

Methanolic extract of Karo-derived *Trigonella foenum-graecum* induces apoptosis and cell-cycle arrest in breast cancer cells through modulation of PI3K/AKT/mTOR signaling

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Bioactive Compound Profile of Methanolic Extract of *Trigonella foenum-graecum* (GC-MS)

Table S1: Analytical characterization of identified phytochemical compounds from GC-MS chromatogram (Figure S1) with Retention Times (RT), precursor ion mass-to-charge ratios (*m/z*), molecular formulas, chemical core structures, CAS identifiers, and associated cytotoxic mechanism/role in breast cancer cells.

Compound Name	<i>m/z</i> [M+H] ⁺ /[M-H] ⁻	RT (min)	Formula	core	CAS ID	Phytochemical Class / Role
Quinic acid	191.05 [M-H] ⁻ 193.07 [M+H] ⁺	1.04	C7H12O6	Cyclohexanecarboxylic acid / Polyol	77-95-2	Cyclic Polyol / Phenolic Precursor; Antiproliferative & antioxidant activity, free radical scavenging.
α -Pinene	137.13 [M+H] ⁺	5.04	C10H16	Bicyclo[3.1.1]heptane	80-56-8	Monoterpenoid; Suppresses tumor cell growth through oxidative stress induction & PI3K/AKT pathway inhibition.
Phytol	297.31 [M+H] ⁺	10.36	C20H40O	Acyclic diterpene alcohol	150-86-7	Diterpenoid Alcohol; Exhibits cytotoxic properties & induces apoptosis in cancer cell lines.
Docosahexaenoic acid (DHA)	327.23 [M-H] ⁻ 329.25 [M+H] ⁺	10.50	C22H32O2	Polyunsaturated fatty acid (22:6 n-3)	6217-54-5	Omega-3 Polyunsaturated Fatty Acid; Antiproliferative & pro-apoptotic effects via cell membrane fluidity alteration.
Campesterol	401.38 [M+H] ⁺	11.23	C28H48O	Ergostane / Cyclopenta[a]phenanthrene	474-62-4	Phytosterol; Alters membrane dynamics & cholesterol metabolism, modulates AKT/mTOR signaling & apoptosis.
Stigmasterol	413.38 [M+H] ⁺	11.44	C29H48O	Stigmastane / Cyclopenta[a]phenanthrene	83-48-7	Phytosterol; Activates death receptors & modulates AKT/mTOR pathways to trigger autophagy and apoptosis.

Sarsasapogenin	417.34 [M+H] ⁺	10.26	C ₂₇ H ₄₄ O ₃	Spirostane (25S spiroketal)	126-19-2	Steroidal Sapogenin; Induces programmed cell death & collapses mitochondrial membrane potential.
Diosgenin	415.32 [M+H] ⁺	10.23	C ₂₇ H ₄₂ O ₃	Spirost-5-en-3-ol	512-04-9	Major Steroidal Sapogenin; Triggers cell cycle arrest (G1/S & G2/M), blocks survival PI3K/AKT/mTOR pathways & induces apoptosis.
Yamogenin	415.32 [M+H] ⁺	10.30	C ₂₇ H ₄₂ O ₃	Spirost-5-en-3-ol (25S epimer)	508-71-4	Steroidal Sapogenin (25S Epimer); Potent pro-apoptotic agent causing mitochondrial dysfunction & ROS generation.
Tigogenin	417.34 [M+H] ⁺	11.66	C ₂₇ H ₄₄ O ₃	5 α -Spirostane	77-60-1	Steroidal Sapogenin; Phytoestrogenic agent suppressing cell viability & promoting apoptosis.
Gitogenin	433.33 [M+H] ⁺	12.58	C ₂₇ H ₄₄ O ₄	5 α -Spirostane-2,3-diol	512-01-6	Di-hydroxylated Steroidal Sapogenin; Cytotoxic metabolite inhibiting breast cancer cell survival.

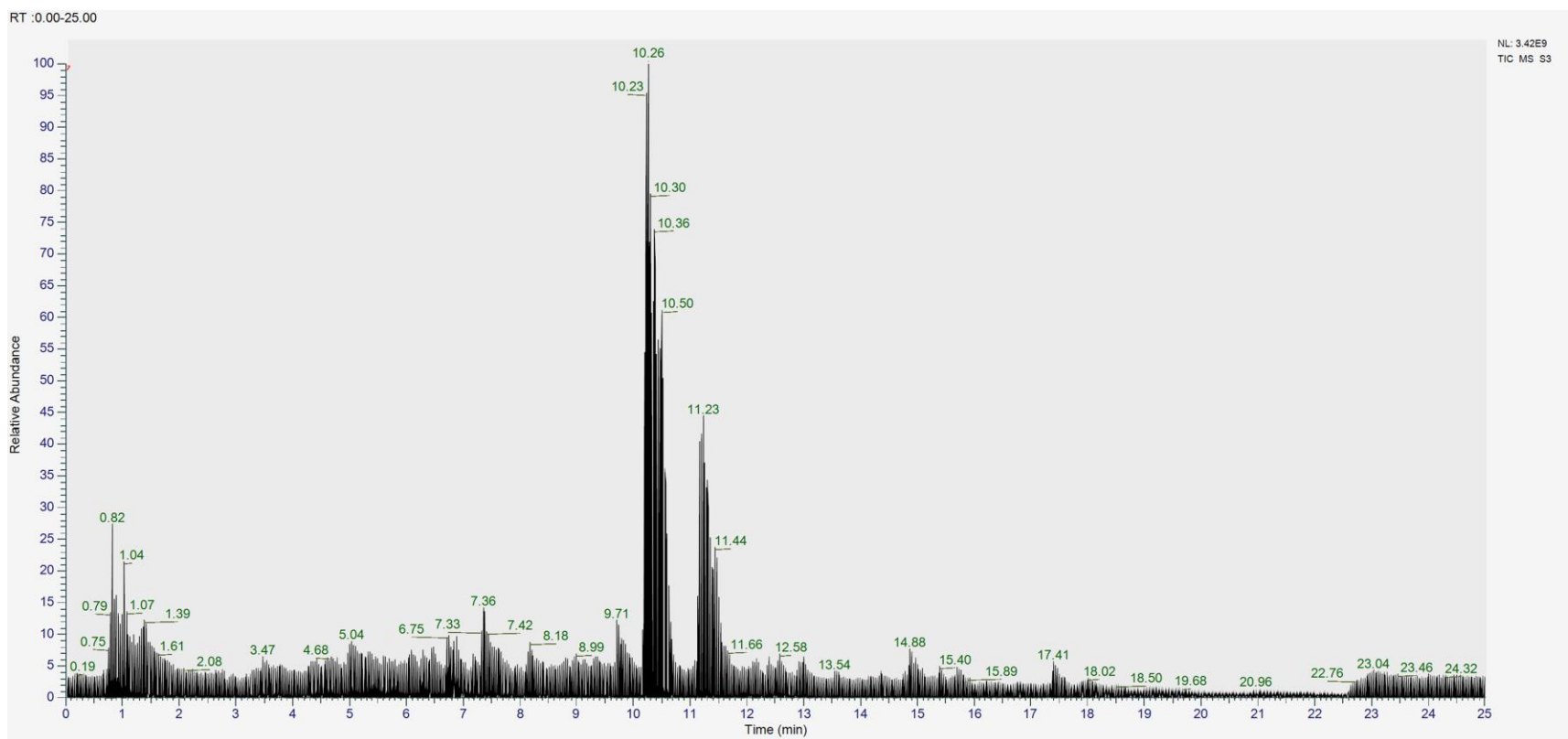


Figure S1. Gas chromatography–mass spectrometry (GC–MS) chromatogram