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Phytochemical Analysis of *Nigella Sativa* and Its Antibacterial and Anticancer Activities

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Abstract:

The present study was aimed at determining the chemical constituents of Nigella sativa seeds and their anticancer and antibacterial activities. The phytochemical screening carried out on methanolic extract of Nigella sativa seeds showed the presence of tannins, alkaloids, proteins and terpenoids in high proportions whereas flavonoids, steroids and cardio-glycosides were present in lesser amount. Five different compounds i.e. Linoleic acid, Thymoquinone, Dithymoquinone, Damasceninine and Tannic acid were isolated from the methanolic extract of Nigella sativa seeds by using column chromatography and later identified by NMR spectroscopy. The result of anticancer activity of isolated compounds performed in bioinformatics lab showed that Dithymoquinone acts as best anticancer agent than the other four compounds. The isolated compounds were also tested for antibacterial activity against different gram positive (Bascillus megaterium and staphylococcus aureus) and gram negative (Pseudomonas aureginosa and Kliesebella pneumoneae) bacteria by disc diffusion method. The antibacterial activity results were expressed in terms of radius of zone of inhibition. The results of antibacterial activity showed that the compounds isolated from methanolic extract of Nigella sativa seeds are highly active against both Gram positive and Gram negative bacteria. Among the compounds, dithymoquinone showed the highest zone of inhibition (2.6cm) against both the gram negative bacteria which is same as shown by tetracycline (standard antibiotic) against the same bacteria.

This study indicated that these compounds have both anticancer as well as antibacterial activities.

Key words: Methanolic extract *Nigella sativa* seeds, antibacterial and anticancer activities.

Introduction

Medicinal plants from the buttercup family (Ranunculaceae) have been applied extensively for many years to treat various diseases, particularly the seeds. Nigella genus consists of about 20 species, including *Nigella glandifera*, *Nigella sativa*, and *Nigella damascena*, which are used in traditional medicine **(Zhang and Chen, 2012)**. Black seed is the medicinal plant which is the main concern of this research study. There aredifferent names used for the herb of black seed. For example, it is named as "Panacea" in old Latin which means "cure all", whereas in Arabic it is known by two names "Habbah Sawda" and "Habbatal Baraka", means "Seeds of blessing". It has given this name due to its strong healing qualities for different diseases **(Aggarwal *et al.,* 2008).** In different research studies, the aqueous and oil extracts of the black seeds have been found to exhibit antioxidant, anti-inflammatory, anticancer, analgesic hypolipemic, immune- regulatory, anti-platelet and antimicrobial activities. Most of these properties have been attributed to thymoquinone which is the active constituents of the seed **(Mehta *et al*., 2009).**

Material and Methods

Preparation of extract

The dried and powdered seeds of *Nigella sativa* were extracted with methanol using soxhlet extractor for 24 h at a temperature not exceeding the boiling point of the solvent (**Linn *et al*.,**

1999). The extracts were filtered and then concentrated to dryness. Yield of the extract obtained was calculated as follows:

$$\text{Yield} = \frac{\textit{weight of extract recovered}}{\textit{weight of dry powder}} \times 100$$

The extract was transferred to glass vials and kept at 4° C before use.

Phytochemical analysis

Phytochemical screening chemical tests were carried out using extract to identify various constituents using standard methods according to **Sofowara, 1993.**

Isolation of the compounds from methanolic extract

Isolation of different constituents was done by using column chromatography.The pure isolated compounds obtained thereby were identified by NMR spectroscopy.

Anti Bacterial Activity

The antibacterial activity of isolated compounds from *Nigella sativa* seeds was done by disc diffusion method **(Bauer *et al*., 1966)**. Whatman No: 1 filter paper discs of 6mm diameter was prepared and autoclaved by keeping in a clean and dry Petri plate. To test the antimicrobial activity on agar plates, LB agar medium was prepared using the ingredients mentioned above. The medium was sterilized at 121°C for 30 minute. The agar test plates were prepared by pouring about 15ml of the medium into 10cm Petri dishes under aseptic condition and left undisturbed for 2hrs to solidify the medium.1ml of inoculums (containing suspension) of *K.pneumonia, B.Subtillis, P.aeruginosa* and *S. aureus* was poured to the respective plates separately containing solidified agar media. Six replicates were maintained. The control petri plate was also maintained for above respective cultures. The inhibition zones were led after 1

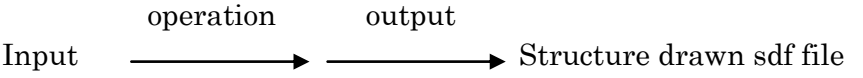
day at 37ºC for bacteria. The diameter of the inhibition zone was measured and recorded with the aid of plastic ruler.

Anticancer activity

The anticancer activity of different constituents of *Nigella sativa* seeds was done by using chemo-informatics. Various steps used in investigating anticancer activity are as follows;

Sketch the structures of various compounds by using PUBCHEM software:

The conceptual framework of this software, that uses these identifiers, is the three part request. (1) Input (2) operation (3) output. The beauty of this design is that each of these three parts of the request is mostly independent allowing a combinatorial expansion of the things that was done in a single request.



Structure of various compounds which were used as a ligand was drawn with the help of PUBCHEM project). 2-dimensional as well as 3-dimensional structures were generated using this software, which was further used in docking.. The structures drawn with the PUBCHEM Sketcher was stored in the sdf format.

Active site Identification

Active site of BCL-2 was identified by using CASTp server. A new program, CASTp, for automatically locating and measuring protein pockets and cavities, is based on precise computational geometry methods, including alpha shape and discrete flow theory. CASTp identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining pockets, pocket openings, and buried cavities; the

volume and area of pockets and cavities; and the area and circumference of mouth openings.

RCSB Protein Data Bank

The RCSB Protein Data Bank is very important software which provides us three dimensional structures of enzymes, proteins and nucleic acids. These are the molecules of life that are found in all organisms including bacteria, yeast, plants, flies, other animals, and humans. Understanding the shape of a molecule helps to understand how it works. It consists of organizations that act as deposition, data processing and distribution centers for PDB data. Then will save the file format as PDB file format. The sequence of BCL-2 was obtained from UNIPROT. The structure was collected from Protein Data Bank. After that the unnecessary chains and hetero atoms was removed using SPDBV software. Later hydrogen's was added to the protein and used for active site identification. Finally, the structure having the least energy with low RMSD (Root Mean Square Deviation) was used for further studies. In this step, the quality of the initial model improves. The final structure was analyzed by Ramachandran's map using PROCHECK (Programs to check the Stereo chemical Quality of Protein Structures) and environment profile using ERRAT graph (Structure Evaluation server). This model was used for the identification of active site and for docking of the substrate with the enzyme.

Docking method

Docking (Genetic Optimization of Ligand Docking) software which was based on genetic algorithm (GA). This method allows as partial flexibility of protein and full flexibility of ligand.

Results and Discussion

Approximately 20 g of finely ground powder of *N. sativa* was placed in a thimble and extracted in an all glass Soxhlet

extractor for 48 h by using 200 ml methanol as solvents. Solvent was removed by rotary evaporation at 40°C under vacuum and the last traces of solvent in the extract were removed by using rotary distillation apparatus to obtaine pure extract. The extract was stored at 4°C until analysis. The phytochemical screening of methanolic extract of *Nigella sativa* seeds was performed by using different tests and the results obtained are shown in table given below.

Phytochemical constituents present in methanol extract of *Nigella sativa* seeds.

	Name of the Phytoconstituent	Result
1	Tannins	+ + +
2	Saponins	+
3	Flavonids	+
4	Alkaloids	+ + +
5	Proteins	+ + +
6	Steroids	+
7	Terpenoids	+ +
8	Anthraquinones	_
9	Cardio glycosides	+

The results of phytochemical screening of methanolic extract of *Nigella sativa* seeds showed the presence of tannins, alkaloids, proteins and terpenoids in high proportions whereas flavoniods, steroids and cardio-glycosides were present in lesser amount. Anthraquinones were absent in the extract.

Test for Alkaloid and Tannins of *Nigella.S* methanolic extract

Test for Saponins of *Nigella.S* methanolic extract

Test for Flavonoids and Terpenoid of *Nigella S.* methanolic extract

Test for Anthraquinones of *Nigella S* methanolic extract

Test for Cardio glycsoside of *Nigella S* methanolic extract

Test for Steroids and Proteins of *Nigella .S* methanolic extract

Isolation and Identification of compounds from Methanolic extract of *Nigella sativa* seeds

The extract obtained by Soxhlet extraction technique was subjected to fractionation on silica gel. A 250 g of activated silica gel was loaded to a column and cleaned with about 100 ml of hexane. About 10 g of the extract was loaded on to the column. The compounds were eluted successively with 500mL each of hexane, diethyl ether, chloroform and methanol. Solvent in the fractions was removed by rotary evaporation at 40^0C under vacuum.

Five compounds were isolated from the methanol extract of *Nigella sativa* seeds by using column chromatography.The isolated compounds were then characterized by NMR spectroscopy (^{1}HNMR and ^{13}CNMR).

COMPOUND-1

C^{13} NMR($CDCL_3$, 400 MHZ): δ(ppm) 14, 23.2 , 24.3, 25.1, 27.4, 29.7,30,30.1,30.3, 32.6, 35.8, 123.8 , 133.2 , 177.

C^{13} NMR spectra showed the expected signals which corresponds to the various functional groups present in the linoleic acid compound .The signals which appeared at, δ 14 indicated the presence of methyl group$\underline{C}H_3$-, and at δ 23.2, 24.3for methylene groups, CH_3-$\underline{C}H_2$-, =CH-$\underline{C}H_2$-CH= respectively. The spectral peaks were observed at δ 25.1, 27.4, 29.7, 30, 30.1,30.3, 32.6, 35.8 for different methylene groups, CH_2-CH_2-$\underline{C}H_2$-CH_2-COOH, -CH_2-$\underline{C}H_2$-CH= ,-CH_2-$\underline{C}H_2$-CH_2-CH_2-COOH, -CH_2-$\underline{C}H_2$-CH_2, -CH_2-$\underline{C}H_2$-CH_2-, -CH_2-$\underline{C}H_2$-, CH_3-CH_2-$\underline{C}H_2$-. CH_2-CH_2-CH_2-$\underline{C}H_2$-COOH, respectively. The spectral peaks were obtained at δ123.8, 133.2 for two ethylene groups-CH=$\underline{C}$H-CH_2, -$\underline{C}$H=CH-CH_2-, respectively, and 177 for carboxylic group CH_2-$\underline{C}$OOH. The $CDCL_3$ has been used as reference material.

^{1}H-NMR (DMSO-d_6-400 MHZ): δ(ppm) 2.5, 3.9, 4.9, 5.34, 5.8, 6.360, 6.7, 9.05.

^{1}H-NMR spectra showed the expected signals which corresponds to the various functional groups present in the linoleic acid compound. The triplet was observed at δ 2.5 for CH_2-CH_3, andat 3.9 the double- doublet of hydrogens atom,=CH-CH_2=CH-. The signals were obtained atδ 4.9indicated the multiplet of hydrogen atoms,CH_2-CH=CH-CH_2 . The signals were obtained at δ 5.34indicated the presence of multiplet for hydrogen atoms, CH_2-CH_2-CH_3. The signals observed atδ 5.8 for the multiplet of hydrogen atoms , CH_2-CH-CH=,and at 6.360 the multiplet of hydrogen atoms, CH_2-(CH_2)$_n$-CH_2 . The signals which appeared at δ 6.7indicated the triplet of hydrogen atoms, CH_2-CH_2. The singlet was observed at δ9.05 for hydrogen atom in hydroxyl group -OH .The DMSO-d_6 has been used as reference material. All the chemical shifts were reported to δ ppm and presence of carbon, hydrogen and oxygen was confirmed by elemental analysis. Anal. Calcd. For compound (C =77.09 % ;H=11.50 %;O=11.41 %)found Molecular Formula; $C_{18}H_{32}O_2$.

.

COMPOUND-2

C^{13} NMR (CDCL$_3$, 400 MHZ): δ(ppm) 20, 28, 40, 58, 129, 148, 198, 201.

C^{13} NMRspectra showed the expected signals which corresponds to the various functional groups present in thethymoquinonecompound. The spectral peak which appeared at, δ20 and 28 for different methyl groups, 2CH_3-CH-CH_3 and -C-CH_3 respectively. The spectral peak at δ 40 indicated the presence of mthylene group -C-CH-CH_3. The spectral peak at δ58, 129, 148,198, for different ethylene groups, O=C-C=C-, O=C-C=CH- , 2 O=C-CH=CH-, $(CH_3)_2$-CH-C=C- , respectively. The spectral peak at δ201 indicated the presence of two

carbonyl groups2 -C=O. The $CDCL_3$ has been used as reference material.

^{1}H-NMR (DMSO-d_6-400 MHZ): δ(ppm) 1.2, 3.6, 5.4, 6.809, 7.311.

^{1}H-NMR spectra showed the expected signals which corresponds to the various functional groups present on thethymoquinonecompound.The singlet was observed atδ 1.2 indicated for C-CH_3, and 3.6 for the multiplet of hydrogen atoms, CH_3-CH-CH_3. The signals appeared at δ 5.4 indicated the presence of doublet of six hydrogen atoms, CH_3-CH-CH_3. The singlet was observed atδ 6.809 indicated for CH=C and 7.311 for thesinglet of hydrogen atom CH=C. The DMSO-d_6 has been used as reference material. All the chemical shifts were reported to δ ppm and presence of carbon, hydrogen and oxygen was confirmed by elemental analysis. Anal. Calcd. For compound (C =73.15% ;H=7.37%;O=19.49 %)found Molecular Formula; $C_{10}H_{12}O_2$.

COMPOUND-3

C^{13} NMR ($CDCL_3$, 400 MHZ): δ(ppm) 20, 26.1, 40, 55,128,158,198, 201.

C^{13} NMRspectra showed the expected signals which corresponds to the various functional groups present in the dithymoquinone compound. The spectral peaks were observed at δ 20 and 16.1 indicated six methyl groups 2(CH_3)$_2$-CH- and 2(CH_3)-C- respectively and at 40, 55 ,128 for different methylene groups CH-(CH_3)$_2$, CH-C, 2-CH-, respectively. The spectral peaks were obtained at δ158, 198 for four ethylene groups 2O=C-C=C-, 2O=C-C=C- respectively, and at δ 201 for four ketone groups 4 -C=O. The $CDCL_3$ has been used as reference material.

^{1}H-NMR (DMSO-d_6-400 MHZ):δ(ppm)6.7 , 7.0 , 7.2 , 7.7 ,8.24.

^{1}H-NMR spectra showed the expected signals which corresponds to the various functional groups present in the Dithymoquinonecompound. The signals which appeared at, δ 6.7indicated for doublet of six hydrogen atoms, 2CH_3-CH-CH_3, and the singlets were observed at δ 7.0 ,7.2 ,8.24, for hydrogens atom, 2C=CH, - C-CH_3 , 2C-H respectively. The signals which appeared at,δ 7.7indicated the presence of multiplet for hydrogen atoms, 2CH_3-CH-CH_3.The DMSO-d_6 has been used as reference material. All the chemical shifts were reported to δ ppm and presence of carbon, hydrogen and oxygen was confirmed by elemental analysis. Anal. Calcd. For compound (C =73.15 % ;H=7.37 %;O=19.49 %)found Molecular Formula; $C_{20}H_{24}O_4$.

COMPOUND-4

C^{13} NMR (CDCL$_3$, 400 MHZ): δ(ppm) 34, 50, 57, 110,113, 130, 131, 141, 168.

C^{13} NMRspectra showed the expected signals which corresponds to the various functional groups present in the damasceninine compound. The spectral peaks which appeared at, δ 34 and 50 indicated for two methyl group NH-CH_3 and CH_3-O-C=O respectively. The spectral peak was obtained at δ57 for methoxy group, OCH_3. The spectral peaks appeared at δ 110, 113, 130 ,131,141,for different carbons atom in aromatic group C=O=Ar-H, CH_3-O-Ar-H, 3Ar-H,respectively, and at 168 for carbonyl group CH_3OC=O-Ar. The CDCL$_3$ has been used as reference material.

^{1}H-NMR (DMSO-d_6-400 MHZ):δ (ppm) 3.588, 3.629, 4.567, 5.00, 5.095, 5.413, 5.491.

^{1}H-NMR spectra showed the expected signals which corresponds to the various functional groups present in the damasceninine compound .The doublet was observed at δ 3.588

for methyl group, NH-CH_3, and the quartet at3.629 for hydrogen atom NH-CH_3. The singlets were observed at δ 4.567,5.0 indicated the presence of hydrogen atom, OCH_3, OOCH_3, respectively and at 5.095, 143, 5.491 singlets for different of hydrogen atoms in aromatic region, 3Ar-H,. The DMSO-d_6 has been used as reference material. All the chemical shifts were reported to δ ppm and presence of carbon, hydrogen, oxygen and nitrogen was confirmed by elemental analysis.Anal.Calcd. For compound (C =61.53 % ;H=6.71 %;O=24.59 % ;N=7.18%)found Molecular Formula; $C_{10}H_{13}NO_3$.

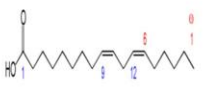
C^{13} NMR (CDCL$_3$, 400 MHZ): δ(ppm) 63, 65.2, 68, 70, 90,110, 111, 111.2,125,136,145.8,164,167.

C^{13} NMRspectra showed the expected signals which corresponds to the various functional groups present in theTaninns (tannic acid) compound. The signals which appeared at,δ 63for carbon atom in sugar molecule and at 65.2 for methylene group ,O-CH_2-Sugar. The spectral peak was obtained at δ 70 for 2- carbon atoms in sugar molecule, and at 90 indicated the presence of ketone group, CO-C-O. The spectral peaks were observed at δ110, 111, 111.2 125, 136,145.8 for different carbon atoms in aromatic region, n(Ar-H) .The signals which appeared at δ 164 and 167, for carbonyl groups, Sugar -O-C=O and 3 O-C=O-Ar, respectively. The CDCL$_3$ has been used as reference material.

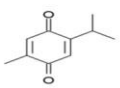
^{1}H-NMR (DMSO-d_6-400 MHZ):δ(ppm)3.4 , 3.631 ,4.7 ,5.094 ,5.495.

^{1}H-NMR spectra showed the expected signals which corresponds to the various functional groups present in the Taninn (tannic acid) compound. The signal which appeared at δ3.4, 3.631 ,4.7 ,5.094 ,5.495, indicated the presence of different singlet of hydrogen atoms -OH- pentose sugar, H -pentose sugar *J*=30.88, C-H, Ar-H, Ph-OH, respectively. The DMSO-d_6 has been used as reference material,all the chemical shifts were

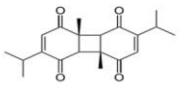
reported to δ(ppm) and presence of carbon, hydrogen and oxygen was confirmed by elemental analysis.Anal.Calcd. For compound (C =51.79% ;H=3.58%;O=44.64%)found Molecular Formula; $C_{34}H_{28}O_{22}$



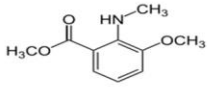
1)-9, 12-linoleic acid



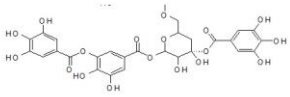
2)-Thymoquinone



3) dithymoquinone



4)-damasceninnine



5)-tannic acid

Antibacterial activity

Antibacterial activity was recorded if the radius of zone of inhibition was greater than 4mm **(Hammer *et al*., 1999).** The antibacterial activity results was considered as inactive if < 4.5 mm; 4.5-6 mm as partially active; while 6.5-9 mm as active and greater than 9mm as very active **(Junior and Zanil, 2000)**. In present study, isolated compounds from *Nigella sativa* seeds were screened for their antibacterial activity by disc diffusion method against four pathogenic bacteria, gram positive *Bacillus megaterium*, *staphylococcus aureus* and gram negative, *pseudomonas aeruginosa, klebsila pneumonia* using tetracycline as standard (4mg/ml in THF) antibiotic.

The isolated compounds showed inhibitory activity against all the four pathogenic bacteria **table 4.3** and **plate. 4.1**.

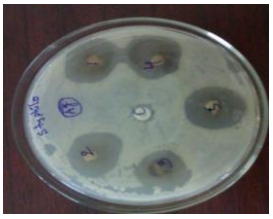
Table 4.3: Antibacterial activity of isolated compounds against selected bacteria.

Micro-organisms	Zone of inhibition(in cm)					
	Linoleic acid	Thymoquinone	Dithymoquinone	Damasceninine	Tannic acid	Antibiotic
B. megaterium	2.4	2.2	1.6	1.8	1.7	2.8
S. aureus	1.9	1.2	2.2	1.6	1.0	2.4

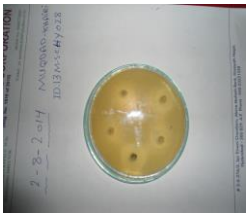
P. aeruginosa	2.4	2.1	2.6	2.4	1.5	2.6
K. pneumonia	1.5	1.2	2.6	2.3	2.0	2.6

The results of antibacterial activity showed that the compounds isolated from methanolic extract of *Nigella sativa* seeds are highly active against both Gram positive and Gram negative bacteria. The radius of zone of inhibition ofthe isolated compounds against different bacteria was close to the tetracycline used as standard antibiotic. Among the compounds, dithymaquinone showed the highest zone of inhibition (2.6cm) against both the gram negative bacteria which is same as shown by tetracycline (standard antibiotic) against the same bacteria.**(Morsi, 2000)** observed that different crude extracts of *Nigella sativa* seeds were more active against gram negative bacteria than the gram positive bacteria. This positive inhibition may be attributed to the important active ingredients of *Nigella sativa* seeds **(Bakathir and Abbas, 2011)**

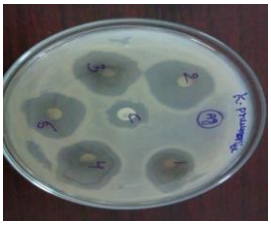
S.aureus



P.aeruginosa



K. pneumoneae



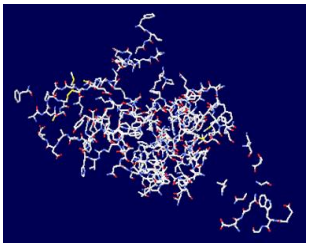
B. megaterium

Plate 4.1: Antibacterial activity of isolated compounds and standard antibiotic (tetracycline)

According to a study by **Kahsai, (2002)** dithymoquinone, a phenolic compound, present in the *N. sativa* exerted a remarkable inhibitory action on different bacterial strains. **(Mohammad *et al*., 2013)** reported that extracts of *N. sativa* showed high inhibitory activity against a range of bacteria resistant to antibiotics. This inhibition is due to the presence of various phenols. Phenols act by inhibiting the synthesis of the cell wall by inducing changes in the structure of the membranes by inhibiting bacterial protein synthesis. The mechanism of inhibition by extracts remains however unclear. Phenolic compounds sensitize membrane phospholipids, which leads to an increase in the permeability and causes a leakage of intracellular components including bacteria enzyme systems **(Singh *et al*., 2002)**.This study revealed that the compounds isolated from methanolic extract of *Nigella sativa* seeds demonstrated strong inhibitory effect on the test organisms. The results therefore established a good support for the use of the compounds in medicinal world to treat the infectious diseases.

Anticancer Activity

In present study, five compounds isolated from methanolic extract of *Nigella sativa* seedswere screened for their anticancer activity by chemo-informatics. BCL-2(1G5M) protein obtained from PDB databank was used during the study.



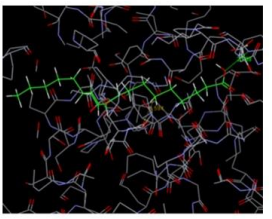
Structure of BCL-2 protein

Active site Identification of BCL-2

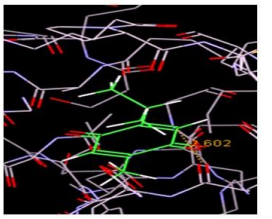
After the final model was built, the possible binding sites of BCL-2 (1G5M) was searched based on the structural Comparison of template and the model build and also with CASTP server shown in Fig. 4.7. Infact from the final refined model of BCL-2 (1G5M) domain using SPDBV program it was found that secondary structures are highly conserved and the residues, ASP-65, CYS-66, CYS-88, TYR-112, LEU-113, LEU-115, ASP-117, ILE-118, GLN-136.

Docking of inhibitors with the active site of BCL2

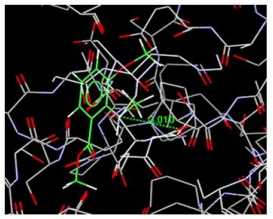
Docking of the inhibitors with BCL-2 was performed using GOLD 3.0.1, which is based on genetic algorithm. This program generates an ensemble of different rigid body orientations (poses) for each compound conformer within the then passes each molecule against a negative image of the binding site. Poses clashing with this 'bump map' are eliminated. Poses surviving the bump test are then scored and ranked with a Gaussian shape function. The binding pocket using the ligand-free protein structure and a box enclosing the binding site were defined. This box was defined by extending the size of a cocrystalized ligand by 4A. This dimension was considered here appropriate to allow, for instance, compounds larger than the cocrystallized ones to fit into the binding site. One unique pose for each of the best-scored compounds was saved for the subsequent steps. The compounds used for docking was converted in 3D with SILVER. To this set, the substrate corresponding to the modeled protein was added. Docking of best inhibitor with the active site of protein showed the activity of the molecule on protein function (Fig 4.9,4.10,4.11,4.12,4.13 and table 4.2)



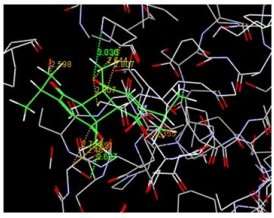
Interaction the $BCL_2$of Conjugated fatty acid



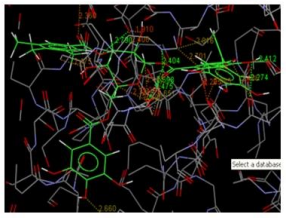
Interaction the $BCL_2$of Thymoquinone



Interaction the $BCL_2$of dithymoquinone



Interaction the $BCL_2$of Tannin



Interaction the $BCL_2$of damasceninine

Gold score fitness function

The docking of drugs into the active site of BCL-2 was performed using the GOLD software and the docking evaluations were made on the basis of GoldScore fitness functions. Gold fitness score is preferred than Chemscore fitness as Gold fitness score is marginally better than Chemscore fitness function.

Gold Score performs a force field based scoring function and is made up of four components.

(i) Protein-ligand hydrogen bond energy (external H-bond) (ii) Protein-ligand vanderwaals energy (external vdw) (iii) Ligand internal vanderwaals energy (internal vdw) (iv) Ligand intramolecular hydrogen bond energy (internal- H- bond). The external vdw score was multiplied by a factor of 1.375 when the total fitness score was computed. This was an empirical correction to encourage protein-ligand hydrophobic contact. The fitness function has been optimized for the prediction of ligand binding positions.

GoldScore = S (hb_ext) + S (vdw_ext) + S (hb_int) + S (vdw_int).

Where S (hb_ext) is the protein-ligand hydrogen bond score, S (vdw_ext) is the protein-ligand van der Waals score, S (hb_int) is the score from intramolecular hydrogen bond in the ligand and S (vdw_int) is the score from intramolecular strain in the ligand.The docking results revealed that Dithymoquinone having more docking score (32.62K.Cal/mol) than other drugs used therefore it has best anticancer activity whereas Tannin have less anticancer activity as it showed less docking score (23.97Kcal/mol). **(Deepika*et al*., 2011)**

Table 4.2: Docking studies of compounds with BCL-2

S.No	Docking Score (K.Cal/mol)	S(hb_ext)	S(vdw_ext)	S(vdw-int)	Ligand name
1	31.62	0.00	40.06	-23.45	Conjugated fattyacid
2	24.23	0.00	25.00	-11.14	Damascenine
3	32.62	0.00	29.61	-8.09	Dithymoquinone
4	23.97	4.24	46.88	-43.72	Tannin
5	27.77	0.00	24.24	-5.56	Thymoquinone

Conclusion

In fact, most of the major anticancer and antibacterial drugs are derived from plant or microganisms. With the advent of biotechnology, chemo-informatics and various tools and techniques available, researchers have been able to elucidate the molecular mechanism of plant products interaction with human body. The frequency of serious cancer diseases are rising continuously, this increases in cancer disease has been accompanied by the development of new and less toxic anticancer agents. Therefore, the present study was designed to isolate different compounds from methanolic extract of *Nigella sativa* seeds and their potential role as anticancer and antibacterial agents. Five different compounds i.e. Linoleic acid, Thymaquinone, Dithymaquinone, Damascenine and Tannic acid were isolated from the methanolic extract of *Nigella sativa*

seeds by using column chromatography and later identified by NMR spectroscopy. The result of anticancer activity of isolated compounds performed in dry lab showed that Dithymaquinon acts as best anticancer agent than the other four compounds. The isolated compounds were also tested for antibacterial activity against different gram positive (*B. megaterium* and *S. aureus*) and gram negative (*P. aureginosa* and *K. pneumoneae*) bacteria by disc diffusion method. The antibacterial activity results were expressed in terms of radius of zone of inhibition. The results of antibacterial activity showed that the compounds isolated from methanolic extract of *Nigella sativa* seeds are highly active against both Gram positive and Gram negative bacteria. Among the compounds, dithymaquinone showed the highest zone of inhibition (2.6cm) against both the gram negative bacteria which is same as shown by tetracycline (standard antibiotic) against the same bacteria whereas tannic acid showed least zones of inhibition against both gram positive and gram negative bacteria. It may be concluded from the present study that all the five compounds isolated from *Nigella sativa* seeds can be used as anticancer as well as antibacterial agents.

REFERENCES

Aggarwal, B.B., Kunnamakkara, A.B., Harikumar, K.B., Tharakan, S.T., Sung, B., and Anand, P.(2008). Potential of Spice -Derived Phytochemicals for Cancer Prevention. *Planta Medicine ;*74(13):1560-9.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., and Turck, M. (1966). Antibiotic susceptibility testing by Standard Single Disc Diffusion Method. *Ame J of Clinical Pathology;* 36:493-496. *BMC Genomics;* 14:(915) 1471-2164.

Kahsai, AW. (2002). Isolation and characterization of active ingredients from *Nigella S* for antibacterial screening.Master's thesis, Department of chemistry, East Tennesse State University.

Linn, J., Opoku, A. R., Geheeb-Keller, M., Hutchings, A.D., Terblanche, S. E., Jager, A. K. and Staden, J. V. (1999) Preliminary screening of some traditional Zulu materials for anti-inflammatory and anti-microbial activities. *J of Ethnopharmacology*.68: 267-274.

Mehta, B.K., Pandit, V., and Gupta, M.(2009).New principles from seeds of *Nigella sativa. Natural Product Research*, Part A; 23(2): 138-48.

Mohammad, H.I., Iffat, Z.A. and Mohammad, T.S. (2013). Antibacterial activity of Nigella S seed in various germination phases on clinical bacterial strains isolated from human patients. *J Biotech. Pharma. Res*. 4(1): 8-13.

Singh, N., Singh, R.K., Bhunia, A.K., Stroshine, R.L.(2002). Efficacy of chlorine dioxide, ozone and thyme essential oil or a sequential washing in killing *Escherichia coli* on lettuce and baby carrots. *F Sci Tech*; 35: 720-729.

Zhang, D. Ge., and Chen, Q. (2012). Flavonol glycosides, nigelflavonosides A-F from the whole plant of *Nigella glandulifera* (Ranunculaceae). *Journal of Natural Medicines*; 66:645–652