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PHYTOCHEMICAL PROFILING OF NIGELLA SATIVA L.

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ABSTRACT

The present investigation was carried out in the plant *Nigella sativa* L. The seeds of *N. sativa* L. were screened for the presence of biologically active compounds by suitable methods. The present investigation deals with the study of phytochemical profiling of a medicinal plant *N. sativa* L. which covers the screening of preliminary phytochemical compounds, analyzing the phytoconstituents using GC-MS, identifying the functional groups using FT-IR and absoption range using UV-VIS. The study was undertaken to identify and analyze the phytochemical constituents which may be responsible for the development of new drug. The preliminary phytochemical analysis showed the presence of major phytoconstituents like alkaloids, phenolic compounds, flavonoids etc. The studies on the bioactive components in the methanolic seed extract of N. sativa L. by GC-MS analysis clearly showed the presence of four bioactive compounds such as 2-hexyldecanoic acid (220.19%), 2-Hydroxy – 14 – methyl – oxa – cyclotetradec – 6 – en – 2 – one (122.04%), 11-Hexadecyn – 1 –ol (622.56%), 6, 9 - octadecadienoic acid methyl ester (226.27%). Infra-red spectrum of methanolic and aqueous seed extracts of N. sativa L. showed the presence of alkyl halide, alcohol, aromatic, acid, alkyne functional group. The results of UV-VIS spectrum analysis for both methanolic and aqueous seed extract of N. sativa showed the absorption at 311 nm (-0.403) and 310 (-0.110) respectively.

KEYWORDS: *Nigella sativa* L., phytochemical profiling, GC-MS, FTIR, UV.

INTRODUCTION

Today there is growing interest in chemical composition of plant based medicines. Several bioactive constituents have been isolated and studied for pharmacological activities. During

the last two decades, the pharmaceutical industry has made massive investment in pharmacological and chemical researches all over the World in an effort to discover much more potent drugs, rather, a few new drugs.[1] Phytomedicine applies scientific research and the highest proficient standards to the practice of herbal medicine.[2] Plant based drugs remain as an important source of therapeutic agents because of the availability, relatively cheaper cost and non-toxic nature when compared to modern medicine and they have received considerable attention in recent years due to their diverse pharmacological properties. There are nearly 2000 ethnic groups in the World and almost every group has its own traditional medical knowledge and experiences.[3], [4]

Therapeutic plants are the most essential component of natural wealth, which serve the mankind and animal kingdom with its active therapeutic agents and its most precious raw therapeutic materials for the synthesis of conventional and modern day medicines.[5] Therapeutic herbs are the best source for the peculiar drugs and that plays a incredible role in the preparation of traditional of modern modecines.[6] The logic behind the usage of this active constituents in therapeutic industry is because of its active constituents.[7]

Nigella sativa L. belongs to the family Ranunculaceae[8], which is established all over the world but specific to Eastern European region, Middle East, and western Asia.[9] It is a shrub with tiny leaves, the flower colour differs from purple to white. Fruits are tiny and dark in colour. Usually seeds are also black in colour.[10] The essential volatile oil and some of the major phytoconstituents like protein, alkaloid and saponins are reported in various studies.[11],[12]

MATERIALS AND METHODS

Preparation of plant extracts

The healthy seeds of *N. sativa* L. was collected and used for the study. Then the seeds were dried in shade at room temperature (31°C) and ground into powder. They were kept in hot air oven for complete drying (80°C). Then it was homogenized and extracted with different types of solvents.

A known quantity (10g) of the dried powdered form of the seeds were taken and soaked in 50ml of aqueous, methanol, ethanol and petroleum ether separately in bottles and closed with corks and were kept for seven days at 31°C (room temperature) for complete extraction. Then the filtrate was evaporated at reduced pressure to remove residual solvent and moisture.

Preliminary Phytochemical Analysis

The screening of qualitative phytochemical constituents of four solvent extracts of the seeds of *N. sativa* L. was carried out according to the standard methods.[13] The extracts prepared for the screening of qualitative phytochemical constituents were namely: Aqueous Extract, Methanol Extract, Ethanol Extract and Petroleum Ether Extract.

Preliminary phytochemical studies includes the test for steroids, reducing sugars, sugar, alkaloids, phenolic compounds, catechins, flavonoids, saponins, tannins, anthroquinones and test for amino acid.

Gas Chromatography-Mass Spectrum (Gc-Ms)

Extraction of plant material

10gm of powdered seeds of *N. sativa* L. was soaked in 20ml of absolute alcohol overnight and then filtered through Whatmann No.1 filter paper along with 2gm Sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wet with absolute alcohol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and was concentrated to 1ml. The extract contains both polar and non-polar phytocomponents.

GC-MS Analysis

GC-MS analysis was carried out on a integrated unit of GC Clarus 500 Perkin Elmer system and mass spectrometer (GC-MS) instrument. Interpretation and identification of mass spectrum obtained from the databases.

Infrared Spectra (FT-IR)

IR spectroscopic analysis is a very useful tool in the detection of functional groups of biomolecules, thus aiding in their structrural elucidation.

Procedure

IR spectra of the plant substance was measured in an automatic recording IR Spectrophotometer either in solution (in chloroform or carbon tetrachloride, 1-5%) as a mull with nujol oil or in the solid state, mixed with potassium bromide. In the latter case, a thin disc was prepared under anhydrous conditions from a powder containing about 1mg of material and 10-100mg potassium bromide, using a mould and press. The range of measurement was from 4000 to 667cm^{-1} (or 2.5 to 15µm) and the spectrum was recorded

within 3 minutes. IR spectoscopy is most frequently used in phytochemical studies as a finger printing device for comparing a natural with a synthetic sample, which is very important in the complete identification of many types of plant constituent. It can also contribute to structural elucidation, when new compounds are encountered in plants.

UV-VISIBLE Spectrum

The methanolic and aqueous seed extract was examined under UV Visible spectrum analysis using standard procedures.

RESULTS AND DISCUSSION

Qualitative or Preliminary Phytochemical analysis

The various solvent extracts of the seeds of *N. sativa* L. also showed the presence of major phytoconstituents (Table-1). The methanolic seed extract of *N. sativa* L. showed the presence of major compounds compare to other extracts used. It revealed the presence of eight compounds such as steroids, reducing sugar, sugar, phenolic compounds, alkaloids, flavonoid, saponins and tannins.

Followed by the aqueous seed extract of *N. sativa* L. showed the presence of six compounds namely steroids, reducing sugar, alkaloids, saponins, tannins and amino acids. Likewise the ethanolic seed extract of *N. sativa* L. showed the presence of five compounds such as steroids, reducing sugar, phenolic compounds, flavonoid, saponins. The petroleum ether seed extract of *N. sativa* L. contains four phytoconstituents such as steroid, sugar, alkaloids and saponins.

Table 1: Preliminary Phytochemical analysis of different solvent extract of *N. sativa* L.

Phytochemicals	Aqueous	Methanol	Ethanol	Petroleum ether
Steroids	+	+	+	+
Reducing sugar	+	+	+	-
Sugars	-	+	-	+
Alkaloids	+	+	-	+
Phenolic compounds	-	+	+	-
Catachins	-	-	-	-
Flavonoids	-	+	+	-
Saponins	+	+	+	+
Tannins	+	+	-	-
Anthraquinones	-	-	-	-
Amino acids	+	-	-	-

+ Present - Absent

These results are in agreement with those of Sania Feroz and Ghias Uddin[14], who investigated the preliminary phytochemical analysis from the crude methanolic extract of *N. sativa* L. seeds and the results showed the presence of various phytochemicals like alkaloids, terpenoids, tannins, reducing sugars, saponins, caumarine etc. Likewise similar reports were found out from the report of Ahmad *et al.*,[15], who performed the qualitative and quantitative phytochemical analysis in *N. sativa* L. seeds and detected various bioactive compounds using TLC.

Gas Chromatography Mass Spectroscopy (GC-MS)

The studies on the bioactive components in the methanolic extract of the seed extract of *N. sativa* L. by GC-MS analysis clearly showed the presence of four bioactive compounds. The active principles presented the experimental plant showed in Table-2.

Table 2: Bioactive Components detected in the methanolic seed extract of *N. sativa* L.

S.No	Retention Time	Name of the Compound	Molecular Formula	Molecular Weight	Peak area
1	20	2-hexyldecanoic acid	$C_{16}H_{32}O_2$	256	220.19
2	21	2-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-one	$C_{14}H_{24}O_3$	240	122.04
3	22	11-Hexadecyn-1-ol	$C_{16}H_{30}O$	238	622.56
4	26	6,9-octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294	226.27

The GC-MS chromatogram of the four peaks of bioactive compounds detected were shown (Figure-1) and their phytoconstituents were identified by the mass spectroscopy.

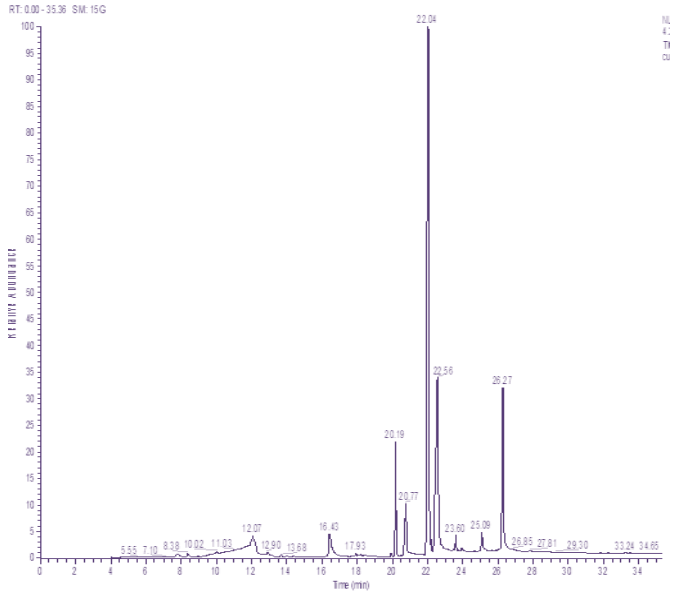

Fig-1: GC-MS chromatogram of methanolic seed extract of *N. sativa* L.

The Table-3 listed out the major phytocompounds and its biological activities obtained through the GC-MS study of the methanolic seed extract of *N. sativa* L. Fig-2, 3, 4 and 5showed the mass spectrum of four bioactive constituents. The biological activities were listed based on phytochemical and ethnobotanical databases.

Table 3: Biological Activity of phytocompounds identified in the methanolic seed extract of *N. sativa* L.

S.No.	Name of the compound	Nature of the compound	Biological Activity
1	2-hexyldecanoic acid	7-isojasmonic-acid	Acidifier, Acidulant, Arachidonic-Acid-Inhibitor, Increase Aromatic Amino Acid Decarboxylase Activity, Inhibit Production of Uric Acid, Urinary-Acidulant, Urine-Acidifier
2	2-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-one	oxacyclonnonadec-10-en-2-one	Decrease Glutamate Oxaloacetate Transaminase, Decrease Oxalate Excretion, Low Oxalate, Decrease Endothilial Leukocyte Adhesion, Decrease Endothilial Platelet Adhesion, Encephalopathic, Endoanesthetic, Endocrinactive Endocrinprotective, Endorphinogenic Endothelium-Dependent, Endothelium-Derived Relaxing Factor Promoter, Enterocontractant, Enterodepressant, Enteromotility- Enhancer, Enterostimulant, Enterorelaxant.
3	11-Hexadecyn-1-ol	cyclo-olivil	Provide Oligosaccharides
4	6,9-octadecadienoic acid methyl ester	octadecadienoic-acid	Catechol-O-Methyl-Transferase-Inhibitor, Methyl-Donor, Methyl-Guanidine-Inhibitor, Acidifier, Acidulant, Arachidonic acid-Inhibitor, Inhibit Production of Uric Acid, Increase Aromatic Amino Acid Decarboxylase Activity, Urinary-Acidulant, Urine-Acidifier

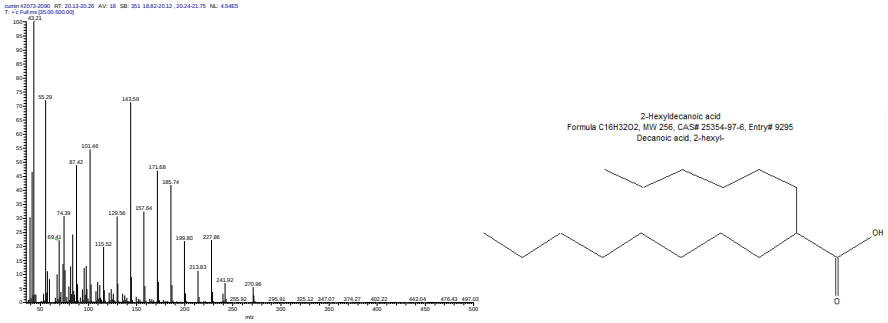

Fig-2: Mass spectrum of 2-hexyldecanoic acid.

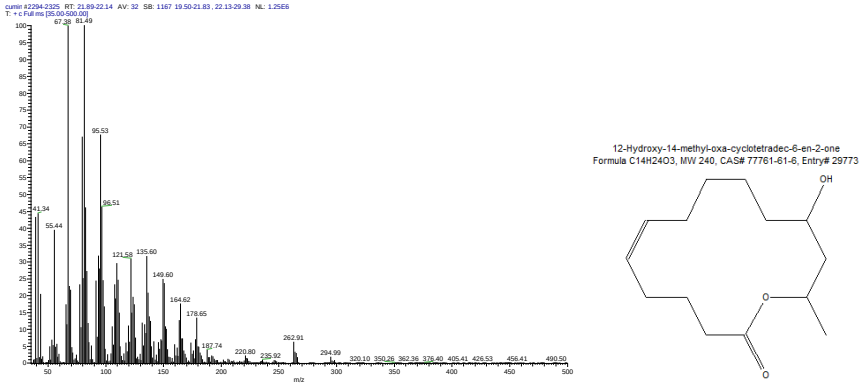

Fig-3: Mass spectrum of 12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-one.

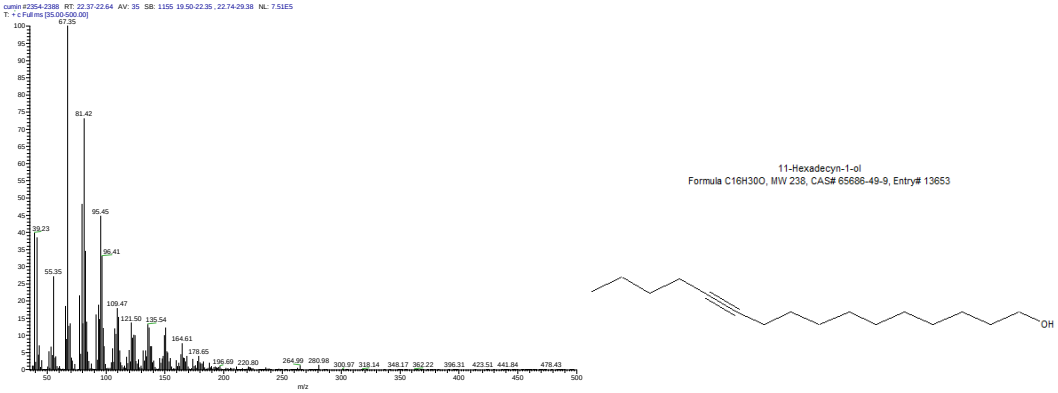

Fig-4: Mass spectrum of 11-Hexadecyn-1-ol.

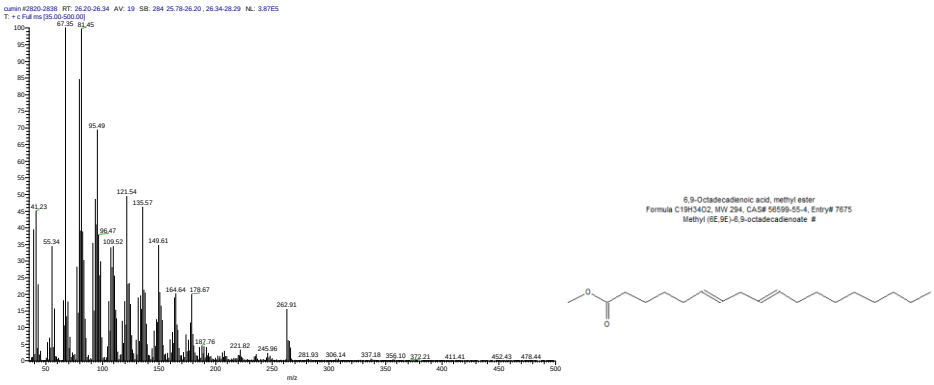

Fig-5: Mass spectrum of 6 9-octadecadienoic acid methyl ester.

The results of GC-MS study was supported by many authors. Krishnaveni and Saranya[16] identified twelve compounds and Hadi *et al*.,[17] identified about twenty eight bioactive phytochemical compounds. Mehta *et al*.[18] studied the GC-MS of *N. sativa* (seeds) and the results of their study revealed the presence of 26 compounds. Nivetha and Prasanna[19], identified nine bioactive compounds in *N. sativa* L. seeds using Gas Chromatography-Mass Spectroscopy (GC-MS).

FT-IR Analysis of phytocompounds

Infra-red spectrum of methanolic extracts of *N. sativa* L. showed the presence of Alkyl Halide, Alcohol, Aromatic, Acid, alkyne functional group. These were the useful compounds identified in *N. sativa* L. (Table-4, Fig-6). The authentication of functional groups and identification of compounds was done with the published article.[20]

Table- 4: FT-IR analysis of methanolic seed extract of *N. sativa* L.

S.No.	Wave number cm^{-1} [Test sample]	Wave number cm^{-1} [Reference article]	Functional group assignment	Possible compounds
1	414.7	~500	C-I Strech	Alkyl Halide
2	445.56	~500	C-I Strech	Alkyl Halide
3	466.77	~500	C-I Strech	Alkyl Halide
4	513.07	500-600	C-Br stretch	Alkyl Halide
5	538.14	500-600	C-Br stretch	Alkyl Halide
6	599.86	500-600	C-Br stretch	Alkyl Halide
7	646.15	600-800	C-Cl stretch	Alkyl Halide
8	1022.27	1000-1400	C-F stretch	Alkyl Halide
9	1112.93	1000-1400	C-F stretch	Alkyl Halide
10	1413.82	1350-1480	-C-H bending	Alkane
11	1452.4	1400-1600	C=C stretch	Aromatic
12	2833.43	2500-3300	O-H stretch	Acid
13	2929.87	2850-3000	C-H stretch	Alkane
14	3331.07	3200-3600	O-H stretch	Alcohol

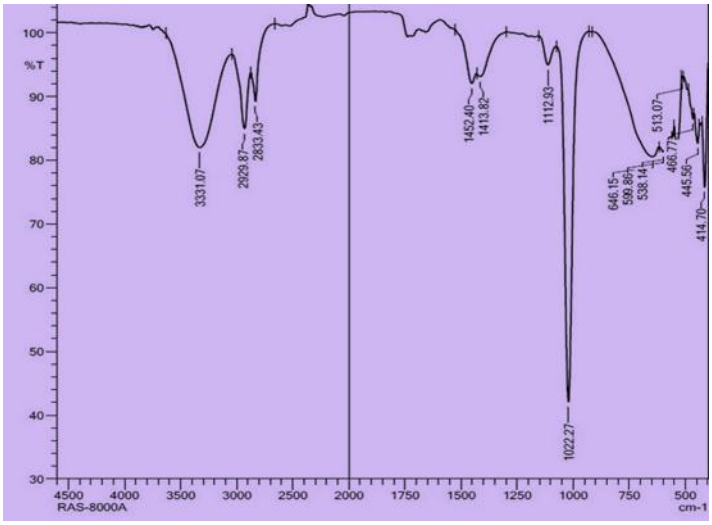

Fig- 6: FT-IR spectrum of methanolic seed extract of *N. sativa* L.

Infra-red spectrum of aqueous extracts of *N. sativa* L. showed the presence of Alkyl Halide, Alcohol, Aromatic, Alkane, alkyne functional group. (Table-5, Fig-7).

Table- 5: FT-IR analysis of aqueous seed extract of *N. sativa* L.

S. No.	Wave number cm^{-1} [Test sample]	Wave number cm^{-1} [Reference article]	Functional group assignment	Possible compounds
1	455.2	~500	C-I Strech	Alkyl Halide
2	553.57	500-600	C-Br stretch	Alkyl Halide
3	597.93	500-600	C-Br stretch	Alkyl Halide
4	653.87	600-800	C-Cl stretch	Alkyl Halide
5	1074.35	1050-1150	C-O stretch	Alcohol
6	1411.89	1400-1600	C=C stretch	Aromatic
7	1637.56	1620-1680	C=C stretch	Alkene
8	2131.34	2100-2260	C≡C stretch	alkyne
9	3331.07	3200-3600	O-H stretch, H bonded	Alcohol

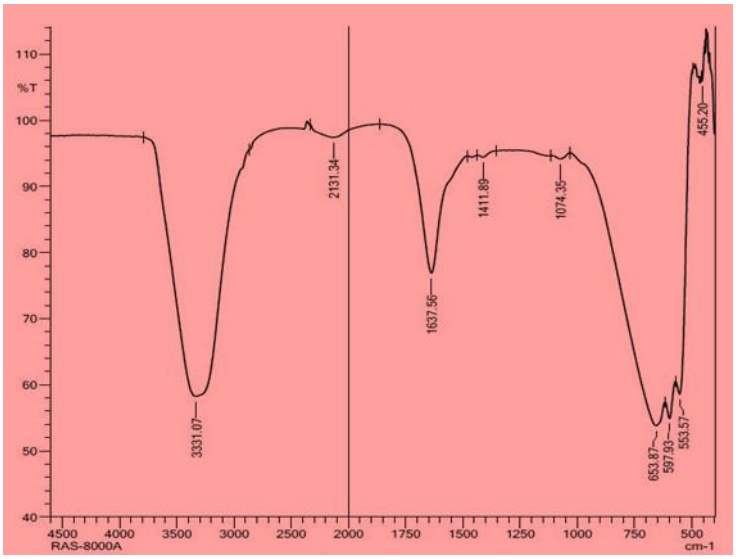

Fig-7: FT-IR spectrum of aqueous seed extract of *N. sativa* L.

Our results are the evidence with the report of some authors[16], who carried out the study of FT-IR which helps in analyzing the phyto-compounds, functional groups present in *N. sativa* L. Similar type of functional groups also identified in different genus like *Micrococca mercurialis*[21], *Solanum torvum.*[22]

UV-VIS Spectrum Analysis

Table-6 showed the result of both methanolic and aqueous seed extract of *N. sativa* L. The results of UV-VIS spectrum analysis for methanolic seed extract of black cumin (*N. sativa* L.) showed the absorption a 311 nm (-0.403). The results of UV-VIS spectrum analysis for aqueous seed extract of black cumin (*N. sativa* L.) showed the absorption at 310 nm (-0.110).

Table-6: UV- VIS Spectra of methanolic and aqueous seed extract of N. *sativa* L.

S.No	Methanol		Aqueous	
	Wavelength	Absorption	Wavelength	Absorption
1.	311	0.403	310	-0.110

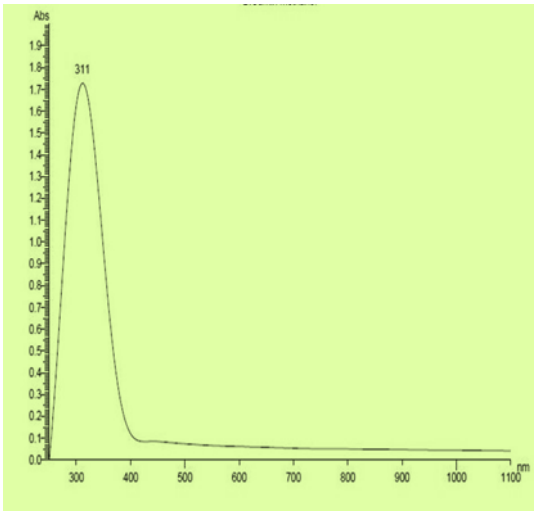

Fig- 8: UV-VIS Spectra of methanolic seed extract of *Nigella sativa* L.

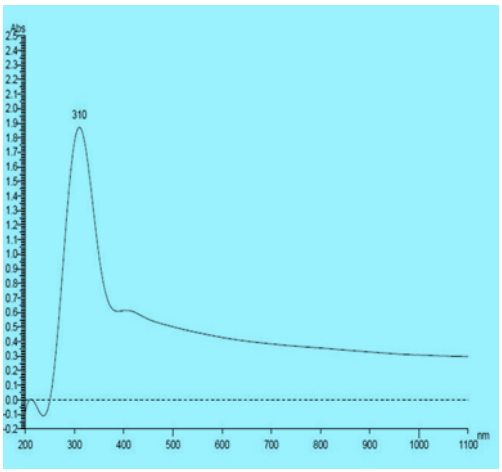

Fig- 9: UV-VIS Spectra of aqueous seed extract of *Nigella sativa* L.

Our findings are concordant with the findings of many authors. The bioactive constituents present in leaf extract of *Meizotropis pellita* using UV-VIS spectroscopy and the results showed different the peaks at different nm with the different absorption.[23] The characterized the bioactive constituents present in different leaf extracts of *Stylosanthes fruticosa*[24] and in *Micrococca mercurialis* using UV-Vis spectrum analysis.[21] Their results showed different peaks ranging from 200-600 nm with different absorption respectively.

Similar results obtained from the findings of some authors.[25] They investigated the phytochemical compounds in the leaf extracts of *Vitex negundo* L. and showed different peaks with different absorption values respectively.

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