids. The data reflects the presence of an array of molecules with biological importance. The method is useful for rapid purification of the molecules of interest with the details of specified parameters. The profile of metabolites stands as a signature of composition of fruits and seeds of G. *xanthochymus.*

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