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GC-MS Analysis and In-silico Activity Evaluation of Isolated Compounds from *Nigella sativa* Seeds on Topoisomerase Alpha II

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Abstract

Plant-based medicines and naturally isolated compounds receive higher demands for the treatment of various ailments including cancer. Fewer side effects and higher therapeutic potential give more scope for drug discovery from herbals. *Nigella Sativa* or Kalonji seeds are well-known medicinal plants in the traditional system of medicine. The essential oil derived from N. sativa has notable biological characteristics. Even so, the essential oil of N. sativa contains several insoluble elements whose activities remain largely unexplored. The compounds identified using GS-MS/MS analysis of the extract identified the presence of around 32 phytoconstituents and literature shows that the isolated compound has potential activity for treating cancer. The *in-silico* docking results confirm the significant topoisomerase alpha II inhibitory activity of these compounds, especially propionic acid, 2-[(2-hydroxy-5-nitrobenzylidene) amino] -3-(1H-indol-3-yl)-, methyl ester with docking score of -10.4. Further isolation and *in-vitro*, *in-vivo* activity evaluation is needed to confirm the anticancer activity.

Keywords:

Full-length article **Corresponding Author, e-mail: spdhanabal@jssuni.edu.in*

1. Introduction

Nigella sativa L., belonging to the Ranunculaceae family, is a renowned botanical species with significant therapeutic properties. It is extensively used in several traditional medical systems, such as Unani, Siddha, and Ayurveda. N. sativa is used for the treatment of many chronic ailments, including diabetes, asthma, hypertension, cardiovascular disease, and cancer [1] , [2], [3]. Previous investigation on N. sativa (NS) has identified and examined the phytochemical components of the plant, as well as explored their pharmacological effects both in laboratory settings and in living organisms. The NS seed has many bioactive phytochemical compounds, like thymoquinone, thymohydroquinone, carvacrol, isoquinolines ρ-cymene, 4-terpineol, t-anethole, nigellicine, nigellidine, and longifolene [4]. The contents of the NS seed show exceptional therapeutic characteristics, which include antiparasitic, antibacterial, antipyretic, anti-inflammatory, analgesic, antioxidant, and anticancer effects.

In addition, several [5] research have shown that the extract from NS seeds may be used to inhibit cough, slow down the development of cancer, break down kidney stones, and treat polio, diarrhea, stomach discomfort, and flatulence [6]. The active components found in NS seeds have a significant impact on preventing the development of cancer and causing the death of cancer cells in different types of cancer, such as cervical cancer, breast cancer, fibrosarcoma, prostate cancer, colon cancer, hepatic cancer, pancreatic cancer, blood cancer, renal cancer, skin cancer, and lung cancer [7]. As an example, dimethylbenz[a]anthracene, which is a key ingredient in NS extract, inhibited the development of skin cancer in mice. Treatment with NS extract resulted in a delay in the creation of papillomas [8]. NS seeds contain specific fatty acids that demonstrated cytotoxicity in Ehrlich ascites carcinoma, sarcoma-180 cells, and Dalton's lymphoma ascites [9].

The combination of N. sativa extract and an oxidative stress agent demonstrated significant anticancer effects in MCF-7 breast cancer cells [10]. Studies reported that administering NS volatile oil orally hinders the development of colon cancer and restrains the growth of colon cancer cells in rats during the post-initiation stage. NS extract inhibited toxicity, renal oxidative stress, and carcinogenesis produced by KBrO3 [11]. Thymoquinone, an active element in NS, triggered apoptosis in myeloblastic leukemia HL-60 cells by caspase-8 activation, independent of the p53 protein [12]. NS seeds have a distinctly acrid flavor and are used as dietary supplements in candy and several other comestible items. In addition, NS seeds are ingested with honey, and milk and included in baked goods or pastries. Nigella sativa (NS) seeds are rich in vital nutrients including essential fatty acids, antioxidants, and a variety of vitamins. They also include important minerals such as calcium, iron, potassium, selenium, magnesium, and zinc [13**]**, [14**]**. They

also contain a variety of fatty acids, including unsaturated ones like linoleic and oleic acid, as well as saturated ones like palmitic acid, palmitoleic acid, myristic acid, linolenic acid, myristoleic acid, margaroleic acid, margaric acid, stearic acid, eicosenoic acid, behenic acid, arachidic acid, and lignoceric acid [15**]**. Furthermore, NS seeds contain sterols like as β-sitosterol, stigmasterol, campesterol, Δ7-avenasterol, and lanosterol [16**]**. The kalonji seed has a chemical composition that is well-suited for the efficient treatment of several ailments. Nevertheless, these seeds also comprise vital oils and very lipophilic substances, and these insoluble elements have not been well examined, either in laboratory experiments or in living organisms.

With around 2.3 million current cases identified in 2020, breast cancer stands as a prevalent form of cancer among women globally. Breast cancer originates in the epithelial lining of the mammary glands and ranks as the second most prevalent form of cancer globally [17]. The treatment of breast cancer involves a range of approaches, such as surgical intervention, chemotherapy, and radiation. However, these techniques are associated with high costs, significant discomfort, and potential adverse consequences [18]. Although there have been advancements in early detection methods and systemic treatment options, the majority of breast tumors exhibit medication resistance [19]. Therefore, it is essential to create innovative and economical treatment modalities or medications that have minimum adverse reactions. Topoisomerase α II (Topo α II) is a well-established anticancer target. The activity of this protein activity has been linked to the sensitivity of the cancer cells, such as increased Topo α II expression is the indication of more aggressive breast cancer and that leads to disease-related deaths.

The well-known anticancer medication, doxorubicin has the Topo α II inhibitory activity [20]. The diverse chemical structures of the phytochemicals present in the novel formulation are expected to have synergistic activity and therefore prevent and treat breast cancer. In this study, we have analyzed the *in-silico* topoisomerase alpha II inhibitory activity of the phytochemicals identified using GC-MS analysis of the novel *Nigella Sativa* formulation using the software Discovery Studio.

2. Materials and Methods

2.1. Gas Chromatography-Mass Spectroscopy Analysis

The GC-MS/MS study was performed by an Agilent 8890 gas chromatography system with a mass selective detector connected to the front injector. For the chromatography, an HP- 5 MS Ultra Inert capillary column (30 m 0.25 m, film thickness M) was employed. The temperature of the oven was programmed to begin at 60 °C, rise to 310 °C at a rate of 10 °C/min, and then be maintained at 200 °C for 5 minutes. The temperature for the injection was fixed at 280°C. Helium was employed as the carrier gas with a split ratio of 30:1, a sample injection volume of 1 l, an injector temperature of 250 °C, and an ion source temperature of 280 °C. The compound's composition percent was calculated using the GC peak areas. Substances were analysed by GC-MS using an Agilent GC 8890 gas chromatography and an Agilent MS/MS 7000 D triple quadrupole mass spectrometer. The identical GC conditions as those listed for GC analysis were applied, along with a 1000 amu column. The unknown component's spectrum was compared to the mass spectra and relative retention periods of the known components found in the 17th version library data of the National Institute of Standard and Technology (NISTGC-MS/MS) system. The GC-MS ran for around 32 minutes in total.

2.2. Molecular Docking

From the data bank of protein, topoisomerase alpha II (RCSB PDB-1CM8) was downloaded, and Discovery Studio 4.1 software was used for docking.

2.3. Preparation of the protein

To find potential issues, protein is produced in this stage. Missing loops are automatically built and fixed, and the side chains of missing residues are optimized.

2.4. Docking by C-Docker Modes

An interface to the LibDock program created by Diller and Merz is the Dock Ligands (LibDock) protocol. A carbon atom is preferred as the acceptor or donor atom for the H bond in polar hot spot compounds. The docking process begins with the calculation of the receptor Hot Spot file. By using docking simulation, the 31 compounds in formulation obtained from GC-MS analysis were created and geometrically optimized. The compounds under investigation were docked using imatinib as a reference into the crystal structure of a topoisomerase alpha II homodimer attached to DNA. The chemicals that were observed were stored in mol2 format. The molecule was imported into Discovery Studio 4.1 after the hydrogen bonds were added. The obtained PDB format was stored. For each position, the CDOCKER interaction energy was computed [21].

3. Results & Discussion

3.1. G-C MS Analysis

Figure (1) depicts the NSF's GC-MS profile, which includes 32 peaks for biomolecules. The phytocomponents, their retention time, peak area percentage, and molecular weight are shown in Table 1. Our findings match previous literature that demonstrated these phytoconstituents have anti-inflammatory, antioxidant, and anticancer properties. 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one (DDMP) is showing antioxidant properties [22], and octanoic acid, a linear saturated fatty acid is exhibiting anticancer activity against colorectal, mammary gland and skin cancer [23]. Dodecanoic acid moiety has anticancer activity in conjugate forms and was reported to have the characteristics of apoptosis induction and cell cycle arrest [24]. Glucobrassicin is a major glucosinolate with anticancer effects and is a precursor of indole-3 carbinol [25].

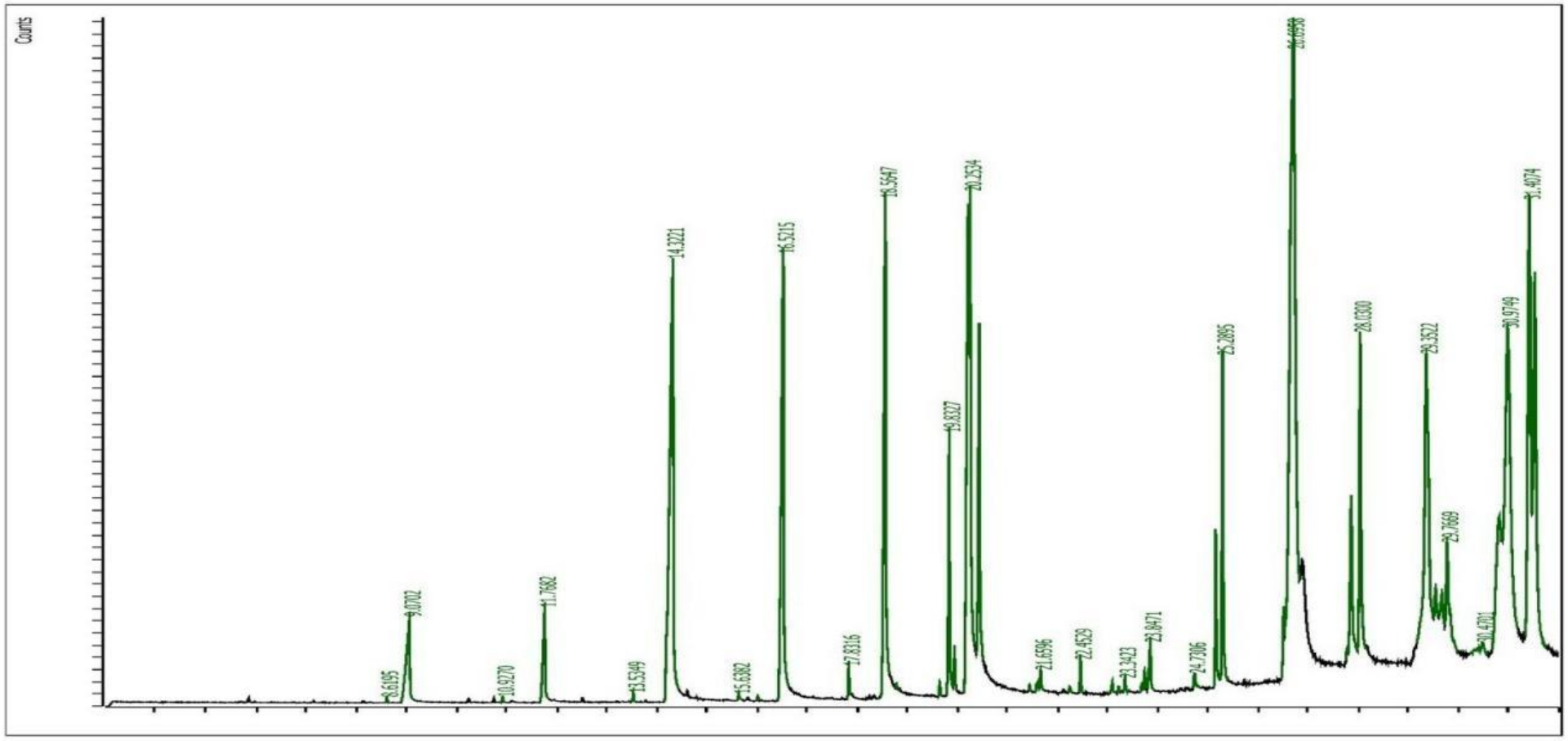


Figure 1

Figure 1. The GC-MS chromatogram of formulation showing compounds with high peak height and peak width.

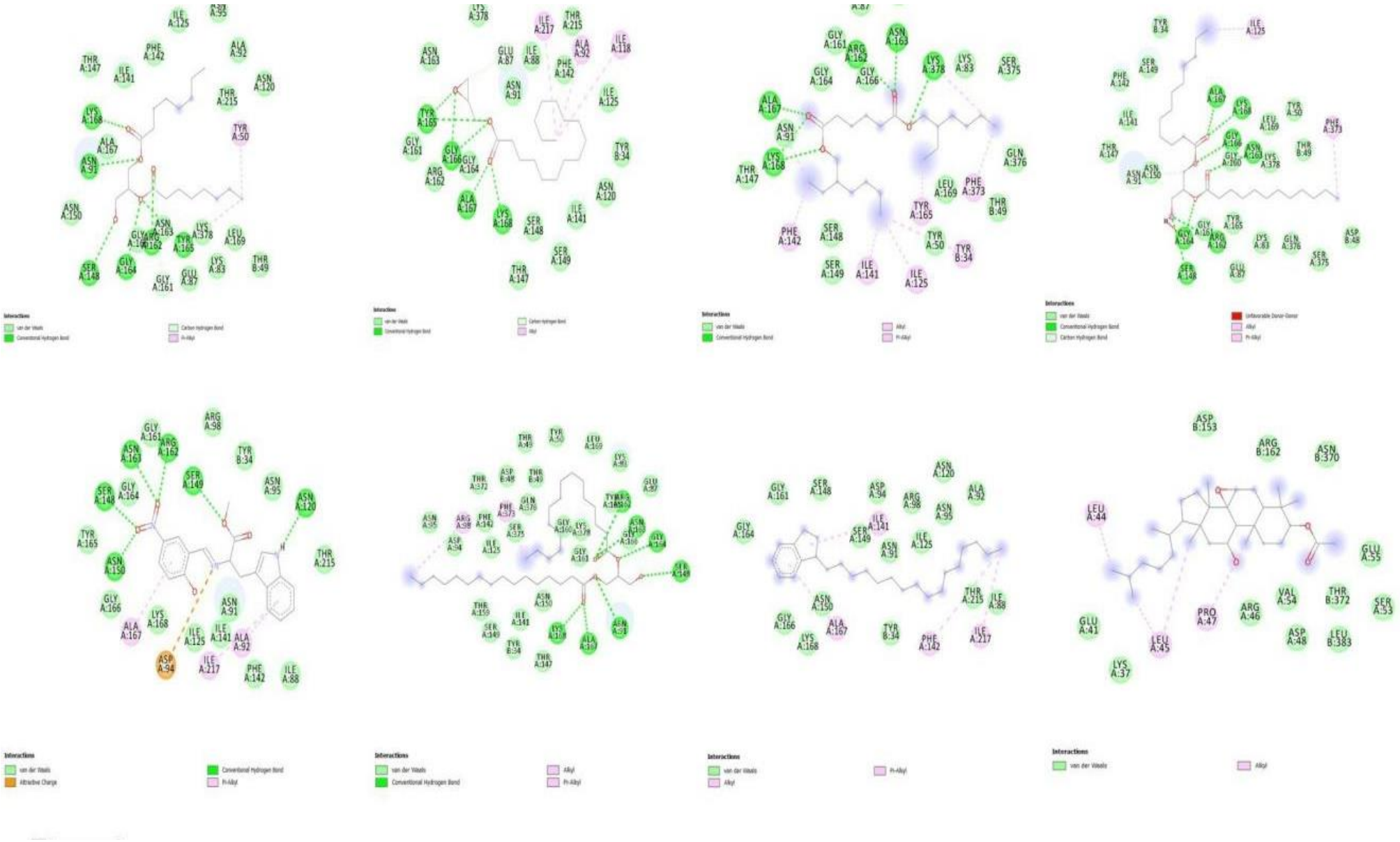
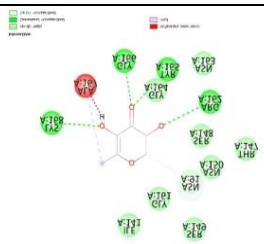
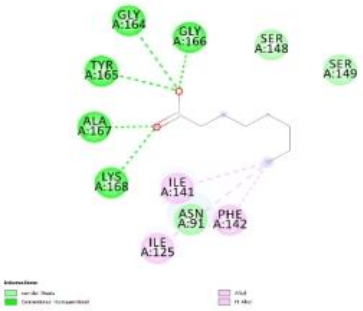
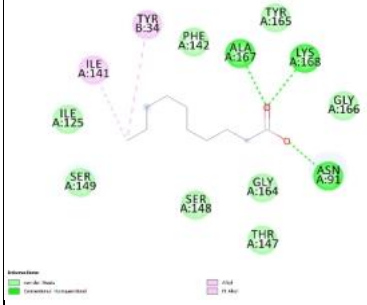
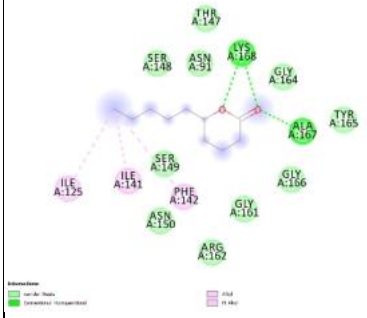


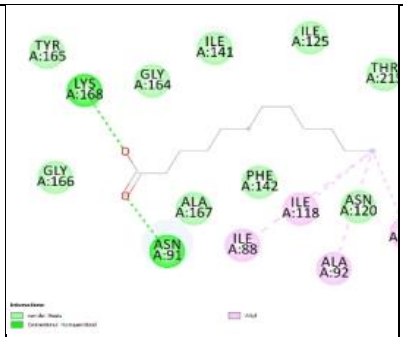
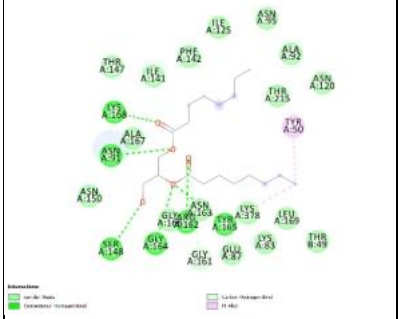
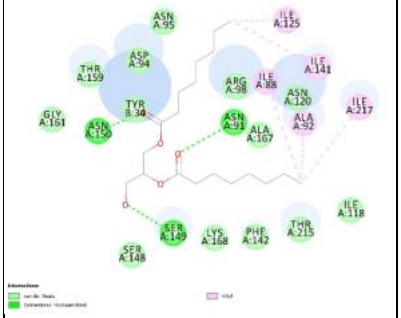
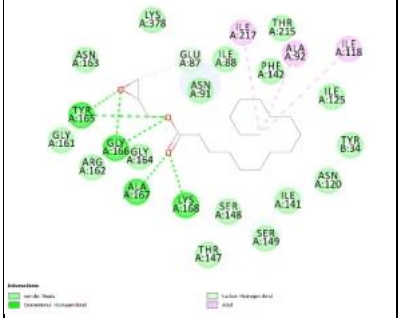
Figure 2: 2 D Binding interaction images of compounds binding interaction > -7 with Topoisomerase alpha II after docking using Discovery studio LibDock program

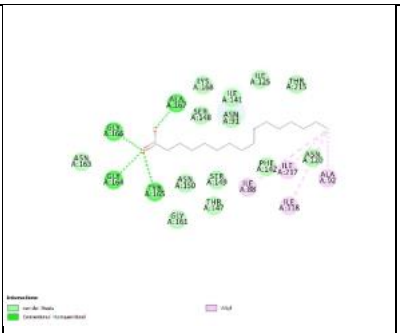
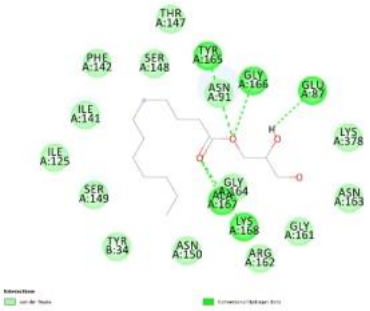
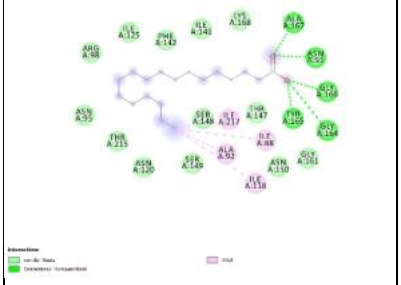
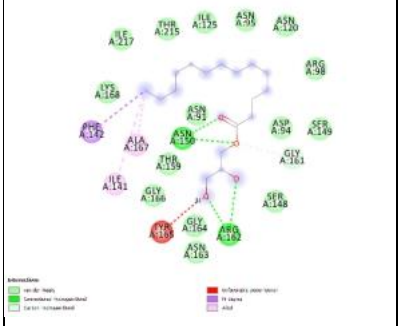
Table 1. List of phytochemicals identified in *Nigella Sativa* Virgin coconut oil formulation, their retention time and peak area% with Structural Interaction with Topoisomerase Alpha II

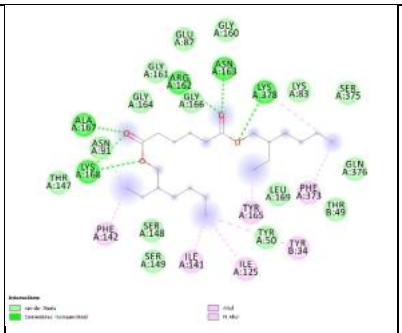
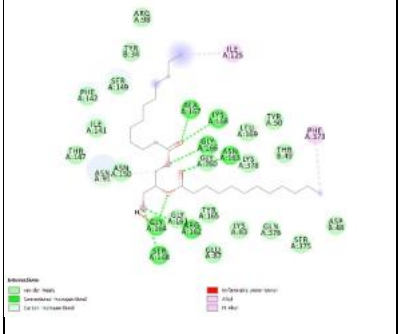
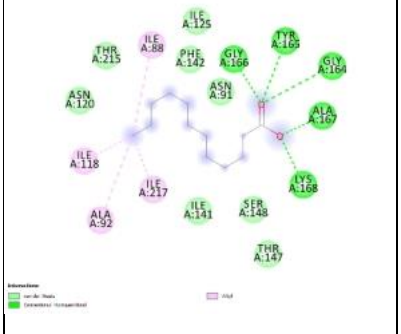
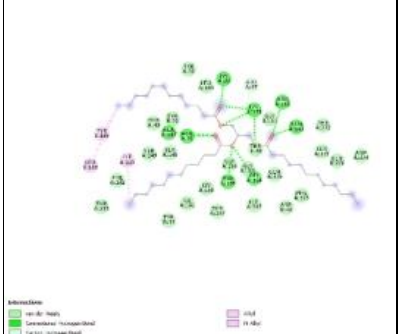
Ligand	Lib Dock Score	Binding Interaction
4r1f_1T1	123.21	-6.5
4r1f_1T2	131.41	-5.2
4r1f_1T3	126.74	-5.6
4r1f_1T4	126.67	-6.1
4r1f_1T5	125.99	-6.2
4r1f_1T6	125.55	-7.5
4r1f_1T7	122.67	-6
4r1f_1T8	122.57	-7.3
4r1f_1T9	119.99	-6.5
4r1f_1T10	119.51	-6.3
4r1f_1T11	118.35	-6.5
4r1f_1T12	118.14	-6.2
4r1f_1T13	116.58	-7.5
4r1f_1T14	112.76	-7.6
4r1f_1T15	111.03	-6.2
4r1f_1T16	110.59	-6.4
4r1f_1T17	109.67	-7.7
4r1f_1T18	108.37	-4.4
4r1f_1T19	106.93	-3.9
4r1f_1T20	104.99	-5.9
4r1f_3T1	91.29	-6.9
4r1f_3T2	90.99	-6.5
4r1f_3T3	90.83	-10.4
4r1f_3T4	85.99	-6.6
4r1f_3T5	52.6	-4.1
4r1f_3T6	85.89	-7
4r1f_3T8	84.63	-7.6
4r1f_3T7	85.77	-10.4
4r1f_3T9	84.56	-7.2
4r1f_3T10	96.52	-4.7
4r1f_3T11	76.66	-6.9
4r1f_3T12	145.23	-7
4r1f_3T13	92.81	-6.9

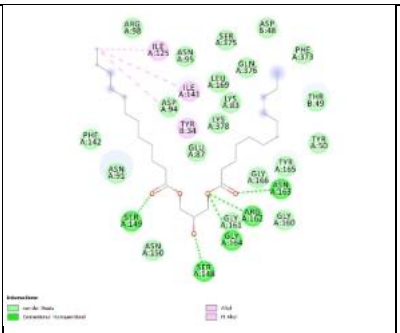
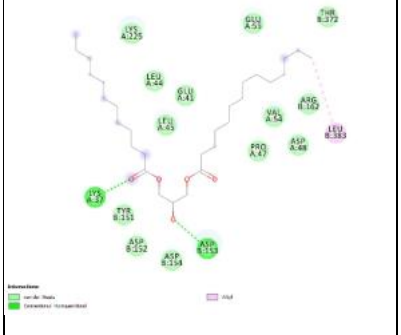
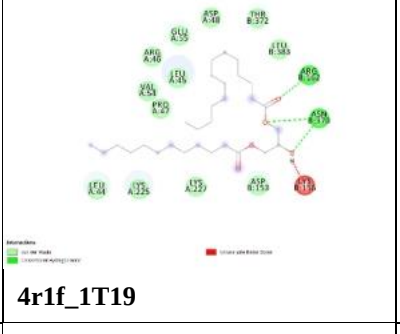
Table 2. List of phytochemicals identified in *Nigella Sativa* Virgin coconut oil formulation, their retention time and peak area% with Structural Interaction with Topoisomerase Alpha II

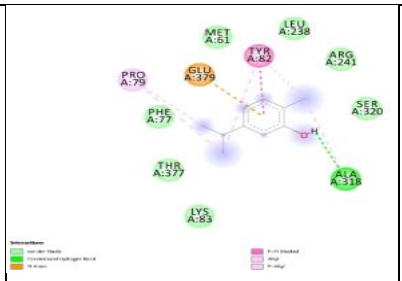
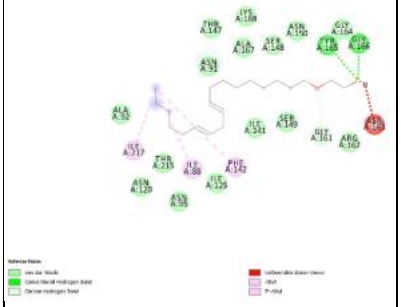
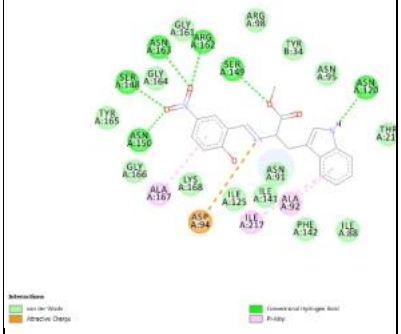
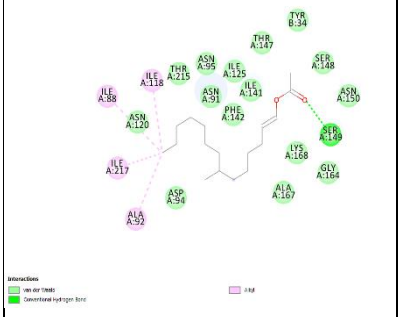
Sl No	Component RT	Compound Name	Structural Interaction with Topoisomerase Alpha II	CAS#	Formula	Component Area	Match Factor	Area % Max. Area
1	8.6194	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	 4r1f_1T1	28564-83-2	C6H8O4	4113529.3	69.0	0.2299
2	9.0941	Octanoic acid	 4r1f_1T2	124-07-2	C8H16O2	225463747.7	91.7	12.6
3	11.7562	n-Decanoic acid	 4r1f_1T3	334-48-5	C10H20O2	81520938.0	90.6	4.555
4	13.5349	2H-Pyran-2-one, tetrahydro-6-pentyl-	 4r1f_1T4	705-86-2	C10H18O2	7377379.8	75.8	0.4122

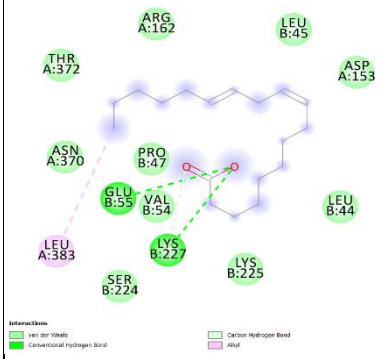
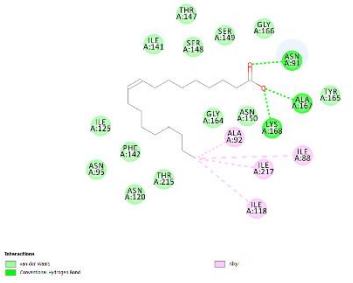
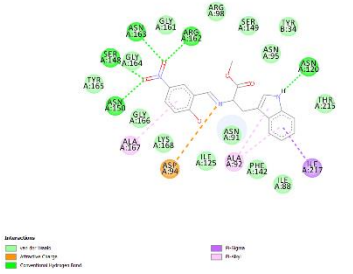
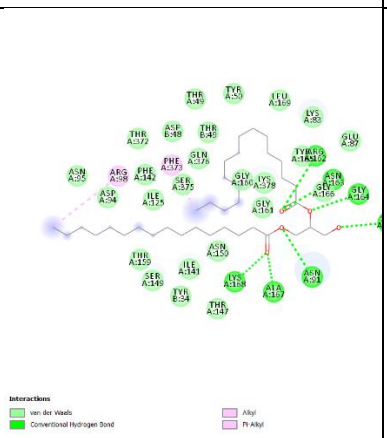
5	**14.2920**	**Dodecanoic acid**	 **4r1f_1T5**	**143-07-7**	**C12H24O2**	**505136806.5**	**94.8**	**28.23**
6	**15.6321**	**Glycerin, 1,2-dicaprylate**	 **4r1f_1T6**	**502-54-5**	**C11H22O4**	**5878657.7**	**71.3**	**0.3285**
7	**16.4733**	**Tetradecanoic acid**	 **4r1f_1T7**	**544-63-8**	**C14H28O2**	**122396309.8**	**93.3**	**6.839**
8	**17.8314**	**Glycidyl palmitate**	 **4r1f_1T8**	**7501-44-2**	**C19H36O3**	**23744070.8**	**78.5**	**1.327**

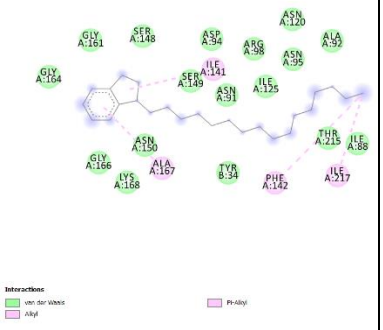
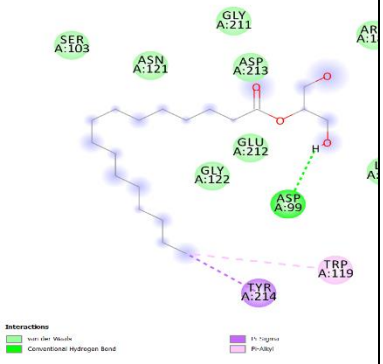
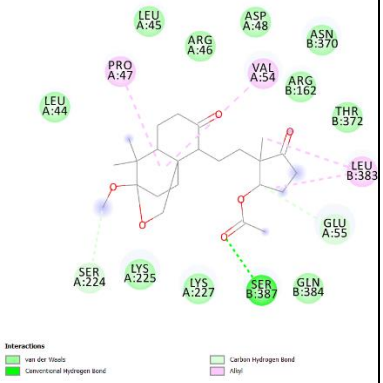
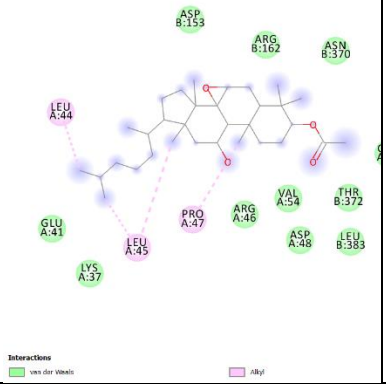
9	**18.5345**	**n-Hexadecanoic acid**	 **4r1f_1T9**	**57-10-3**	**C16H32O2**	**185648544.2**	**93.1**	**10.37**
10	**19.8265**	**Dodecanoic acid, 2,3-dihydroxypropyl ester**	 **4r1f_1T10**	**142-18-7**	**C15H30O4**	**97270568.2**	**92.0**	**5.435**
11	**20.4034**	**Octadecanoic acid**	 **4r1f_1T11**	**57-11-4**	**C18H36O2**	**94390816.2**	**92.8**	**5.275**
12	**21.6534**	**Tetradecanoic acid, 2,3-dihydroxypropyl ester**	 **4r1f_1T12**	**589-68-4**	**C17H34O4**	**11411251.7**	**74.6**	**0.6377**

13	**22.4526**	**Hexanedioic acid, bis(2-ethylhexyl) ester**	 **4r1f_1T13**	**103-23-1**	**C22H42O4**	**26668553.8**	**87.4**	**1.49**
14	**23.8469**	**Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester**	 **4r1f_1T14**	**17598-94-6**	**C27H52O5**	**43280746.9**	**67.4**	**2.419**
15	**25.2892**	**1,3-Dioctanoin**	 **4r1f_1T15**	**1429-66-9**	**C19H36O5**	**221808730.1**	**73.5**	**12.39**
16	**26.6955**	**Dodecanoic acid, 1,2,3-propanetriyl ester**	 **4r1f_1T16**	**538-24-9**	**C39H74O6**	**1789552101.6**	**68.5**	**100**

17	**28.0297**	**Rac-glycerol-1,3-dilaurate**	 **4r1f_1T17**	**539-93-5**	**C27H52O5**	**20968848 6.1**	**89.3**	**11.72**
18	**29.7666**	**1-Dodecanoyl-3-myristoylglycerol**	 **4r1f_1T18**	**91925-73-4**	**C29H56O5**	**34655715. 5**	**79.0**	**1.937**
19	**30.9686**	**1-Dodecanoyl-3-myristoylglycerol**	 **4r1f_1T19**	**91925-73-4**	**C29H56O5**	**10330934 71.4**	**65.3**	**57.73**
20	**31.4074**	**2-(Octanoyloxy)propane-1,3-diyl bis(decanoate)**	 **4r1f_1T20**	**33368-86-4**	**C31H58O6**	**64021265 6.5**	**78.8**	**35.78**
21	**10.9270**	**Phenol, 2-methyl-5-(1-methylethyl)-**	**4r1f_3T1**	**499-75-2**	**C10H14O**	**3975996.0**	**75.8**	**0.1834**

								
22	18.7810	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	 4r1f_3T2	17367-08-7	C20H38O2	5149133.1	61.0	0.2375
23	19.6464	Propionic acid, 2-[(2-hydroxy-5-nitrobenzylidene)amino]-3-(1H-indol-3-yl)-, methyl ester	 4r1f_3T3	1000296-35-4	C19H17N3O5	8897456.6	60.1	0.4103
24	19.9409	7-Methyl-Z-tetradecen-1-ol acetate	4r1f_3T4 	1000130-99-6	C17H32O2	27898099.4	72.4	1.287

25	20.2113	9,12-Octadecadienoic acid (Z,Z)-	 4r1f_3T5	60-33-3	C18H32O2	594315638.2	93.1	27.41
26	20.2534	9-Octadecenoic acid, (E)-	 4r1f_3T6	112-79-8	C18H34O2	5118769509	78.1	23.61
27	21.4372	Propionic acid, 2-[(2-hydroxy-5-nitrobenzylidene)amino]-3-(1H-indol-3-yl)-, methyl ester	 4r1f_3T7	100029635-4	C19H17N3O5	5180063.1	55.0	0.2389
28	21.6055	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	 4r1f_3T8	761-35-3	C35H68O5	11459065.0	70.8	0.5284

29	23.2101	1H-Indene, 1-hexadecyl-2,3-dihydro-	 4r1f_3T9	55334-29-7	C25H42	4862930.5	53.5	0.2243
30	23.3423	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	 4r1f_3T10	23470-00-0	C19H38O4	11648572.7	74.7	0.5372
31	24.7306	8,14-Seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3.beta.-methoxy-4,4-dimethyl-	 4r1f_3T11	10001 95-87-0	C24H36O6	21912324.8	74.0	1.01
32	30.4701	7,8-Epoxylanostan-11-ol, 3-acetoxy-	4r1f_3T12 	10001 87-60-9	C32H54O4	38696002.5	65.5	1.784

Bio-assay-guided fractionation of Hexadecenoic acid and its activity in colorectal cancer cells showed significant cytotoxicity in HCT-116 cell lines [26]. The essential oil component of NS, Phenol, 2-methyl-5-(1-methylethyl)-

commonly known as Carvacrol also reported to have anticancer properties [27]. Table 1 also lists the chemical makeup of the active ingredients and their interactions with topoisomerase alpha II.

3.2. Molecular docking

Based on their predicted behavior, we selected 32 phytoconstituents from GC-MS data for docking investigations using the Discovery Studio Lib Dock program. All these compounds interacted with the active site of the targeted protein topoisomerase alpha II. Although there are 10 molecules (Figure 2) having significant interactions with protein that range from -7 to -10.4 and significant roles score for LibDock that range from 84.56 to 145.23. The topoisomerase alpha II receptor served as a molecular docking target for the screening of plant phytochemicals. Figure 2 displays the top ten active substances along with their docking scores, and 2D molecular docking interactions with the topoisomerase alpha II protein.

4. Conclusions

The evidence that we acquired on NS Formulation indicates that leveraging knowledge of conventional medicinal systems may facilitate the bioprospecting, identification, development, and commercialization of novel medicinal sources that are both safe and efficient. Within this context, we have created NS formulations that exhibit varying phytoconstituents, determined by GC-MS/MS analysis of oil and the current study provides evidence that these natural compounds from NS formulation have significant topoisomerase alpha II inhibitory activity. Further investigations into NSF have the potential to provide more evidence for its efficacy in targeted therapeutic applications for cancer.

Conflict of interests

Authors confirm that there is no conflict of interest regarding this study.

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