ABDULLAHI GARBA USMAN

QUANTITATIVE DETERMINATION OF THYMOQUINONE USING HPLC TECHNIQUE AND DETERMINATION OF ITS ANTI-CANCER ACTIVITY IN HUMAN COLON CANCER CELL LINE FROM NIGELLA SATIVA L. SEEDS OF DIFFERENT GEOGRAPHICAL REGIONS: A CHEMOMETRICS BASED APPROACH

PhD THESIS

YEAR 2021

NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF ANALYTICAL CHEMISTRY

QUANTITATIVE DETERMINATION OF THYMOQUINONE USING HPLC TECHNIQUE AND DETERMINATION OF ITS ANTI-CANCER ACTIVITY IN HUMAN COLON CANCER CELL LINE FROM *NIGELLA SATIVA L.* SEEDS OF DIFFERENT GEOGRAPHICAL REGIONS: A CHEMOMETRICS BASED APPROACH

PhD. THESIS

Abdullahi Garba USMAN

Nicosia

January, 2022

NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF ANALYTICAL CHEMISTRY

QUANTITATIVE DETERMINATION OF THYMOQUINONE USING HPLC TECHNIQUE AND DETERMINATION OF ITS ANTI-CANCER ACTIVITY IN HUMAN COLON CANCER CELL LINE FROM *NIGELLA SATIVA L.* SEEDS OF DIFFERENT GEOGRAPHICAL REGIONS: A CHEMOMETRICS BASED APPROACH

PhD. THESIS

Abdullahi Garba USMAN

Supervisor

Assist. Prof Dr. Selin IŞIK

Nicosia

September, 2021

Approval

Thesis defense was held online. The Jury members declared their acceptance verbally, Which is recorded,

Examining Committee	Name-Surname
Head of the Committee:	Prof. Dr. Dudu Ozkum
Committee Member*:	Assoc. Prof. Dr. Hayati Celik
Committee Member*:	Assist. Prof. Dr. Hurmus Refiker
Committee Member*:	Assist. Prof. Dr. Sani Isah Abba
Supervisor:	Assist. Prof. Dr. Selin IŞIK

Approved by the Head of the Department

…../…../20…

……..

Prof. Dr. Selin IŞIK

Head of Department

Approved by the Institute of Graduate Studies

…../…../2022…

Prof. Dr.Kemal Hüsnü Can Başer

Head of the Institute

Declaration

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

Name and Surname of the Student

Abdullahi Garba USMAN

…01../…02../…2022..

Day/Month/Year

Acknowledgments

In the name of Allah, the most beneficial the most merciful. Praise be to Allah (SWT), lord of the world. Thanks and Glory to him for his endless blessings throughout my life. I will never relent by saying 'Alhamdulillah' for giving me this opportunity to complete my non-stop academic journey up-to the PhD level successfully.

My PhD journey history can never be complete without mentioning the hardworking supervisor Assist. Prof. Dr. Selin IŞIK, who always strive hard to see that the journey was successful through her daily mentorship, enthusiasm, patience, assistance and motivation. I will equally like to thank my tutors that gave me their best during the PhD course work. I will not relent to thank my PhD. Jury members for their profound support and recommendations in order to enhance the quality of my research; Prof. Dr. Dudu Ozkum, Assoc. Prof. Dr. Hayati Celik, Assist. Prof. Dr. Sani Isah Abba and Assist. Prof. Dr. Hurmus Refiker. Moreover, I will also like to use this opportunity to thank the Dean faculty of pharmacy Prof. Dr. Ihsan Calis as well as the director of graduate school institute Prof. Dr. Kemal Husnu Can Baser for their support and motivation throughout my Master and PhD programs.

At this point, I will be glad to say a special prayer to my late father Engr. Garba Usman for doing his best to see that I make it in life, may your gentle soul keep resting in peace and may Allah make Jannah as your final abode. My special thanks goes to my Mom for doing her best to see that I make it, I think she deserve more credit than any person as far my academic career is concern through her mentorship, assistance, encouragement and more importantly her prayer. My special thanks also goes to my siblings for their emotional support throughout this journey. I will always be grateful to my research teams as well as my academic network more especially; Dr. Sani Isah Abba, who mentored and collaborate with me to develop different chemometric and AI-based articles with high impact factor that can be beneficial to the academic circle. My special thanks also goes to my friends, academic peers and colleagues whom have always been source of inspiration to me through their emotional and social support. It's equally a pleasure to show my appreciation to Kano state government as well as Near East University for providing me with the scholarship opportunity.

Name and Surname of Student

Abdullahi Garba USMAN

Abstract

Quantitative determination of Thymoquinone using HPLC technique and determination of its anti-cancer activity in human colon cancer cell line from Nigella sativa L. Seeds of different geographical regions: a chemometrics based approach

Usman, Abdullahi Garba, Assist. Prof. Dr. Selin IŞIK

PhD, Department Analytical Chemistry

……02…. (Month), …2022… (Year), ……95…… (number) pages

Thymoquinone (TQ) is considered to be the main active ingredient of Black cumin. This thesis involve the quantitative determination of TQ from black cumin of different geographical regions including; India, Syria, Saudi Arabia, Iraq and Turkey. The obtained results showed that the highest amount of TQ was found in Iraq and Indian samples. Although the amount of TQ in all samples varied. TQ was detected in all samples; the amount of TQ was 0.031, 0.030, 0.022, 0.005 and 0.001% in the Iraq, India, Saudi Arabia, Syria and Turkey samples, respectively. Different geographical factors such as rainfall, seasonal variations and soil plays a vital role in the total amount of TQ present in each sample as indicated in the literature. Subsequently, the anticancer activity on human epithelial colon cell line of TQ from black cumin of different geographical regions was tested using different cytotoxity analysis. The amount of TQ affected the cytotoxic potential of the black cumin extract on Caco-2 cells. Indian black cumin were found to induce highest cytotoxic effect with highest TQ levels. This preliminary experiment provides evidence that the geographical differences were found to have an effect on the efficacy of the anticancer potency of black cumin. Finally, the chemometric applications of both artificial intelligence (AI) based models as well as ensemble machine learning approaches as one of the recent employed chemometrics methods for modelling chromatographic behavior of different analytes. The obtained results from indicates the ability of these models in predicting both the qualitative and quantitative properties of TQ.

Key Words: Anti-cancer activity, HPLC, Black Cumin, Thymoquinone, chemometrics

Ozet

Timokinon (TQ), siyah kimyonun ana etken maddesi olarak kabul edilir. Bu tez, aşağıdakiler dahil olmak üzere farklı coğrafi bölgelerdeki çörek otundan TQ'nun nicel olarak belirlenmesini içermektedir; Hindistan, Suriye, Suudi Arabistan, Irak ve Türkiye. Elde edilen sonuçlar, en yüksek TQ miktarının Irak ve Hindistan örneklerinde bulunduğunu göstermiştir. Tüm numunelerdeki TQ miktarı değişse de. Tüm numunelerde TQ tespit edildi; Irak, Hindistan, Suudi Arabistan, Suriye ve Türkiye örneklerinde TQ miktarı sırasıyla 0,031, 0,030, 0,022, 0,005 ve 0,001 idi. Yağış, mevsimsel değişimler ve toprak gibi farklı coğrafi faktörler, literatürde belirtildiği gibi her numunede bulunan toplam TQ miktarında hayati bir rol oynar. Daha sonra, farklı coğrafi bölgelerdeki çörek otundan elde edilen TQ'nun insan epitelyal kolon hücre hattı üzerindeki antikanser aktivitesi, farklı sitotoksite analizi kullanılarak test edildi. TQ miktarı, siyah kimyon ekstraktının Caco-2 hücreleri üzerindeki sitotoksik potansiyelini etkiledi. Hint çörek otu en yüksek TQ seviyeleri ile en yüksek sitotoksik etkiyi indüklediği bulundu. Bu ön deney, coğrafi farklılıkların siyah kimyonun antikanser potansiyelinin etkinliği üzerinde bir etkiye sahip olduğunun bulunduğuna dair kanıt sağlar. Son olarak, hem yapay zeka (AI) tabanlı modellerin kemometrik uygulamaları hem de farklı analitlerin kromatografik davranışlarını modellemek için son zamanlarda kullanılan kemometrik yöntemlerden biri olarak topluluk makine öğrenimi yaklaşımları. Elde edilen sonuçlar, bu modellerin TQ'nun hem nitel hem de nicel özelliklerini tahmin etme yeteneğini gösterir.

Anahtar Kelimeler: Anti-kanser aktivite, HPLC, Çörek Otu, Thymoquinone, kemometri

Table of contents

CHAPTER IV

CHAPTER V

CHAPTER VI

List of Tables

List of Abbreviations

TQ	Thymoquinone
MDA	Malondialdehyde
UV	Ultra-violet
NSA	Non-starch polysaccharides
PC	Pancreatic cancer
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
OD	Optical density
IC_{50}	50% inhibitory concentration
LC_{50}	50% lethal concentration
AML	Myeloid leukemia
LDH	Lactate dehydrogenase
SPE	Solid phase extraction method
LLE	Liquid-liquid extraction
KD	Concentration factor
AI	Artificial intelligence
PLS	Partial least squares
PCR	Principal components regression
ANNs	Artificial neural networks
SWLR	Step-wise linear regression
LR	Linear regression
PCA	Principal component analysis
SVM	Support vector machine
LSSVM	Least square-support vector machine

KKT	Karush-Kuhn-Tucker
ANFIS	Adaptive-neuro fuzzy inference system
MFs	Membership functions
LSQ-BOOST	Least square boost
GBM	Gradient boosting system
SAE	Simple average ensemble
WAE	Weighted average ensemble
NNE	Neural network ensemble
MSE	Mean square error
RMSE	Root mean square error
CC	Correlation co-efficient
NSE	Nash-Sutcliffe efficiency
tR	Retention time
PA	Peak area
RSD	Relative standard deviation
LOD	Detection limit
LOQ	Limit of quantitation
R^2	Determination co-efficient value
SHS	Switchable hydrophilicity solvents
DES	Deep eutectic solvents
(HHO)	Harris Hawks' optimization algorithms
(PSO)	Particle swam optimizations

CHAPTER I

Introduction

Nigella sativa (Black cumin) is a native plant of found in different part of the world such as Middle East region, Southwest Asia, North Africa and Southern Europe. Although the amount of *N. sativa* seeds varies (Ramadan and Mörsel, 2003). Thymoquinone (TQ) (30-48%), thymohydroquinone, dithymoquinone compounds are the main active ingredients. However, there are also other analytes like α-hederin and alkaloids present in this plant. Black cumin has been applied traditionally in treating various diseases such as in liver diseases, asthma, cardiovascular diseases, skin diseases etc (Kouidhi et al., 2011). However, there are also various articles in the technical literature on cardioprotective, antimicrobial, antihistaminic, antidiabetic, antihyperlipidemic, antihypertensive, antidiarrheal, antioxidant and anticancer effects (Woo, Kumar, Sethi, & Tan, 2012a).

The effect of cancer, which is accepted as an emerging disease recently that causes increasing mortality all over the world, and equally disrupts human lifestyle and general well-being. Mostly, anticancer agents are used naturally to support and improve the immune system, inhibits certain side effects resulted due to the use of chemotherapeutic agents and serve as auxiliary agents to various medical treatments.

80% of anti-cancer drugs are derived from plants. In addition, 177 cancer drugs used in the Indian traditional medicine system contain more than 70% derived from natural sources. Therefore, research of plant extracts as new drug led continues. Many plants, including extracts from *N. sativa* and active substances found in the plant, have been investigated as potentials in cancer therapies (Woo et al., 2012a).

Different researchers have studied the fact that various phytochemicals, which are present in plants, are responsible in preventing different diseases as well as promoting health status of human widely in order to establish facts regarding their efficacy as well to know their mechanism of action (S. H. Lee & Min, 2019). These studies consists of isolation and identification of these chemical compounds, establishment and elucidation of their pharmacological potency in both in vivo and in vitro studies. Looking at pharmacological applications of major active ingredients of *N. sativa* especially TQ, it is significant to determine both the quantitative and qualitative properties of TQ using a suitable analytical technique

(Gheshlaghi, Scharer, Moo-Young, & Douglas, 2008). The High performance liquid chromatography (HPLC) is one of the useful analytical tools that can be used in the elucidation of the chemical behaviour of various analytes extracted from different medicinal plants (Marrero-Ponce, Barigye, Jorge-Rodríguez, & Tran-Thi-Thu, 2018), (Wu et al., 2015). The chromatographic optimization of different parameters such as the mobile phase composition, column temperature, column type and flow rate are very important in order to achieve sufficient chromatographic resolution (Khader et al., 2017).

Based on the large number of factors affecting the analysis of different plants and phytochemical determination using the chromatographic technique, it is considered to be time consuming and difficult, especially through the use of the traditional single factor optimization method, whereby a parameter will be changed within a time while other parameters are kept constant within that time (Wang et al., 2018). In recent times, the coupling of the experimental approach and chemometrics-based approach was proposed using universal a optimization technique. The coupling is done using proper experimental method followed by modelling using chemometric based models by employing various dependent and independent variables (Novotná, Havliš, & Havel, 2005). The application of chemometrics has numerous application in the field of chromatography.

Methanolic extracts obtained from *N. sativa* seeds obtained from different geographical regions within the scope of this study were compared in terms of their TQ content and cytotoxic activity of the extracts in cancer cell line (human epithelial colon cancer cell line (CaCO-2), mitochondrial (MTT), lysosomal (Neutral Red) and cell membrane permeability (lactate dehydrogenase) was investigated. In addition, malondialdehyde (MDA) level, which is one of the oxidative stress parameters, was also studied.

High performance liquid chromatography (HPLC)

High-pressure liquid chromatography (HPLC) or high performance liquid chromatography (HPLC) is one of the major significant type of liquid chromatography technique, which consists of a solid and liquid stationary and mobile phase respectively(Ostertag et al., 2020). The major designed of HPLC technique is based on the idea of providing an effective separation technique, which can be attained using a well-packed column, hence, aid in substantial analysis time reduction (Sparkman, 2008). Furthermore, it involves the use of a pump that delivers the mobile phase (which carries the target compound/analyte) into the stationary phase that can

only be done using sophisticated technology. Therefore, this lead to the birth of HPLC instrumentation (Adamson et al., 1999).

Liquid chromatography is divided into 4 different categories; partition chromatography, adsorption chromatography, ion-exchange chromatography and size-exclusion chromatography (Biswas et al., 2007). Currently, the partition chromatography is the widely used chromatographic method, which is also further sub-classified into reverse and normal phase chromatography according to the polarity of their respective mobile phases (Zapata, Rodríguez and Garrido, 2000).

Figure 1

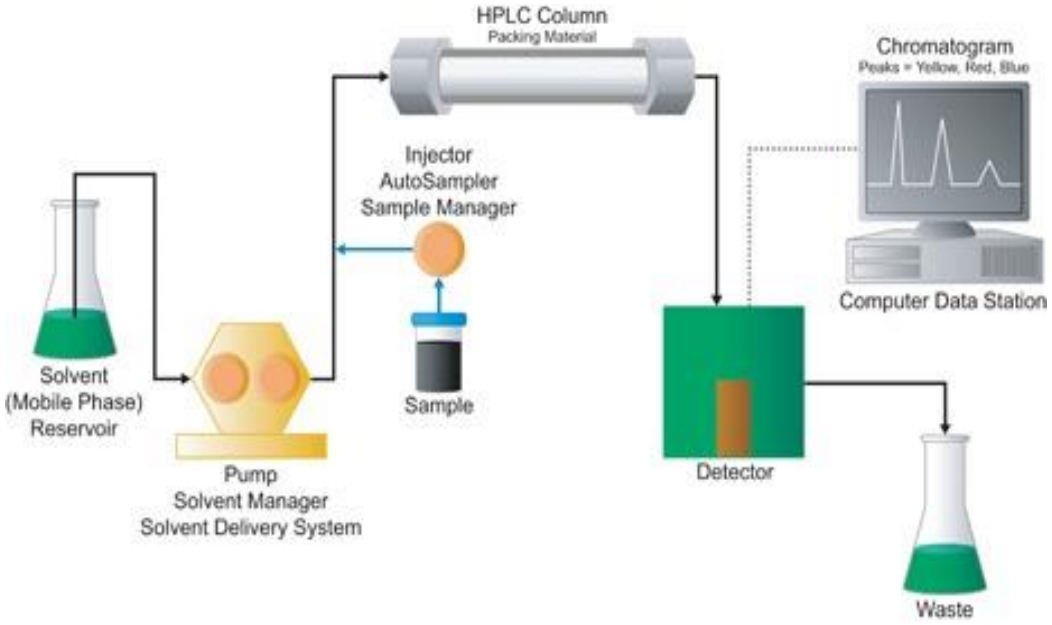
High-pressure liquid chromatography (HPLC) instrument

HPLC instrumentation

Commonly, the HPLC instrumentation consists of seven main components as demonstrated in Figure 2, which are; HPLC solvent (eluent), HPLC pump, sample injector(mostly DAD, MS and Fluorescence), HPLC column, detector, waste or sometimes sample collector and data processing unit. Moreover, other more extra accessories may be integrated to HPLC instrument in order to enhance its performance efficiency such as column oven (column thermostatic jacket), quaternary pump, guard column, degasser, auto sampler and fraction collector (Henri, 2018).

Figure 2

HPLC instrumentation



Modes of elution in HPLC

There are two kinds of elution modes that are generally used in HPLC method development, which are; isocratic and elution gradient systems. Isocratic elution mode consists of delivering the composition of the mobile phase in a constant manner throughout the analysis. While the gradient elution system composed of varying the composition of the phase during the analysis. The commonly used elution mode is the isocratic system as compared with the gradient system due to the fact that it does not need a quaternary pump (Ehlert, Trojer, Vollmer, Van De Goor, & Tallarek, 2010). Moreover, the factors that influence the chromatographic applications of the isocratic system can be understand easily. However, the isocratic system can easily be affected with the 'general elution problem' (which are; poor resolution and longer analysis time). Therefore, the gradient elution mode can be employed in order to solve these types of problems with higher resolution by varying the mobile phase composition during the analysis. Even though, some basic gradient scan can be done if we plan to use the isocratic elution system in order to replace the gradient elution and this will give some useful information that be utilized by chromatographers, which can be used in analyzing the peaks by some mathematical

calculations in order to finalize whether the isocratic can be used as a substitute or not (Nagesh, Ch, Ch, & Narayana, 2016).

Scope of study

This study focuses on three different approaches including:

1. TQ quantitative determination from *Nigella sativa* L. using the chromatographic approach
2. Determination of anti-cancer activity of the bioactive compound
3. Chemometric application of Ensemble machine learning on predicting the bioactive compound.

Aim and specific objectives

The major aim of this work is the quantitative determination of TQ from *Nigella sativa* L. (Black cumin) Seeds of different geographical regions using HPLC method development as well as the elucidation of its anti-cancer activity in Human Epithelial Colon Cancer Cell Line. The quantitative determination was equally simplified using recent and emerging chemometrics based approaches.

Limitations

The major limitations of this research is the use of a single cancer cell line and the applicatication of classical extraction method.

CHAPTER II

LITERATURE REVIEW

Phytochemistry

Phytochemistry involve the study of biologically active ingredients that are natural chemical compounds present in plants. The study of Phytochemistry equally provides health assistance to human health more than the micro and macronutrients found in plants. The Phytochemistry equally involves the understanding of the chemical compounds known as Phytochemicals and their possible applications and uses ranging from the protection they gave plants against potential damage and disease up to the protection they gave to the environment against some hazard such as UV exposure. Generally, the major essence of Phytochemistry is the elucidation of various chemical compounds from plants that can serve as lead in the protection of human health especially if their dietary intake is significant (Kurmukov, 2013).

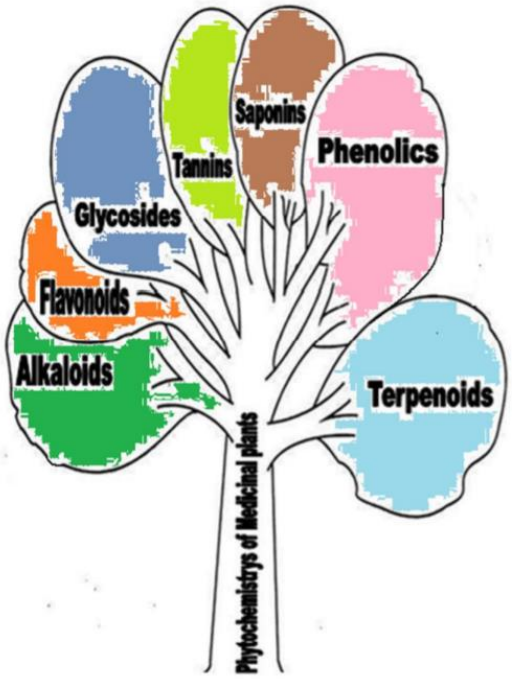
Phytochemicals

The word Phytochemical is believed to have been originated from a Greek word namely, ‘phyto’ that literally means plants with active biological, natural chemical compounds that are found in plants. Generally, the chemicals found in plants that protect the plant cells against hazards from the environment such as Pathogenic attack, drought, stress, pollution and UV exposure are also considered as phytochemicals (Wei et al., 2019). Almost 4,000 phytochemicals have been reported, characterized, assessed and cataloged, which are then classified based on different properties such as chemical characteristics, physical characteristics and by protective functions. Phytochemicals are found in wide-range dietary products such as herbs, spices, fungi, seeds, nuts, whole grains, legumes, vegetables and fruits. Legumes, beans, soy foods, raspberries, strawberries, cherries, grapes, tomatoes, whole wheat bread, garlic, onions, carrots, cabbage and broccoli are also common sources of phytochemicals. These active ingredients generally accumulate in various parts of the plants such as seeds, fruits, flowers, leaves, stems and roots. Most of these phytochemicals, most especially the coloring fragments, are seldom concentrated in distinct botanical tissues' outer layers. Depending on the processing, type, cooking, and growing conditions, the levels vary from plant to plant. Supplementary phytochemicals are also available, albeit there is no solid scientific evidence that they provide the same health advantages as dietary phytochemicals (Kurmukov, 2013). These chemical combinations are considered as secondary metabolites and possess wide range of biological activities such as anticancer property, modulation of hormone

metabolism, decrease of platelet aggregation, stimulation of the immune system, modulation of detoxification enzymes, antimicrobial effect and antioxidant activity. There are currently over a thousand known phytochemicals and a large number of unknown phytochemicals. It is well-known that numerous plants produces these chemical compounds in order to protect themselves against damages and diseases, but recent studies indicates that various phytochemicals can equally serves as protecting agents to man against various diseases (Kurmukov, 2013).

Figure 3

Medicinal plants Phytochemistry



Generally, these chemicals are not major nutrients needed by human body to sustain life, but possess vital role in fighting and preventing some common diseases. Most of these roles suggest viable benefits by phytochemicals in the treatment and prevention of diseases. Owing to these properties, various scientists have been reported to reveal different beneficial pharmacological properties of phytochemicals (Valerio et al., 2010).

The trend in determination of Phytochemicals from medicinal plants

Assessment regarding the previous impact and trend of research in the field of medicinal plants is significant prior to regarding the current inclinations (Saito, 2013). Subsequently, numerous

years regarding empirical applications and usage of herbal products, the isolation of active ingredients of alkaloids such as quinine, strychnine and morphine was first conducted in the 19th century. This subsequently indicated a new era for the application of various medicinal plants as well the beginning of the modern Phytotherapy (Das, Tiwari, & Shrivastava, 2010). In the 1945, more emphasis was shifted from medicinal plant based drugs to microbial fermentations and synthetic pharmaceutical chemistry. Different metabolites from plants were generally investigated from chemotaxonomic and phytochemical point of view at that period. Over the course of last decade, there is increase in the production and uses of plant and animal based drugs. Whereby, the use of medicinal plants has almost doubled in the Western Europe at that time. Factors such as ecological awareness, various phytopharmaceutical formulations with high efficacy such as valerian, garlic and ginkgo as well increased in interest of some major pharmaceutical industries in the use of some medicinal plants as main sources of lead compounds are the major reasons for this rise in the phytochemicals renewal of interest (Das et al., 2010). Owing to the development of different fields such as pharmacognosy and chemical science, physicians starts to extract chemical compounds from different kinds of medicinal plants. Some kind of the phytochemicals extracted from medicinal plants consists of isolation of quinine from Cinchona by the French pharmacists, Peletier & Caventou. In the mid-19th century, a German chemist known as Hoffmann obtained Aspirin from the bark of the willow. Using the concept of active principles in medicinal plants isolation and identification, plant-based drugs started to be substituted gradually with pure substances that are easier and have more power in terms of administering and prescription (J. L. Yang, Wang, & Shi, 2011). Phytomedicine and Phytotherapy almost went into extinction in the beginning of the 21st century owing to the applications of more potent and powerful synthetic drugs. Nonetheless, because of the multiple side effects of the synthetic drugs, the utilization and value of the medicinal plants bounce again and still being rediscovered owing to the fact that some of them proved to have similar efficacy with the synthetic drugs with no or less side effects (J. L. Yang et al., 2011). Lastly, it has been shown that though the effects of phytochemicals seems to be slower, the final results are usually better on the long run more especially on chronic and critical diseases (Das et al., 2010).

Biological Activities of Phytochemicals

The fact that various bioactive ingredients found in plants are used in curing different diseases as well as promoting health status of human has been studied widely by different researchers

in order to establish facts regarding their efficacy as well to know their mechanism of action (Moghadamtousi, Goh, Chan, Shabab, & Kadir, 2013). These studies consists of isolation and identification of these chemical compounds, establishment and elucidation of their pharmacological potency in both in vivo and in vitro studies (Wangchuk et al., 2011). One of the studies indicated that phytochemicals could minimize coronary heart disease risk through prevention of LDL level, minimizing the absorption or synthesis of saturated fatty acid, controlling clotting as well as increasing arterial elasticity. Phytochemicals has the ability of detoxifying chemical compounds that can lead to cancer (Wangchuk et al., 2011). They equally have the ability of neutralizing some radical compounds, activate enzymes that detoxify poisons and inhibit enzymes, which activate toxic chemical compounds. For instance, based on Table 1, it can be observed that some chemical compounds have the ability of inhibiting the production cancer cells. The physiological activities of some bioactive ingredients are known and most of them focused on their potency in treating and preventing heart and cancer diseases (J. L. Yang et al., 2011). They are equally used in the prevention of high blood pressure, macular degeneration and diabetes. Even though phytochemicals are classified based on their functions, one compound may possess numerous pharmacological activity such as antibacterial and an antioxidant agent for example. Some disease preventing and bioactive phytochemicals present (Ma, Chen, Chen, Li, & Chen, 2017)in medicinal plants are presented in Table 1.

Table 1

Some bioactive Phytochemicals in medicinal plants

Classification	**Main groups of compounds**	**Biological function**
Non-starch polysaccharides (NSA)	Lignin, pectins, mucilages, gums, hemicellulose,cellulose	Binding bile acids and toxins, delay in nutrient absorption and water holding capacity
Antifungal and Antibacterial	Phenolics, alkaloids, terpenoids	Minimize the risk of fungal infection, inhibits micro-organism
Antioxidants	Ascorbic acids, tacopherols, carotenoids, flavonoids, polyphenolic compounds	Quenching of oxygen free radicals, lipid peroxidation inhibition
Anticancer	Flavonoids, curcumine, polyphenols, carotenoids	Tumor inhibition, inhibition of metastatic effect, inhibits the development of lung cancer
Detoxification agents	Cyanates, retinoids, carotenoids, flavones, coumarins, aromatic isothiocyanates, indoles, phenols, tocopherols, reductive acids	Inhibits tumourogenesis, inhibits procarcinogen activation, induces drug binders of carcinogens
Others	biogenic amines, volatile flavor compounds, terpenoids, Alkaloids,	cancer chemoprevention, anti-oxidants, Neuropharmacological agents

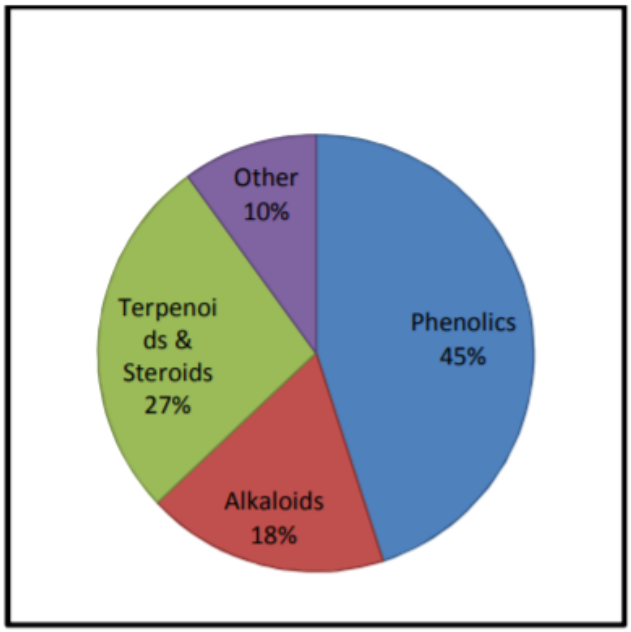
Classification of Phytochemicals

Currently, there is no exact and specific classification for phytochemicals, due to the wide variety of these chemical compounds (Tierno et al., 2016). However, recently, phytochemicals are categorized into primary and secondary constituents, which depends on their role in plant metabolism (Deepak, Rupali and Mahesh, 2016). The primary constituents composed of pyrimidines of nucleic acids, purines, chlorophyll's, proteins, amino acids and common sugars. While the secondary constituents are other chemicals from plants such as glucosides, flavonoids, phenolics, saponins, curcumines, plant steroids, lignans, flavonoids, terpenes and alkaloids (Tierno et al., 2016). Based on a survey conducted in the recent published technical

literature it was found that phenolic compounds are the major widely found phyto-constituents (see Figure 4).

Figure 4

Major constituents of phytochemicals from medicinal plants



Phenol (C6H5OH) is considered as the simplest group of natural chemical compounds in which (-OH) is bonded directly to the hydrocarbon group (Zou et al., 2019).

The phenolic groups are vast and complex class of chemical compounds present in different part of various plants. They are generally considered as secondary metabolites of plants that plays significant roles in protecting the plants. They equally demonstrates significant role to humans such as anti-oxidant activity, which is vital in giving protection against free radicals-based diseases (Hoda, Hemaiswarya, & Doble, 2019). More also, Flavonoids are considered as the largest chemical compound group found in plant that composed of the phenolic group, which are equally the most studied group. Phenolic acids also constitutes of a diverse groups, which composed of the widely distributed hydroxycinnamic and hydroxybenzoic acids (Hoda et al., 2019).

Black cumin (Nigella Sativa L.)

Black cumin (*Nigella sativa L.)* is considered a holy and revered medicinal seed in the history. The seeds were best produced in Cairo under suitable in the oases, which will be sprinkled till

the seedpods are developed (Zou et al., 2019). Even though, the medicinal benefits of this seed is mentioned by Prophet Muhammad (may peace be upon him) as well as in the Bible, it was not researched carefully until the last four decades. Since that time, various researchers conducted numerous studies on it. The seed is considered to be popular owing to the conviction that the plant has multiple medication for different illnesses. Actually, the seed was considered to be noble and novel regarding its high biological applications (Mohamed Fawzy Ramadan, 2007). Thus, it is studied extensively particularly to justify its broadly traditional medicinal and therapeutic application. The main reason can be due to the complex composition of the seed's chemical nature. The seed has more than 100 various chemical constituents, which include numerous fatty acid groups. However, its oil is widely used for medicinal purpose; the seeds are also used as curries, Mediterranean cheeses and pastries owing to its spicy nature (Mohamed Fawzy Ramadan, 2007). The seeds have minimum aroma though they are carminative, which tend to aid in digestion of complex food materials as well get rid of vapors in the intestines and the stomach. They also help in elimination and peristalsis. The crucial oils present in black cumin has anti-microbial activity that aid in getting rid of intestinal worms (Mohamed F. Ramadan & Mörsel, 2002). The black cumin seeds have been widely applied in promoting health as well as fighting various diseases foe centuries in the Middle East and Southeast Asia. In the Arab region, it is also known as Haba-Al-Barka or Al-Habat-El-Sauda. The plant is grown widely in various parts of the world as an annual herb (Shabana, El-Menyar, Asim, Al-Azzeh, & Al Thani, 2013). Black cumin has significant focus on research for many year ago, still possess numerous traditional applications, and hence has been studied extensively owing to its chemical composition and biological properties (Takruri & Dameh, 1998).

Figure 5

A black cumin plant and seeds

Local uses of Nigella sativa L. seed

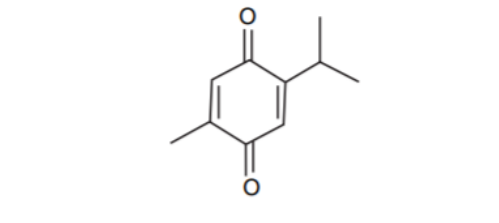
This plant composed of numerous pharmacological and dietary uses. It can equally be an additive in coffee, tea, breads or casseroles, can be used in canning as well as extracted in vinegar or wine. The seed could be equally sprinkled on salad or mixed with honey (Srinivasan, 2018). Moreover, many people that seek for black cumin benefits take its oil in capsule form. Nevertheless, some people make use of the oil for external purpose in beauty and for the treatment of skin conditions such as eczema and psoriasis (Guler, Dalkiliç, Ertas, & Çiftçi, 2006). A mixture of the oil together with beeswax can be used as anti-wrinkle agent, joint pain reliever, moisturizers, skin infections and skin burns. Traditionally, they are equally used as diaphoretic, diuretic, liver tonic, digestive and stomachic. The seeds of this plant can be used as a confection together with other ingredients in the treatment of dyspepsia, sour belching, breath deodorizer and diarrhea (Elahi et al., 2014). The seed are also taken with buttermilk in order to treat vomiting, obstinate hiccups, loss of appetite, dropsy and puerperal diseases. The herb is also considered as an important remedy in digestive and hepatic disorders and also work as a stimulant in different conditions related to cold humours (Elahi et al., 2014). They have equally been used extensively in the treatment of chronic migraine and headache. Also used in leprosy, sores and mercury poisoning. When brayed in water, it can be used in the removal of swellings from feet and hands. It can also be used in the curing of pimples, freckles, eczema, alopecia and leukoderma. The black cumin seeds has been used widely as an antibacterial and anthelmintic agent (Hajhashemi, Ghannadi, & Jafarabadi, 2004).

Profile of phytochemicals

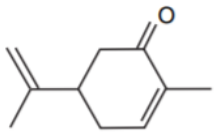
Considering the wide range of pharmacological applications of black cumin, it undergone various phytochemical analysis and different chemical compounds have been isolated (Mashayekhi-Sardoo, Rezaee, & Karimi, 2020). The seed composed of fixed oil (35.6– 41.6%), yellowish volatile oil (0.5–1.6%), proteins (22.7%), reducing sugars, amino acid (such as tyrosine, tryptophan, threonine, serine, proline, aspartic acid, glutamic acid, cystine, phenylalanine, alanine, glycine, valine, isoleucine, leucine, and lysine), arabic acid, moisture, melanthigenin, melanthin resembling helleborin, glycosidal saponins, bitter principles, metarbin, toxic glucoside, resins, tannins, organic acids, alkaloids and mucilage (Tradit, Altern, Kurt, Dede, & Ragbetli, 2015). The seed equally contains crude fibre, minerals (such as Ca, P, Zn, Na, and Fe) as well as vitamins such as folic acid, pyridoxine, niacin, thiamine and ascorbic acid. It equally composed of fatty acids (such as palmitic acid, linoleic acid, and oleic acid), unsaturated hydroxy ketones, aliphatic alcohols and terpenoids. The seed oil also contains acylated steryl glucosides, steryl glucosides, steryl esters and free sterols. A novel nigellicine (alkaloid), nigellimine (isoquinoline alkaloid) and nigellidine (indazole alkaloid) were extracted from the seed of the plant. The active ingredients of the seeds composed of volatile oil containing carvone, p-cymene, a-pinene, d-limonene and an unsaturated ketone. The crystalline active principle known as nigellone, is regarded as the only carbonyl oil's fraction c (Lutterodt et al., 2010). The bioactive ingredients of the volatile oil are thymoquinone, thymol, thymohydroquinone and dithymoquinone (see Figure 6).

Figure 6

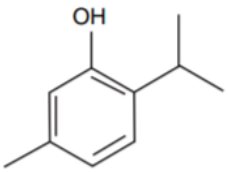
The major active ingredients presents in volatile oil of black cumin



Thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone)



Carvone



Thymol (5-methyl-2-(1-methylethyl)phenol)

Thymoquinone

Thymoquinone (TQ) is the major bioactive ingredient present in black cumin (*Nigella sativa L.*). It is considered as an ingredient that is cultivated in the Mediterranean are as well as in Western part of Asian countries such as Afghanistan, Pakistan and India (Darakhshan et al., 2015).

The seed is reported in folklore medicine to have possess diverse pharmacological effects to obesity, hypertension, eczema, gastrointestinal problems, headache, dysentery and bronchial asthma (Woo et al., 2012b). Recently, various therapeutic applications of TQ is reported in the recent technical literature such as its chemo-preventive potential and chemo-sensitizing TQ to gemcitabine and oxaliplatin in pancreatic cancer (PC) (Darakhshan et al., 2015). The clinical efficacy TQ to gemcitabine and oxaliplatin in cancer of the prostate gland was equally reported to show antiproliferative activity on cells derived from osteosarcoma, breast, myeloblastic leukemia, lung, larynx, ovary and colon as well as has the ability of inhibiting the hormone refractory prostate cancer through targeting transcription factor E2F and androgen receptor (Badary et al., 2003).

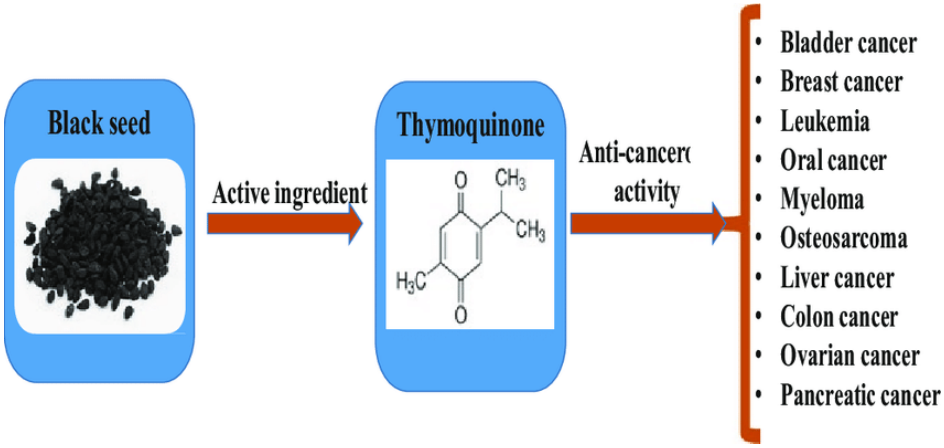
Figure 7

Thymoquinone colour and texture

As proposed by various researchers TQ is reported to have induced apoptosis in tumor cells via downregulating NF-κB, Akt activation as well the signaling pathway of the extracellular signal-regulated kinase and tumor angiogenesis inhibition (2,10–12) (Badary et al., 2003).

Figure 8

Major properties of Thymoquinone



Biological properties of black cumin seed

Antioxidant action of volatile oil

Thymoquinone as well as other volatile component of black cumin are said to show peroxidation in the phospholipid liposomes of the ox brain. More also, TQ showed protective properties on the hepatotoxicity induced by ter-butyl-hydro-peroxide as well as

hepatoprotective properties on the toxicity induced by carbon tetrachloride in rats and mice (Mansour et al., 2001). Moreover, TQ equally exhibits renal protective activity in rats via its antioxidant property. scavenging of free radical activity of dithymoquinone, TQ and thymol were studied based on reaction leadin to formation of reactive oxygen species like hydroxyl 2-radical, singlet oxygen and superoxide anion radical with the help of spectrophotometric and chemiluminescence method (Jang, Piao, Kim, Kwon, & Park, 2008). The hepatoprotective activities provide protection against doxorubicin-induced nephropathy were found through the antioxidant mechanism. In contrast, the effect of TQ on benzopyrene induced on forestomach tumors in rats, whereby its anti-tumor effect on the fibrosarcoma tumorigenesis induced by 20-methylcholanthrene was demonstrated to be partially via its anti-oxidant property. The potential mechanism of the protective activity of TQ on colitis mice induced by acetic acid was equally partly due to its antioxidant activity (Grosso et al., 2009).

Hypoglycemic effects

Based on the folk medicine application for the treatment of diabetes using different plant extract in middle East, in 1987 various researchers conducted a research using different plant extracts mixture containing of; Gum asafetida, Gum olybanum, Myrrh and black cumin in order to check for anti-diabetic effects on rat, which was found to be effective. Another independent research indicated that the blood sugar lowering ability by inhibiting the ability of the hepatic gluconeogenesis of the plant mixtures, which showed that the mixture can be used in the curing of type II diabetes mellitus(non-insulin dependent) (Al-Ali et al., 2008). A black cumin extract mixture was shown to have significantly minimize the blood sugar upon oral administration. Administration of the volatile oil in an intraperitoneal way also formed a hypoglycemic property in alloxan-induced diabetic and normal rabbits. A recent work showed that the seed extract upon oral administration reduced the high glucose level in alloxan-induced diabetic rabbits after 2 months of treatment (Abdelmeguid et al.,2010). An additional research was also designed so as to elucidate the potential insulinotropic effects of the plant seed oil in Streptozotocin plus Nicotinamideinduced diabetes mellitus in hamsters. In the 4^{th} week of the treatment, there is a reduction in the blood sugar level as well as increase in the serum albumin level observed. The result indicated that the hypoglycemic activity of the oil was partly, owing to the stimulatory activity on the beta cell function which later increase the serum insulin level and hence the type II-like model contain an insulinotropic properties. The hypoglycemic activity of black cumin was equally needed to be mediated through extra pancreatic activity

rather than through the release of the stimulated insulin. More also, the influence of the seed oil on blood sugar concentration was equally investigated in Streptozotocininduced diabetic rats. Recently, a clinical based research was conducted on human volunteers indicated that black seeds taken daily at 1g lead to reduction of blood sugar level after 14 days (Tavakkoli et al., 2017).

Anti-cancer activity

Black cumin oil and seed are used traditionally as tonic for promoting health as well as in disease prevention. They are also reported to shown immunomodulating, immunopotentiating and interferon-like properties (Majdalawieh, Fayyad, & Nasrallah, 2017). The ethanol extract of the seeds were shown to inhibit endothelial cells and limits the progression of cancer cells in vitro. The nutraceutical activity of black cumin was also investigated on the carcinogenesis and ability to induce oxidative stress via methylnitosourea in aprague Dawley rats, which was found to form about 80% protection on methylnitrosourea-induced oxidative stress, carcinogenesis and inflammatory response (Edris, 2009). The alcohol extracts were found to exhibit cytotoxic effect and was showed to have the ability of curing oral cancers. A mixture containing of crude gum, two black cumin seed's component, a fixed oil, dithymoquinone and TQ were assayed in an in vitro study to check for their cytotoxic property for various multi-drug and parental resistant human tumor cell lines. Even though about 1% w/v of the oil or gum do not possess cytotoxic effect, both dithymoquinone and Thymoquinone were shown to have cytotoxic effect for different kinds of tumor cells. The protein present in black cumin extract, which are fractioned through ion exchange chromatography were equally found to have immunomodulatory activity (Bashmail et al., 2018). The activity of these protein extracts on the cytokine synthesis was evaluated further with specific enzyme-linked immunosorbent assay. The findings showed that the protein fraction was considered less effective as compared to the whole protein. The topical administration of the seed lipid extracts inhibits the skin carcinogenesis in mice as well as causes delay in the onset of 1 papilloma formation upon intraperitoneal application (100 mg kg) 1 body weight) (Bashmail et al., 2018). A study conducted usingthe model of murine cytomegalovirus, upon intraperitoneal application of oil reduced the viral load in spleen and liver substantially. Whereby, there in increase in CD4+ T cells, macrophages and interferon and reduction in both function and number of NK cells. In which on the 10^{th} day, the virus titre was subsequently undetectable in the liver and spleen of the infected mice, while still in the positive controls. The ethanolic extract fraction of the black

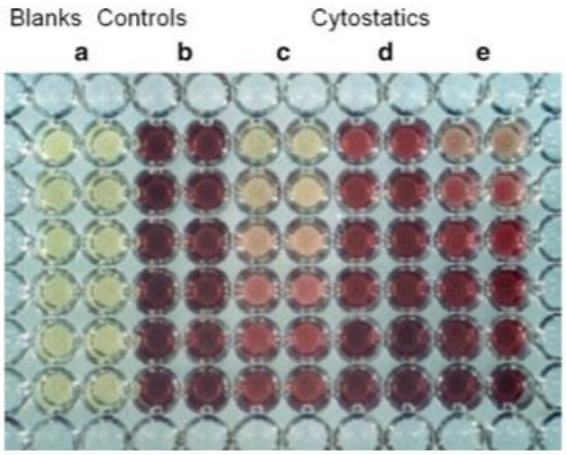
seed was examined in mice against intraperitoneal implantation of murine P388 leukaemia (Woo et al., 2012b).

Although the lewis cells of the lung carcinoma cimplanted subcutaneously. The life time of the mice treated was raised by 153% as compared with the control mice treated with dimethyl sulphoxide. Hederin, which is a kind of triterpene saponin that is isolated from the fraction gives a vital tumor inhibition rates; in which its anti-tumor is yet to be fully understood. Another study indicated that, the stimulating activity of hederin upon release of nitric oxide as well as upregulation of gene expression induced by nitric oxide synthase in mouse macrophages were examined (Paramasivam et al., 2016). Hence, indicated that the mechanism leading to its biological activity such as anti-tumor activities can be fully understood. Another independent study equally demonstrates that the inhibition of tumor activity by TQ was examined both in vitro and in vivo in fibrosarcoma male Swiss albino rats on that was induced by 20-methylcholanthrene and was found to have anti-tumor activity by inhibiting both tumour burden and tumor incidence significantly (Paramasivam et al., 2016).

MTT assay: The 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide popularly known as MTT assay is done based on the idea of converting MTT by living cells into formazan crystals, and subsequently determine the activity of mitochondria. Since majority of the cell populations, the overall mitochondrial activity is associated with the number of the viable cells. Therefore, this assay is categorically used in measuring the cytotoxic activities of drugs and other agents on primary patient cells and cell lines in vitro. The main reason for conducting MTT assay is to measure the viable cells using high throughput technique such as the 96-well plates that do not need the cell counting in an elaborate manner.

Figure 9

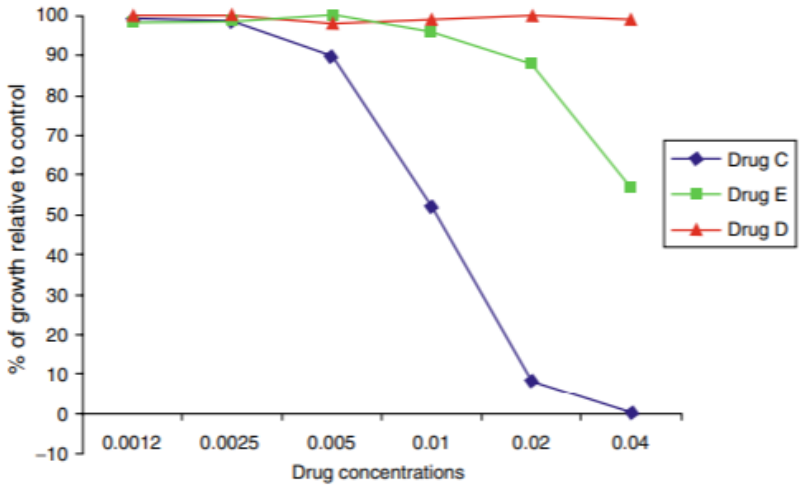
The 96-well plate's set-up indicating the conversion of the MTT salt into the formazan crystals



Therefore, it is commonly used in the determination of cytotoxity of various agents at variable concentrations. Moreover, the mitochondrial activity is shown by converting MTT salt into the crystals of formazan that can also be solubilized during homogenous measurements (Van Meerloo, Kaspers, & Cloos, 2011). Hence, any decrease or increase of the number of the viable cells can be indicated through measuring the concentration of the formazan reflect in optical density (OD) with the help of a plate reader at 540 and 720nm. Furthermore, regarding dividing cells, which are mostly cell lines, the reduction in the cell line number indicates inhibition of the cell growths as well as the drug sensitivity, which is usually shown as the concentration of the agent needed to achieve 50% inhibition of the cell growth in comparison with the untreated control growth (50% inhibitory concentration, IC_{50}). For non-dividing, which are mostly primary cells, the sensitivity of the drug is measured as the ability of enhancing the cell kill of the treated cells in comparison to the loss of cells that is frequently seen in untreated cells (50% lethal concentration, LC_{50}) (Van Meerloo et al., 2011).

Figure 10

Dose–response curve of cell lines C, D, and E. (C) cell line with drug C with a dose–response curve from control growth without drugs (set at 100%) to complete cell kill at the highest drug concentrations, (D) cell line with drug D with a dose–response curve showing no growth inhibition, (E) cell line with drug E with a dose–response curve showing dose-dependent modest growth inhibition (IC_{50} is not reached).

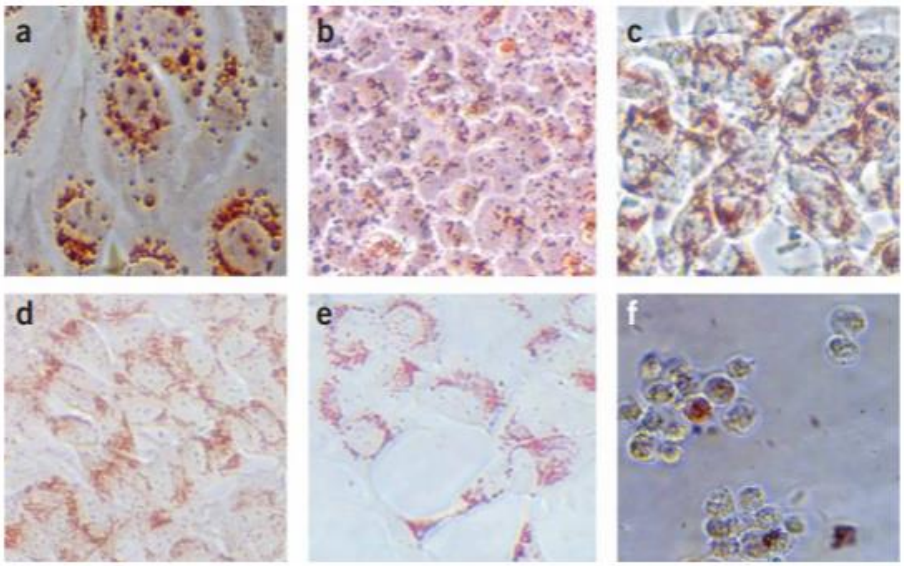


For other different type of cells like the myeloid leukemia (AML) cell the median survival of the cell is 100% and is considered as no issues as far as the treated cells are compared with the controls (Langdon, 2004).

Neutral Red assay: This assay gives a quantitative idea for the determination of the viable cells number present in a culture (Repetto, del Peso, & Zurita, 2008). It is considered as one the most applied cytotoxic assays with several different biomedical, phytochemical as well as environmental uses. Its basic idea is based on the capability of the viable cells to integrate and bind with the supravital dye neutral red in the lysosomes. Most of the cell lines and primary cells from different origin can be used successfully. The cells are nurtured in the 96-well tissue culture plates, which are then treated until the suitable period is reached. The plates are subsequently incubated for about 2 hours in the neutral red medium (Fotakis & Timbrell, 2006).

Figure 11

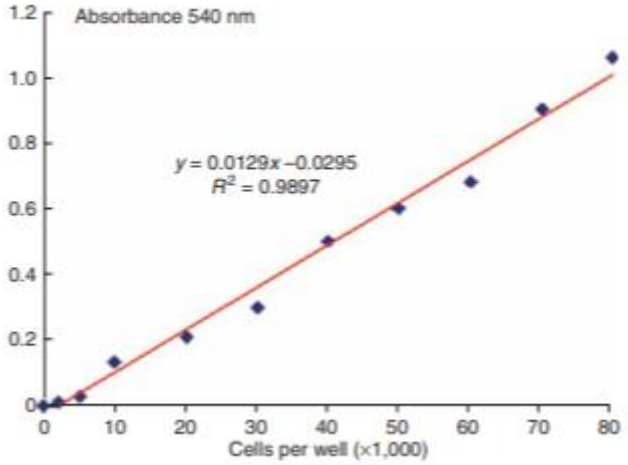
Cultured cells stained with neutral red. (a) Morphology of different cells stained with neutral red (1,600), including the RTG-2 salmonid fish cell line derived from the gonad of rainbow trout Oncorhynchus mykiss, (b) the PLHC-1 cells derived from a hepatocellular carcinoma of the topminnow Poeciliopsis lucida, (c) the SH-SY5Y human neuroblastoma cell line, and the same human cells after 24-h exposure to (d) 1, (e) 20 and (f) 50 mg ml 1 zinc chloride



The cells are then washed, the absorbance will then be determined using a spectrophotometer using the extracted dye from each well.

Figure 12

A graph indicating the correlation between the absorbance at 540 nm and the number of cells after the neutral red assay was conducted

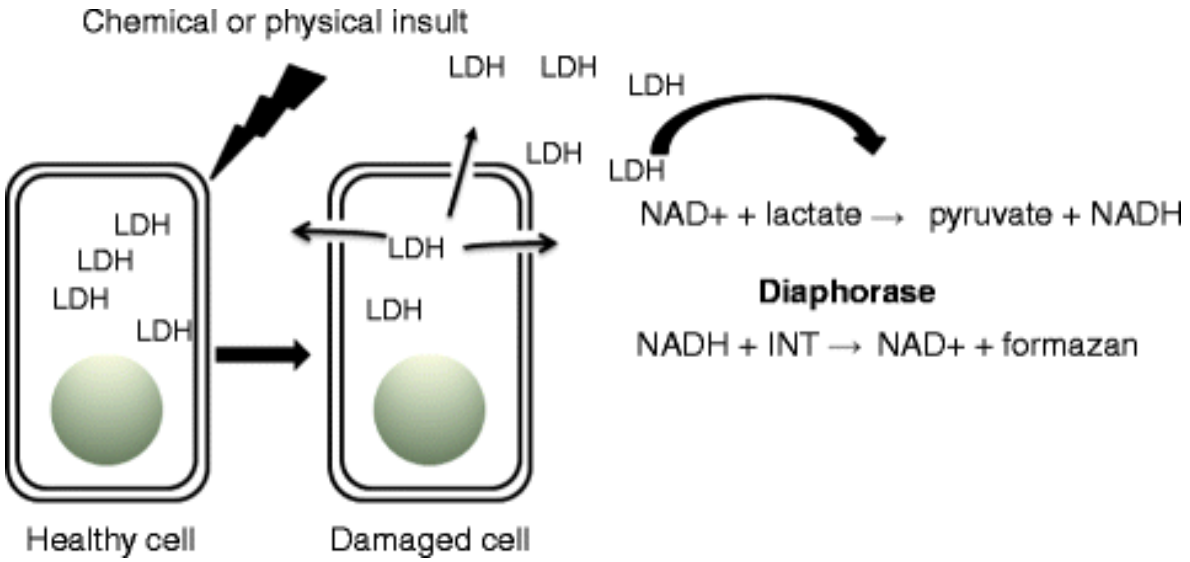


The technique is more sensitive than other various cytotoxity assays as well as cost-effective (enzyme leakage, tetrazolium salts, or protein content). Immediately the cells are treated, the test can then be completed in 3 hours (Fotakis & Timbrell, 2006).

LDH (Lactat Dehydrogenase) assay: One of the commonest method used in the determination of cytotoxicity depend on the effect of the damaged cells released by cytoplasmic enzymes. Lactate dehydrogenase (LDH) is considered as a form of stable cytoplasmic enzyme, which is present in all living cells. The LDH is quickly released to the supernatant of the cell culture immediately the plasma membrane is destroyed (Han et al., 2011). The activity of LDH can be quantified easing using NADH produced when lactate is converted to pyruvate in order to reduce a subsequent compound in a coupled reaction into a product with properties that are quantitated easily. This procedure measures the reduction a yellow colour terazolium salt, INT using the NADH to a rae water-soluble formazan dye at an absorbance of 492 nm. Whereby, the amount of the formazan is directly proportional to the total amount of the LDH utilized in the culture, which is equally proportional to the total number or damaged or dead cells (Han et al., 2011).

Figure 13

Formation of formazan dye by LDH using NADH



Sample pre-treatment methods

Most of the techniques used for the determination of phytochemicals such as TQ depends on accurate extraction method and a clean-up of the sample. These preliminary steps are significant stages for a successful analysis as well as for a good result. However, they are tedious and time consuming with some expensive steps sometimes. Even though, it's worth doing as it affect the final choice regarding the detection technique that can be used during the analysis (Bermejo-Barrera, Moreda-Piñeiro, & Bermejo-Barrera, 2002).

These steps are vital for an efficient protocol, owing to the fact that constitute of almost two-third of the time used in the overall analysis in a certain study. The type of extraction method that can be used in the extraction of a certain analyte depends solely on the physical and chemical properties of the analyte. Mostly, polar and intermediate polarity analytes like TQ needs polar extractants or solvents such as ethanol, acetonitrile, methanol, water and other organic solvents . While non-polar analytes are mostly extracted using hydrophobic organic extractants such as chloroform, N-hexane, petroleum ether and some higher group alcohols which can be through direct extraction or derivatization using another kind of solvent that can be used in partial cleaning up of the matrix in order to get rid of the excessive component of the biological material (Tessini et al., 2010). The sample matrix also plays a key role in

determining a well suitable extraction solvent. The use of chlorinated extractants for extraction is currently minimized drastically owing to their hazardous effect to man and the environment. Sample clean-up and preparation are considered as the 'bottle neck' of any study as they easily influence the purity of the sample materials, which in turns affects the sensitivity, and selectivity of the analysis (Moreda-Piñeiro & Moreda-Piñeiro, 2015).

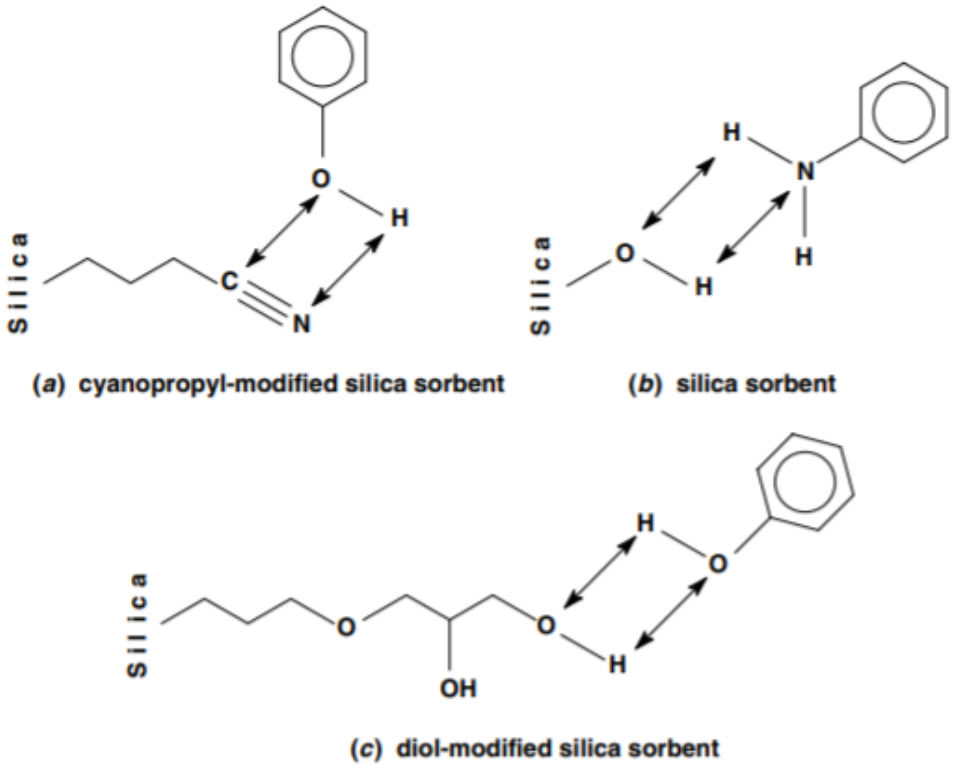
Solid phase extraction method (SPE)

Different researchers traced the history regarding the development of solid-phase extraction (SPE). SPE was long known during the latency period (from biblical times to 1977) though not used frequently in practiced (Zwir-Ferenc & Biziuk, 2006). The breakthrough in the technological advancements of sorbents as well as devices accelerates the growth of SPE that continues to be used currently. The major reported advantage of SPE, which resulted in its early advancement over LLE are reduction in analysis time, reduced labour and cost (due to the fact that SPE is quicker and need less or no manipulations), also reduced consumption and disposal of organic solvents. This in turn leads to reduction in analyst exposure to hazard, which equally minimized the potentials for emulsion and smog formation (Buszewski & Szultka, 2012). Automation potentials shown by SPE is an indication that showed that it has the ability of increasing productivity as multiple and simultaneous extraction can be done at the same time. It leads to higher concentration factor (KD) when compared with LLE, which can also be used in storing analytes in form of a vehicle in the sorbed state for chemical derivatization. It equally provides higher selectivity than LLE as it is considered as a multi-staged separation technique. SPE is equally considered as non-equilibrium that involves the exhaustive removal of various chemical constituents from a moving liquid sample through retention on a solid sorbent, which then recover the selected chemical components via elution from the sorbent (Calderilla, Maya, Leal, & Cerdà, 2018).

The commonest sorbents used are mostly polar (normally known as normal-phase SPE) such as magnesium silicate (MgSiO3 or Florisil), silica (SiO2) x, and alumina (Al2O3), whereby, the bonded silica found in the sorbet will interact with most polar functional groups present in the sample material to produce cyanopropyl [(SiO2)xa(CH2)3CN], aminopropyl [(SiO2)xa(CH2)3NH2]-, and diol [(SiO2)xa(CH2)3OCH2CH(OH)CH2(OH)]- modified silica sorbents as seen in Figure 14.

Figure 14

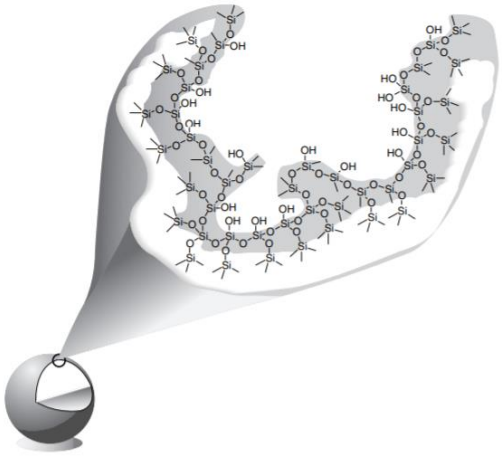
Interactions between analytes and polar sorbents through hydrogen bonding or dipolar attraction.



The polar sorbent are usually applied in the removal of matric effects from organic extracts present in animal tissues and plant materials (Faraji, Yamini and Gholami, 2019).

Figure 15

Demonstration of the unbounded silica material



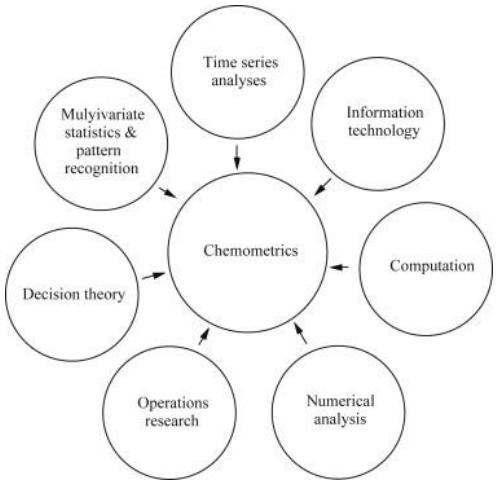
Chemometrics

Chemometrics is a branch of chemistry that deals with the applications of statistical techniques to chemical data analysis. Additionally, it involves borrowing various techniques from the engineering and mathematical literatures, chemometrics has led to the birth of new innovative data analytical techniques (Iwaniak et al., 2015).

Recently, statistical approaches were used in various ways to solve many kind of chemical problems. For instance, experimental design methodologies showed high impact on understanding as well as improving the industrial chemical procedures. In recent times, the field of chemometrics has emerged as an area that involves the analysis of observational data from analytical and organic chemistry, environmental research and food studies. The data is generally characterized by numerous measured variables on few observations (Frank & Friedman, 1993). Once the total number of the variables (p) exceeds greatly the observation count (N). Therefore, there will be a high degree of correlation among the variables, which are sometimes digitisations of analog signals. Most of the tools used by chemometricians are same with those used in other areas that led to the production and analysis of observational data, which are mostly well known by statisticians and mathematicians than any other professionals (Iwaniak et al., 2015).

Figure 16

The major scope of chemometrics



These tools generally composed of data exploration via cluster and principal component analysis, the recent computer graphics, as well as artificial intelligence (AI). Predictive modelling as well as simulation (classification and regression) is equally considered as a vital goal in most of the applications. However, based on this chemometricians have developed their own methodologies based on intuitive ideas and heuristic reasoning, and there is strong indications regarding empirical evidences that showed they perform with higher efficiencies in different situations. One of the common regression technique in chemometrics is the partial least squares (PLS) and major components regression (PCR) (Iwaniak et al., 2015).

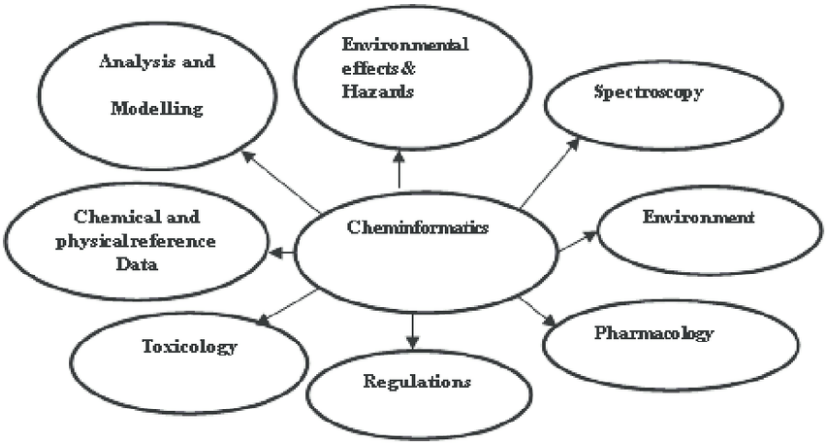
Different researchers considers chemometrics as a chemical discipline that involve the use of mathematics, formal logics and statistics to i) select and/ or design an optimal condition for an experimental method, (ii) Give the highest relevant chemical information based chemical analysis of the data (iii) generate chemical knowledge regarding the data system. Chemometrics as well as other scientists that employed the use of the word ‘metrics’ such as technometrics, econometrics, sociometrics, psychometrics and biometrics involve the use of statistical and mathematical procedures for elucidating and solving adequate issues related to their subject areas (Kumar, 2017).

The spectrum of the chemometrics applications in the contemporary science has a wide range and involve different issues related to multivariate data analysis. Recently, chemometrics is

employed in petroleum industries, pharmaceuticals, bioscience and has now become a well-known and interesting tool for research in metabonomics (Wegner et al., 2012). Chemometrics is also related with cheminformatics (or sometimes called as chemoinformatics), which is considered as a discipline that deals with the application of information technology using computer for solving chemical issues. Cheminformatics emphasizes more on chemical data collection, analysis, manipulation and storage. The main interest of cheminformatics is deposited on minute molecules, more especially their properties as well as their chemical structures (Wegner et al., 2012).

Figure 17

Overview of cheminformatics and chemometrics



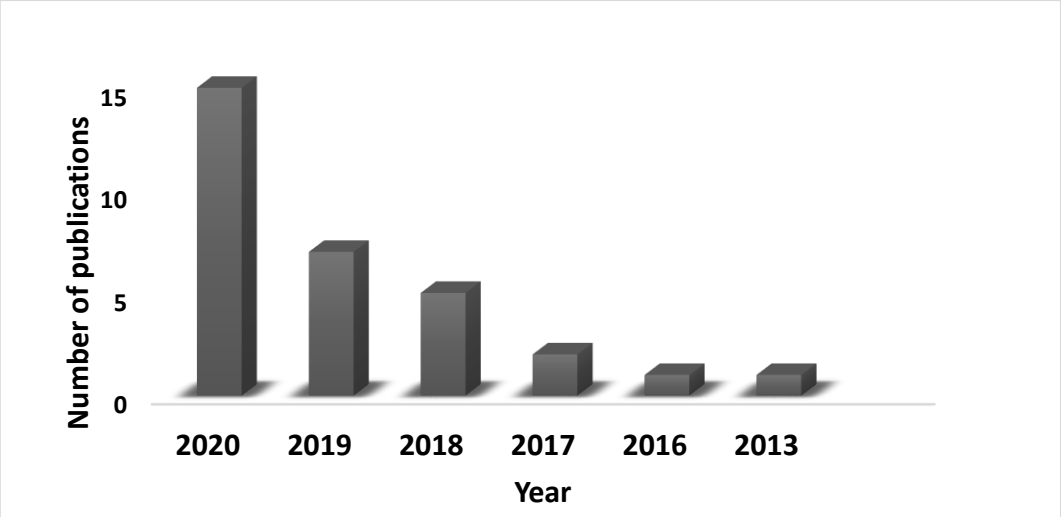
The major fields use in chemometrics involves the analysis of molecules to predict their biological potency considered their descriptive properties. The activity and properties of molecules describing them are considered as their endogenous and exogenous variables, respectively (Prakash & Gareja, 2010). In order to elucidate the biological activity using the above-stated variables, distinctive mathematical methods inform of models are constructed that are based on regression analysis (Prakash & Gareja, 2010). The commonest techniques used in chemometrics in practice for constructing, analysis and identifying models consists of; partial least squares (PLS) regression, principal component analysis (PCA) and artificial neural networks (ANNs) using different data bases and tools such as Mat lab, Python, PubChem,

ChemSpider and AAindex database. Various applications of AI has been demonstrated by various chemist recently in different field of chemistry such as chromatographic applications like HPLC, GC-MS, Spectroscopy and electrochemistry (Hassan, Brown, Varma-O'Brien, & Rogers, 2006).

Currently, there is quick and rapid progress in the total number of articles published using AI technique in chromatography with about 32 published articles in the last five years as indicated in Figure 18.

Figure 18

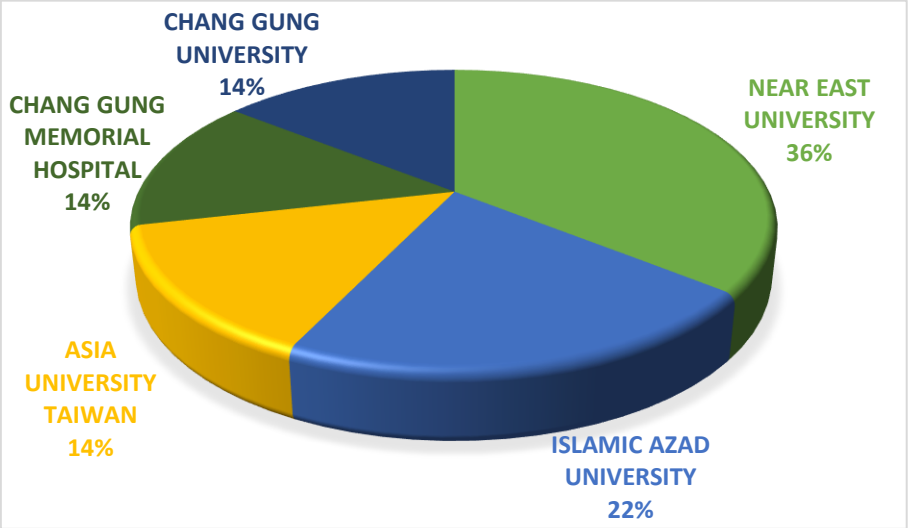
Total number of articles published using the hyphenation of AI and HPLC technique (Web of Science, November 2021).



To have a better understanding of this coupling, Figure 19 demonstrates the main affiliations that employ the application of AI in HPLC method development using various analytes from different sample matrices with Near East University emerging as the first then followed by Islamic Azad University.

Figure 19

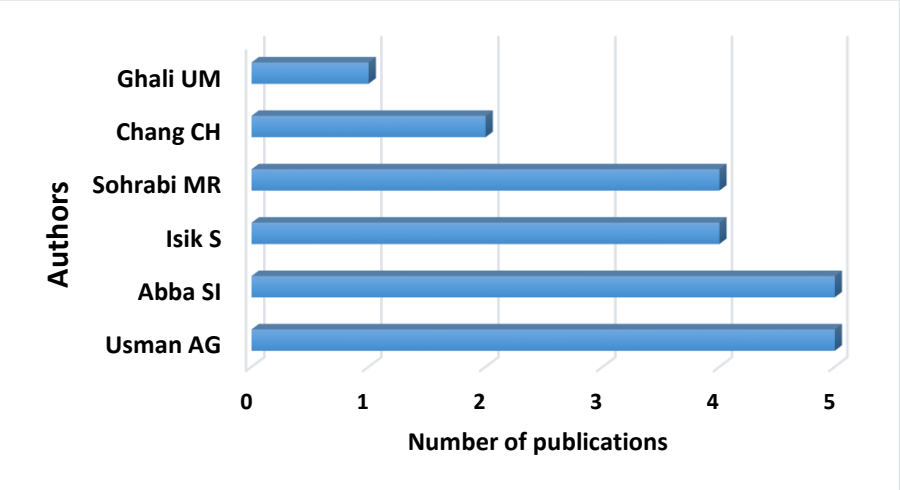
The major affiliations contributing in the hyphenation of AI and HPLC in the last five years (Web of Science, November 2021).



More also, Figure 20 indicates the major contributors of AI-HPLC coupling in the last five years.

Figure 20

Major authors contributed to artificial intelligence (AI) with HPLC method development coupling in the last five years (Web of Science, November 2021).



Step-wise linear regression (SWLR)

Mostly, the linear regression (LR) is among the major chemometric methods using in simulation of different dependent and independent variables. It is important to understand that significant inter-relation between multiple and single variables so as to determine the set of optimal parameters that provide the strongest prediction performance (Abba et al., 2020). Numerous chemometricians highlights the systematic regression as an advanced form of selection, which employs the optimal set of the independent variables through adding or deleting variables by the residual sum of the squares influence (Abba et al., 2020).

The Step-wise linear regression (SWLR) obeys the systematic approach of changing the variables through their impact checking. Any variable which do not contributes as well as not satisfies the modelling will be deleted and eliminated step-wise to remove its impact (Lee et al., 2017).

Principal component regression (PCR)

Principal component analysis ((Solgi, Pourhaghi, Bahmani, & Zarei, 2017)) is one of the oldest and popular chemometric approach inform of multivariate statistical method, which is used by in almost all disciplines. In reality, its origin can be traced back to Pearson or even Cauchy (Solgi et al., 2017). However, its modern application is based on Hostelling formalization, who equally coined the name principal component. PCA generally analyses a set of data that represents experimental observations described by different dependent variables, which are generally inter-correlated (Ringnér, 2008). Its objective is to extract the significant information from the data set and then express the information as a set of orthogonal variables referred to as principal components (Celeste et al., 2019). More also, this technique can be based on regression approach and hence it is called Principal component regression (PCR) (Celeste et al., 2019).

Least square-support vector machine (LSSVM)

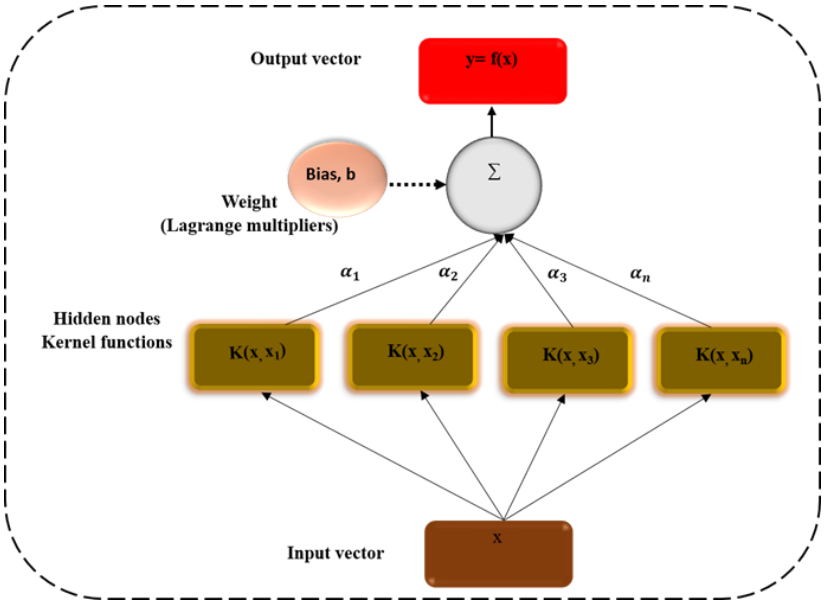
This algorithm consists of an improved standard Support vector machine (SVM) algorithm that gives computational applications over the latter by reduction of computational burden through conversion of the problems during the optimization of quadratic into a system of various linear equations (Zhou, Wang, & Yang, 2017). IN LSSVM technique, a solution is generally obtained through solving linear equations instead of quadratic programming problems that involves standard SVM. It can equally be used in solving both regression and classification problems (Nourani, Elkiran, & Abba, 2018a). A short explanation of the LSSVM is demonstrated below;

Considering the output y_i and inputs x_i. Based on the LSSVM, the non-linear LSSVM functions can be shown as follows;

$$f(x) = w^T \varphi(x) + b \quad (1)$$

Figure 21

LSSVM model architecture



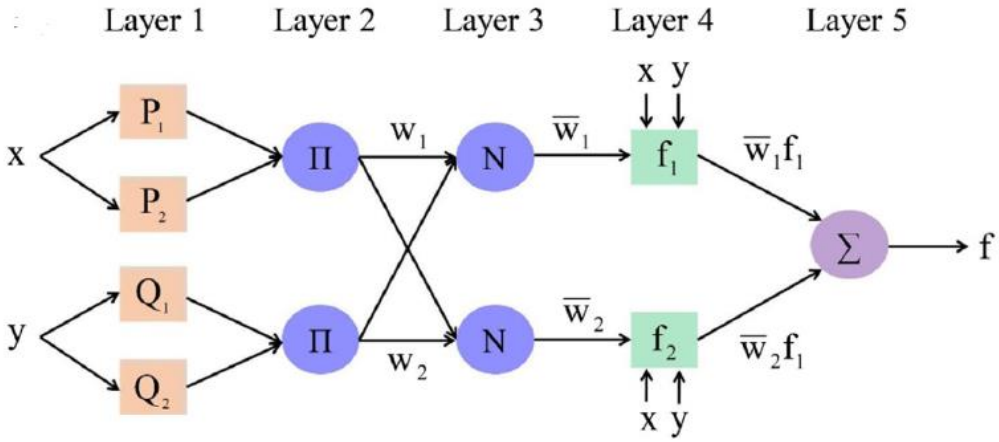
Adaptive-neuro fuzzy inference system (ANFIS)

This is a combination of fuzzy inference system and Artificial Neural Network (ANN). Thus, it couples the learning abilities of ANN as well as handling data uncertainty of fuzzy logic system (Yaseen, Ghareb, et al., 2018).

Even though various classes of ANFIS were into existence since such as TSumoto, Sugeno and Mamdani (Sanikhani & Kisi, 2012). The architecture of ANFIS model composed of nodes that acts as membership functions (MFs). Different kinds of membership functions are available such as gaussian, sigmoid, triangular and trapezoidal. Mostly, the Gaussian function provides higher performance based on previous studies.

Figure 22

Architecture of ANFIS model with two inputs and five layers (Yaseen, Ramal, et al., 2018)



Least square boost (LSQ-BOOST)

Least square boost is a technique that involves controlled learning, which minimize variance and prejudices (Freund, Y.; Schapire, R.; Abe, 1999). LSQ-BOOST converts a slow learner to a solid one. A weaker is partially related with the true classification (Kearns & Valiant, 1994). The definition of LSQ-BOOST was proposed by Friedman as the one that broadens the regression through implementing the gradient boosting system (GBM) technique (Yi-ming et al., 2015). The gradient boosting generally develops an estimating model based on an ensemble of weak prediction models and in a step-wise manner. This assumption is performed to improve the functions of the arbitrary losses (Huerta, Leiva, Liu, Rodríguez, & Villegas, 2019).

Simple average ensemble (SAE)

Based on the proposed ensemble technique, the SWLR, PCR, ANFIS and LSQ-BOOST AI-based models, are first calibrated and tested separately; afterwards, average of the output of the predicted single models is compared with the experimental values (Abdullahi Garba Usman, Işik, Abba, & Meriçli, 2021). The overall formula for SAE is shown as;

$$P_{(t)} = \frac{1}{N}\sum_{i=1}^{N} p_i\,(t) \qquad (2)$$

Where N is considered as the no. of learners (here N=4) and p_i is considered as the output of the single models (SWLR, PCR, ANFIS and LSQ-BOOST) within a time *t*.

Weighted average ensemble (WAE)

This kind of ensemble machine learning is mostly determined by assigning different weights to individual predicted values of the simulated single models based on their individual

importance of the predicted values (Selin & Abba, 2020). The weights are allocated to each of the outputs based on their relative importance. In addition, this is contrary to the case of the single AI-based models.

The WAE is described as;

$$P_{(t)} = \sum_{i=1}^{N} w_i \, p(t) \quad (3)$$

Where w_i is define as the applied weight on the output of the i^{th} model, The weight can be determine according to the model performance as:

$$w_i = \frac{DC_i}{\sum_{i=1}^{N} DC_i} \quad (4)$$

DC_i is the i^{th} single model performance efficiency.

Neural network ensemble (NNE)

In this type of technique, non-linear averaging is done by calibrating another new neural network. The outputs of the previously conducted single (SWLR, PCR, ANFIS and LSQ-BOOST) models were loaded into the input layer of the neural network, in which everyone will be assigned to a single neuron in the input layer. In NNE just line traditional ANN, the tangent sigmoid was employed as the activation function for both the output and hidden layers. The network training was done with the use of back propagation algorithm. The best architecture of the network was chosen based on trial and error method (Abba et al., 2021a).

Adaptive-neuro fuzzy inference system ensemble (ANFIS-E)

In general, Adaptive-neuro fuzzy inference system ensemble (ANFIS-E) is conducted by taking single models average in a non-linear manner. Whereby, new ANFIS model will be calibrated using the values predicted previously using (SWLR, PCR, ANFIS and LSQ-BOOST) models as input variables, which will subsequently be modelled to predict new simulated values. The predicted values will then be compared with the measured values using various evaluation metrics (U. M. Ghali et al., 2020).

CHAPTER III

Methodology

MATERIALS

The chemicals used during this study composed of TQ standard, de-ionized water, Methanol, isopropanol and ethanol were of HPLC-grade and purchased from Sigma Aldrich.

Instrumentation

The HPLC analysis was done using an (Agilent Technologies 1200 series, USA) instrument, which composed of an auto sampler, a binary pump, diode array detector (DAD) and a vaccum degasser. The analyte TQ was determined using an Eclipse XDB-C18 (150 mm×4.6 mm, 5 μm) reversed phase column. The mobile phase involve the use of an isocratic elution system consisting of de-ionized (D.I) water: methanol: 2-propanol (Mp-A: Mp-B: Mp-C). The optimum flow rate was found to be 0.9 mL min^{-1} with 20 mL as the injection volume. The analytical wavelength for the determination of TQ was set at 254 nm with 16 min as the analysis time. The determination of the analyte was done by retention time comparison of the pure standard with the real samples.

Sample preparation

The black cumin seeds were collected from six different geographical regions; Turkey, India, Saudi Arabia, Syria, and Iraq as gift from undergraduate students while coming back to school during winter after the summer holiday. The seeds were further dried, grounded, powdered and then weighed. The sample was extracted using 100 mL methanol and stirred on a magnetic stirrer for 2 hours. The extract obtained was evaporated with the aid of a rotary evaporator. The residue was subsequently dissolved using ethanol and filtered using a solid phase extraction (SPE) cartridge (C8) before analysis with HPLC instrument.

HPLC Quantification

For the quantitative analysis; TMQ solutions, which have 10 different concentrations between 1 to 1000 ppm, were prepared and area of these concentrations were used for calibration.

Chemometrics and models conceptualization

In this study, the application of chemometrics is employed in two different scenarios. First, the chromatographic behaviour of TQ inform of retention time and peak area was simulated using five different AI-based models; SWLR, PCR, LSSVM, ANFIS and LSQ-Boost using different independent variables. Second, the application of ensemble machine learning inform of SAE, WAE, NNE and ANFIS-E to boost the performance activity of the AI-based models. The chromatographic behaviour of TQ inform of retention time and peak area was modelled using different concentrations of the standard solution of the analyte, flow rate and mobile phase consisting of de-ionized (D.I) water: methanol: 2-propanol (Mp-A: Mp-B: Mp-C).

Phase 1: Data acquisition

The complete data set was obtained from our experimental studies, based on calibration of standard solution of TQ. Furthermore, the data points were divided into 70% for the calibration stage and 30% for the testing stage. The data was subsequently validated so as to check and control potential modelling problems such as overfitting and under fitting (Gozen, Nourani, and Abba, 2019).

Phase 2: Data Normalization, correlation and statistical analysis

Both the input and output variables used in this study were normalized based on equation 12 into a range of 0 to 1. The major advantages of normalization before modelling is to reduce the data redundancy as well as the minimize large numerical errors.

$$y = \left(\left(\frac{x - x_{min}}{x_{max} - x_{min}} \right) \right) \quad (5)$$

The correlation and statistical analysis were done using excel 2016 with a confidence limit of 95%.

Phase 3: Simulation using single models

The single AI-based models (PCR, LSSVM, ANFIS and LSQ-Boost) together with the traditional linear regression SWLR were all conducted on MATLAB 9.3 (R2020a).

Phase 4: Ensemble machine learning techniques

The ensemble machine learning techniques involves the assembling, addition and integration of both the non-linear and linear models (PCR, LSSVM, ANFIS, LSQ-Boost and SWLR) to boost the single models performance efficiency.

Phase 5: Performance evaluation metrics

For any kind of data-driven technique, the performance evaluation metrics is investigated using various criteria by experimental and simulated values comparison. Both the performance of the single models as well as the ensemble machine learning techniques developed in this work were checked in both calibration and verification phases using four different performance evaluation criteria.

These are as follows; mean square error (MSE), root mean square error (RMSE), correlation co-efficient (CC) and Nash-Sutcliffe efficiency (NSE) given respectively as Equations 13-16.

$$\text{MSE} = \frac{1}{N}\sum_{i=1}^{N}(Y_{obsi} - Y_{comi})^2 \quad (6)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}(Y_{obsi}-Y_{comi})^2}{N}} \quad (7)$$

$$CC = \frac{\sum_{i=1}^{N}(Y_{obs}-\bar{Y}_{obs})(Y_{com}-\bar{Y}_{com})}{\sqrt{\sum_{i=1}^{N}(Y_{obs}-\bar{Y}_{obs})^2 \sum_{i=1}^{N}(Y_{com}-\bar{Y}_{com})^2}} \quad (8)$$

$$NSE = 1 - \frac{\sum_{j=1}^{N}\left[(Y)_{obs,j}-(Y)_{com,j}\right]^2}{\sum_{j=1}^{N}\left[(Y)_{obs,j}-\overline{(Y)}_{obs,j}\right]^2} \quad (9)$$

Cell Culture Conditions

Human epithelial colon cancer cells (CaCO-2) (ATCC) in 75 cm^2 flasks (Sarstedt, Nümbrecht, Germany), 10 mL DMEM containing 10% FBS (Gibco) and antibiotics (penicillin-streptomycin, Gibco) in a 37°C indicator of carbon dioxide (Dulbecco's Modified Eagle Medium). One day before the cell viability tests, the cells in the flask were lifted with 1 mL of Trypsin-EDTA (Gibco) and transferred to 96-well plates. The cells transferred to the plates were grown in a 37°C carbon dioxide incubator for 48, 72, 96 hours, and *Nigella sativa* extract was made ready for application.

Cell Viability Tests

Nigella sativa extracts were applied to cell culture at 12 half dilutions for cell viability assay analysis. Extracts, 25 µL were applied to the cells in 100 µL medium and each application was repeated three times. Cells were incubated for 48 hours (Larrosa, Tomás-Barberán, & Espín, 2006), 72 and 96 hours (ATCC) in a 37°C carbon dioxide incubator after application of the extracts. Extract concentration ranges to which cells are exposed in cell culture are 3.20-6540

μg/ml, 3.40-6920 μg/ml, 4.54-9300 μg/ml, 3.83-7860 μg/ml for India, Iraq, Turkey, Syria and Saudi Arabia, respectively.

MTT Test

After incubation, the medium in the wells was emptied and 15 μL of MTT working solution (5 mg/mL PBS) was added. After two hours of incubation, where the formation of formazan was observed microscopically, 100 μL of 1% SDS (Sodium Dodecyl Sulphate) was added. Since it is a light sensitive method, the whole study was carried out in a dark environment. After the plates were mixed on a horizontal mixer (100 rpm, 20 min), their optical densities were read on a microplate reader at a wavelength of 540 nm (Mitra et al., 2016).

LDH (Lactate Dehydrogenase) Test

The test was performed using the ChronoLab Quantitative Detection of Lactate Dehydrogenase kit (Barcelona, Spain). Accordingly, following the incubation of the drug-administered cells, the cell media were separated; cell fraction was used for MTT analysis and media for LDH analysis. As stated in the kit, 50 μL of separated cell medium was added to 1.5 mL of fresh working solution (Buffer- Imidazole and pyruvate, Substrate- NADH; 4:1, v:v) prepared in the amount to be used in the research. After vortexing, it was transferred to 96-well plates and read in a microplate reader at 340 nm.

Neutral Red Experiment

Neutral red assay was conducted according to the method of Repetto et al., (2008). After incubation, the medium was decanted and the plates were washed with 200 μL of PBS. 100 mL of neutral red reagent was added to the wells (40 μL/ml in DMEM), and they were kept in the culture medium for 2 hours. After washing with 150 μL of PBS, destain solution (49% deionized water, 50% ethanol, and 1% glacial acetic acid) was added as a dye and shaken for 10 minutes, and the neutral red test results were determined by reading at 540 nm optical density.

Cytotoxicity Assessment

Cytotoxicity in the sample treated cells is expressed as percentage and compared to the positive control group (Maximal Viability, Max V) considered 100% viable and the negative control group (Minimal Viability, Min V) treated with Triton-X, which is considered 0%

viable. Calculated using the formula. IC50 evaluation was performed using GraphPad Prism4 and NCSS software according to classical sigmoid-dose modeling.

$$\text{Cytotoxicity (\%)} = [1 - (\text{test} - \text{MinV}) / (\text{MaxV} - \text{MinV})] \text{ X } 100 \quad (10)$$

Malondialdehyde (MDA) Assay

After performing cytotoxicity tests, MDA level determination from oxidative stress parameters in the cell line was made using Cayman Chemical TBARS Assay Kit.

CHAPTER IV

FINDINGS AND DISCUSSION

HPLC Linearity Results

The performance of the chromatographic method was accessed by plotting the aqueous calibration curve graph, through the preparation of the standard solution of TQ between 0 to 1000 ppm.

Figure 23

The calibration curve of the experiment

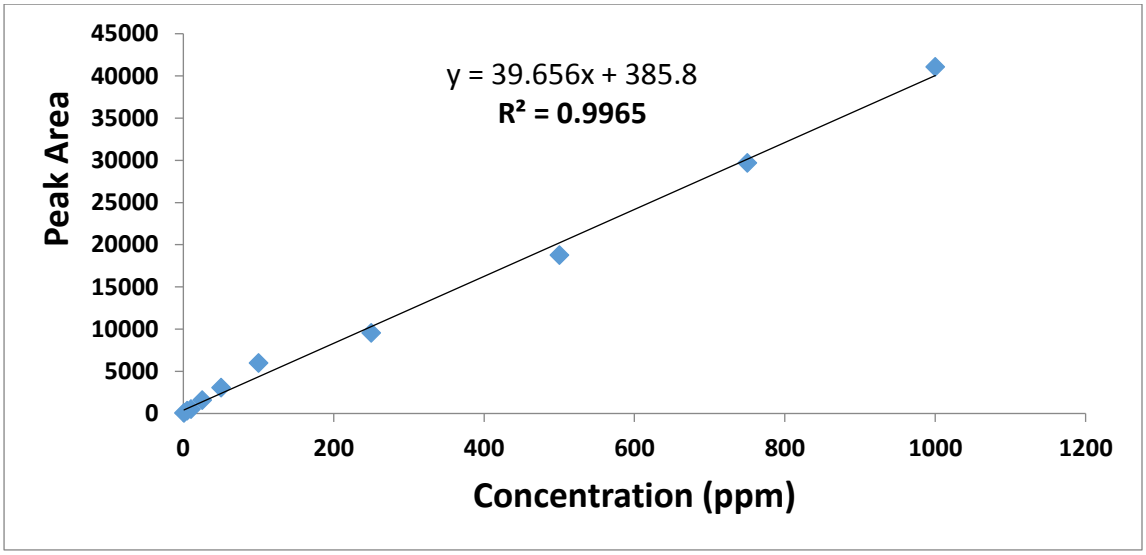


Table 2

Linear equation, LOD, LOQ, R2, and RSD values of TQ calibration

Linear equation	LOD	LOQ	R^2	RSD
y = 39.656x + 385.8	0.102	0.309	0.9965	1.50

Figure 24

The chromatogram of the standard TQ analyte

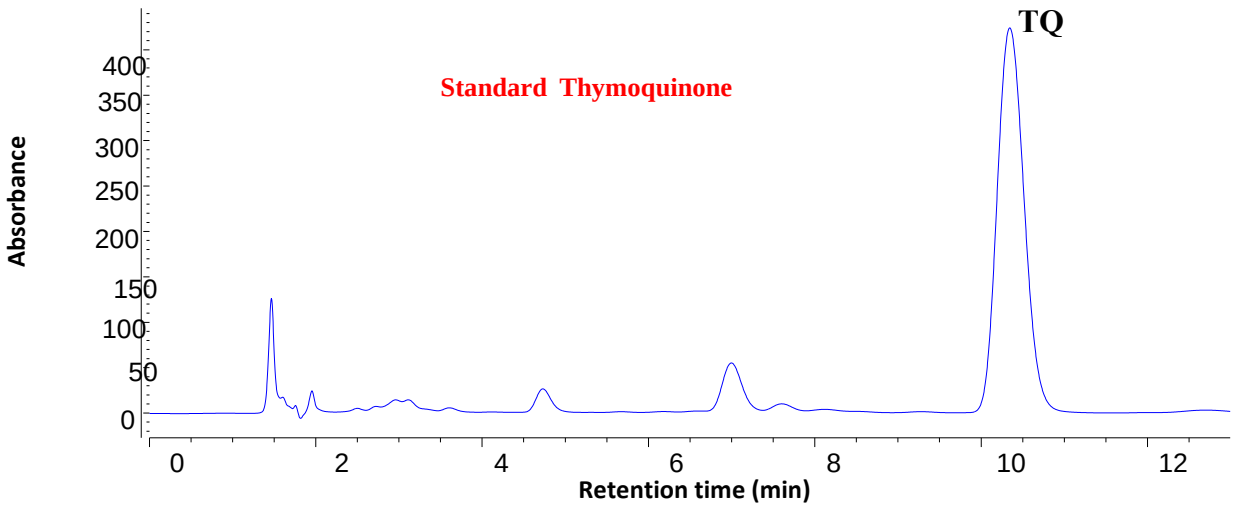


Figure 25

The chromatogram of the Indian sample

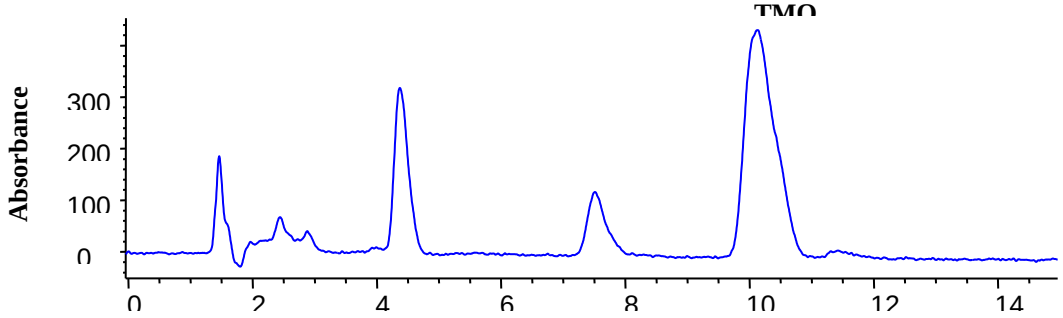


Figure 26

The chromatogram of the Iraq sample

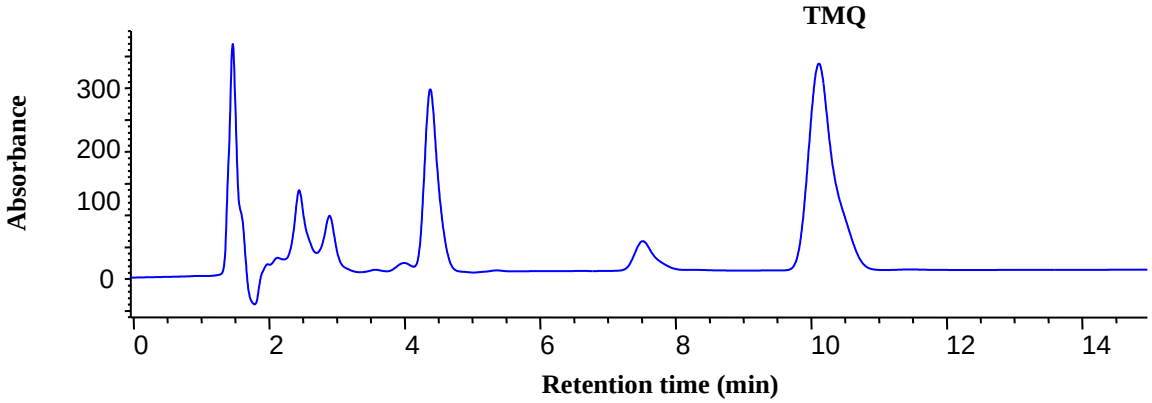


Figure 27

The chromatogram of the Saudi Arabian sample

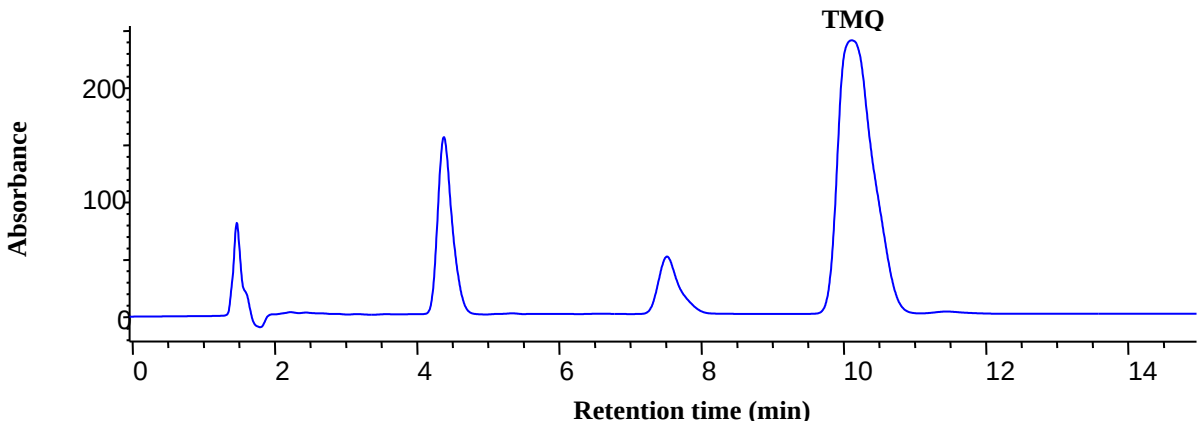


Figure 28

The chromatogram of the Syrian sample

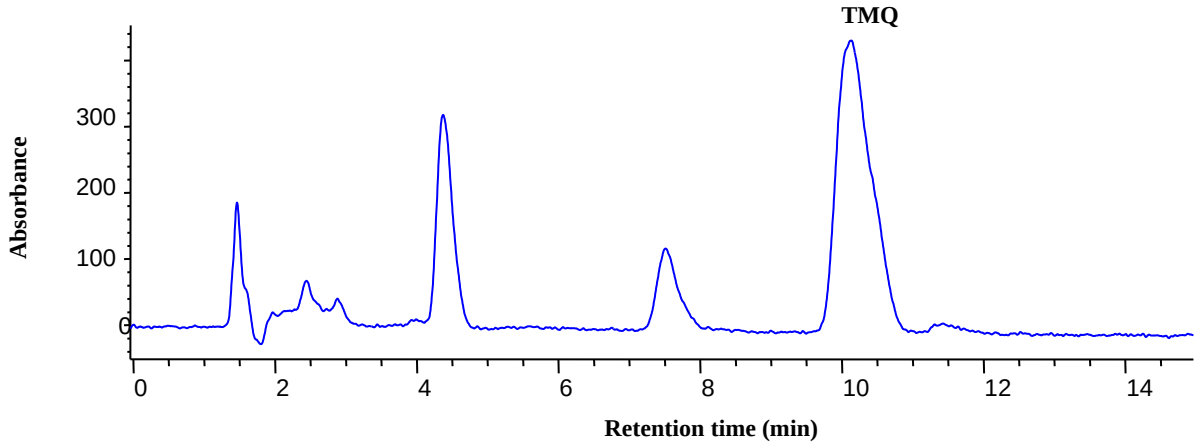
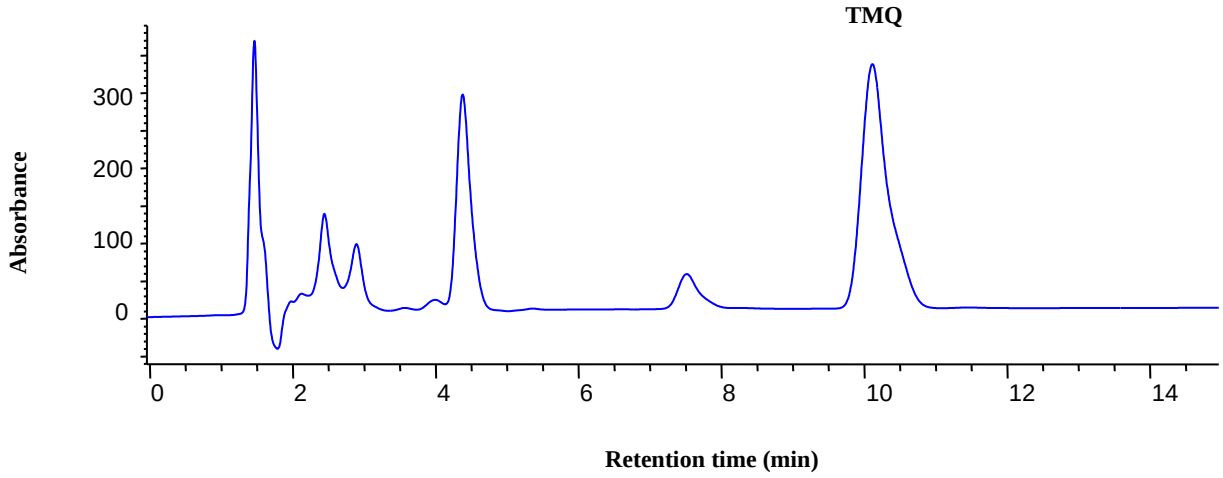


Figure 29

The chromatogram of the Türkey sample



The amounts of Thymoquinone found in different black cumin seeds of different geographical regions are calculated using the obtained calibration equation, are summarized in Table 3.

Table 3

Thymoquinone amounts in Nigella sativa seed extracts from different countries

Sample Name	Concentration (ppm)	% Thymoquinone amount
Iraq	31.26	0.031
India	30.35	0.030
Saudi Arabia	22.15	0.022
Syria	5.47	0.005
Turkey	1.39	0.001

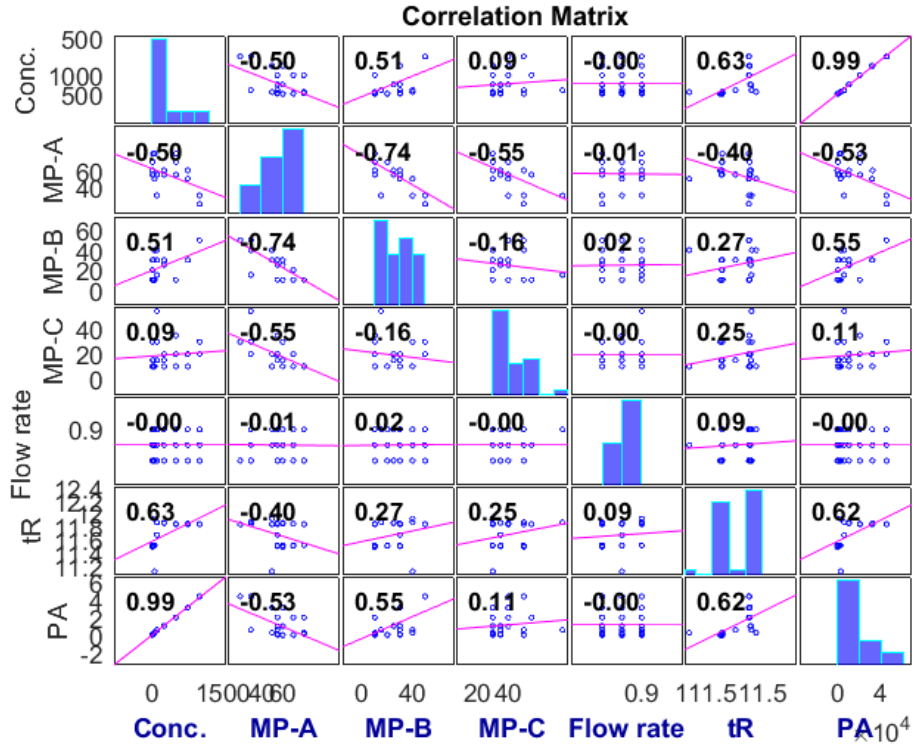
Table 4

Descriptive statistics results of both the independent and dependent variables

	Concentration	MP- A	MP-B	MP-C	Flow rate	tR	PA
Mean	278.34	55.86	25.34	18.79	0.90	11.68	12274.37
Median	100.00	55.00	25.00	15.00	0.90	11.84	6055.80
Mode	5.00	60.00	10.00	10.00	0.90	11.52	#N/A
Standard Deviation	349.38	15.76	13.02	10.58	0.08	0.20	15029.98
Kurtosis	-0.27	0.00	-0.72	3.49	-1.52	0.19	0.06
Skewness	1.09	-0.58	0.43	1.65	-0.07	-0.76	1.18
Range	999.00	60.00	40.00	45.00	0.20	0.81	44626.10
Minimum	1.00	20.00	10.00	10.00	0.80	11.12	49.30
Maximum	1000.00	80.00	50.00	55.00	1.00	11.93	44675.40

Figure 30

The correlation analysis performance of the variables



Performance of the chemometrics based models

Table 5

Performance of the single models (PCR, LSSVM, ANFIS, LSQ-Boost and SWLR)

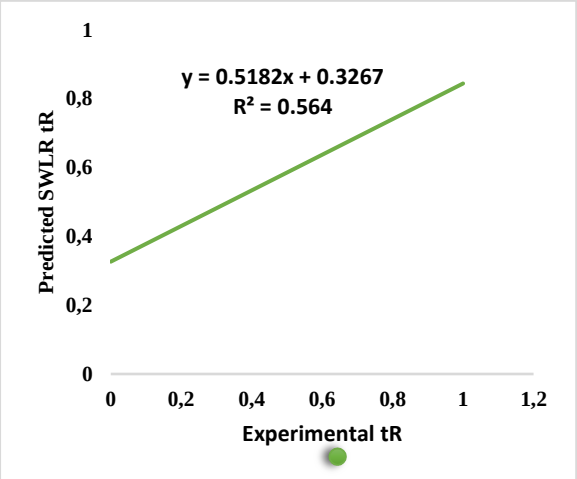
	Training				Testing			
	NSE	**CC**	**RMSE**	**MSE**	**NSE**	**CC**	**RMSE**	**MSE**
SWLR-tR	0.362	0.601	0.192	0.037	0.220	0.469	0.004	0.000
PCR-tR	0.701	0.837	0.131	0.017	0.586	0.766	0.003	0.000
LSSVM-tR	0.173	0.416	0.218	0.048	0.016	0.128	0.005	0.000
ANFIS-tR	0.999	1.000	0.001	0.000	0.999	1.000	0.000	0.000
LSQ-Boost-tR	0.613	0.783	0.149	0.022	0.651	0.807	0.003	0.000
SWLR-PA	0.999	1.000	0.005	0.000	0.987	0.994	0.009	0.000
PCR-PA	0.674	0.821	0.126	0.016	0.234	0.483	0.070	0.005
LSSVM-PA	0.923	0.961	0.022	0.000	0.983	0.991	0.029	0.001
ANFIS-PA	0.999	1.000	0.000	0.000	0.999	1.000	0.005	0.000
LSQ-Boost-PA	0.765	0.875	0.039	0.002	0.567	0.753	0.146	0.021

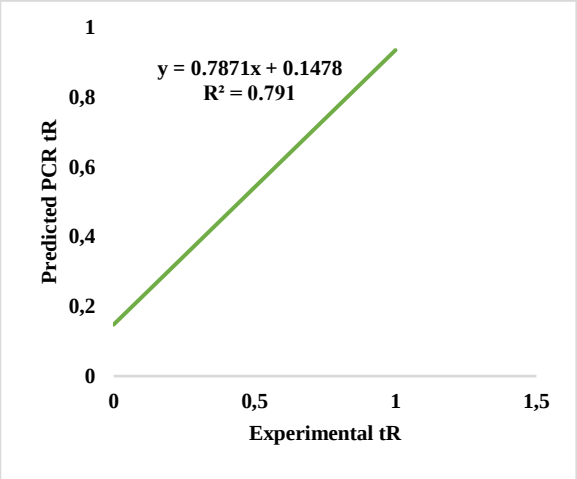
The comparative performance of the models can be shown graphically using scatter plot as shown in **Figure 31 and 32**.

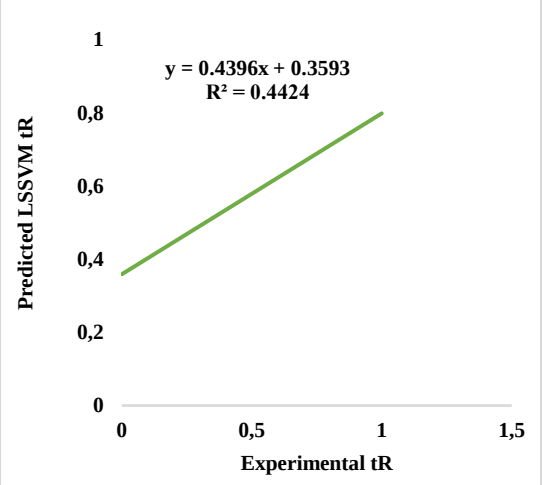
Performance of the single models (PCR, LSSVM, ANFIS, LSQ-Boost and SWLR)

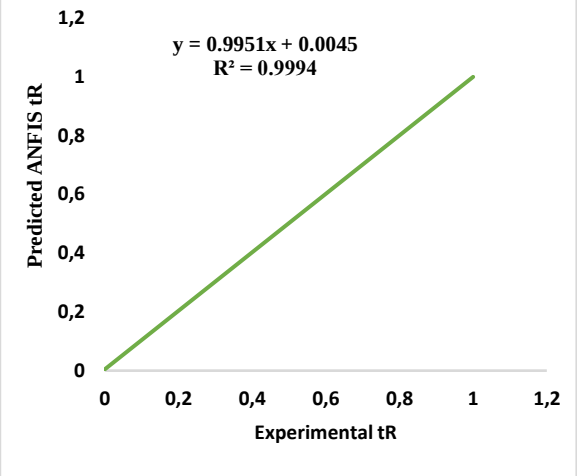
Figure 31

Scatter plots of the single models for their respective retention time (tR)


y = 0.5182x + 0.3267
R² = 0.564
Predicted SWLR tR
Experimental tR
0
0,2
0,4
0,6
0,8
1
1,2


y = 0.7871x + 0.1478
R² = 0.791
Predicted PCR tR
Experimental tR
0
0,2
0,4
0,6
0,8
1
0,5
1,5


y = 0.4396x + 0.3593
R² = 0.4424
Predicted LSSVM tR
Experimental tR
0
0,2
0,4
0,6
0,8
1
0,5
1,5


y = 0.9951x + 0.0045
R² = 0.9994
Predicted ANFIS tR
Experimental tR
0
0,2
0,4
0,6
0,8
1
1,2

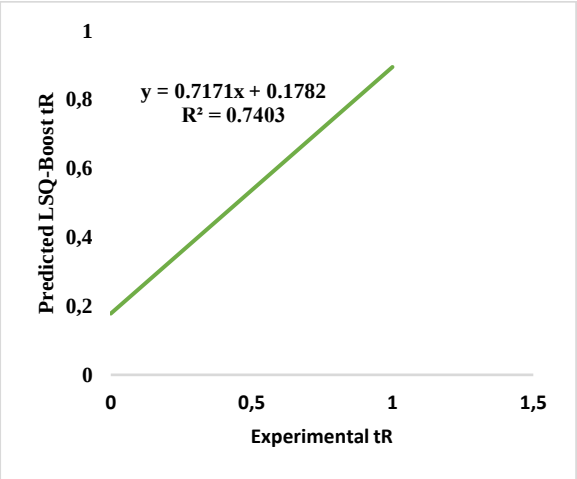
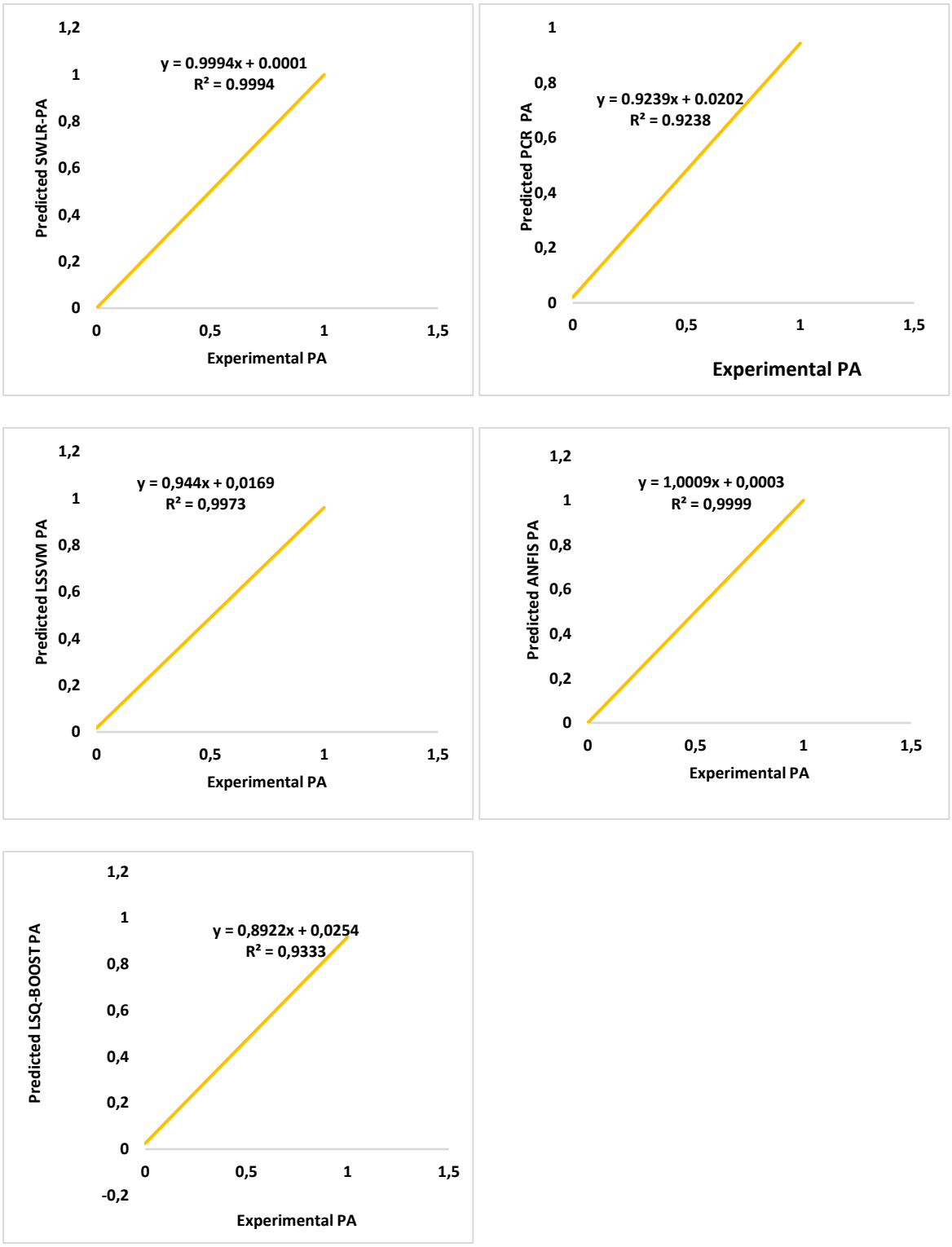

y = 0.7171x + 0.1782
R² = 0.7403
Predicted LSQ-Boost tR
Experimental tR
0
0,2
0,4
0,6
0,8
1
0,5
1,5

Figure 32

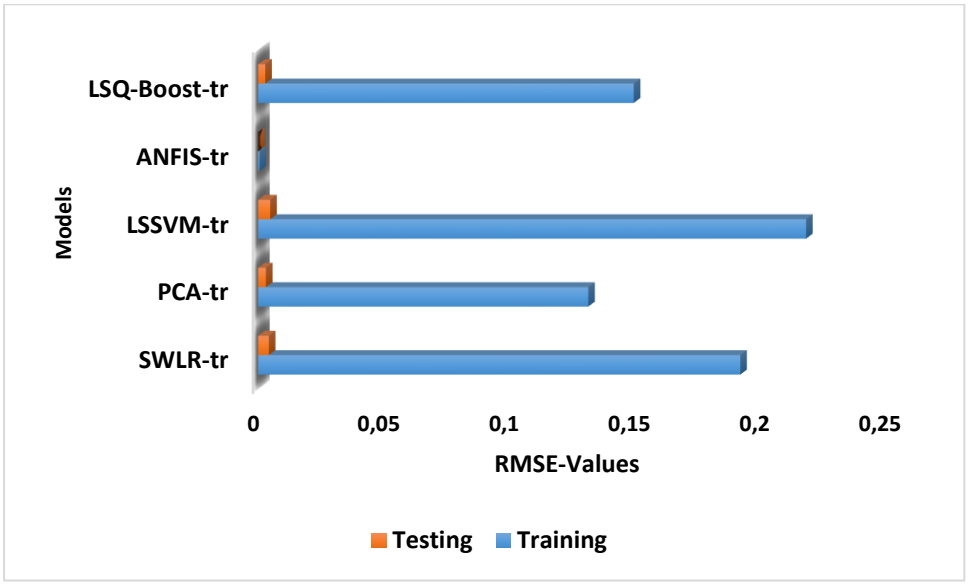
Scatter plots of the single models for their respective Peak area (PA)

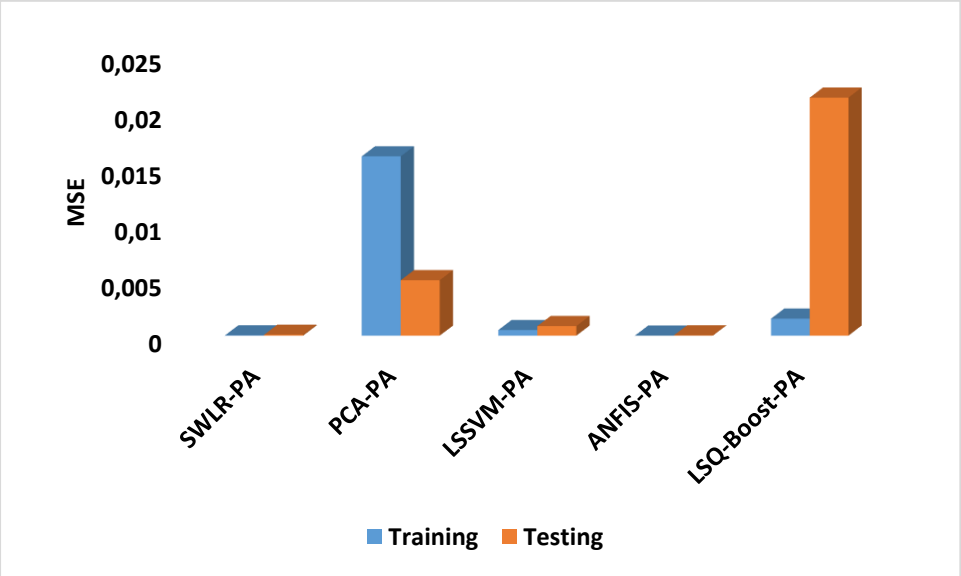


The performance of these plots is in line with the results demonstrated in **Table 5.** Moreover, the comparative performance efficiency of these models can be demonstrated based on their respective error-values. The lower the MSE and RMSE-values the higher the performance of the models and the higher the MSE and RMSE-value the lower the performance of the models and vice-versa. Figure 33 indicates that ANFIS outperformed all the other single models based on their RMSE-vales in modelling tR and MSE-values for modelling PA respectively.

Figure 33

Error performance of the single models in terms of RMSE for retention time (tR) modelling and MSE for peak area (PA) modelling respectively





The response surface of the TQ, which composed of tR and PA were further comparatively shown in Figure 34. Based on the performance inspection, it can be noted that two AI-based models (PCR and ANFIS) emerged as the reliable and satisfactory models, which is equally in line with **Table 5.** The AI-based models predictive performance was equally proved in numerous technical literature and published articles including (Abdullahi Garba Usman, Ahmad, Danraka, & Abba, 2021), (Shojaeimehr & Rahimpour, 2018), (J. Yang et al., 2002), (Nourani, Elkiran, & Abba, 2018b) and (Korany, Mahgoub, Fahmy, & Maher, 2012). In addition, the correlation co-efficient (R) shown in **Table 5** indicates the merit of these two data driven approaches over the others. Mostly, SWLR and other linear models fails when it comes to modelling highly complex and non-linear data as it obeys the regression technique and least-squares method. The radar plot can be used in demonstrating the correlation co-efficient performance of the models as shown in Figure 34.

Figure 34

The comparative models performance based on their respective correlation co-efficient in both the training and testing stages

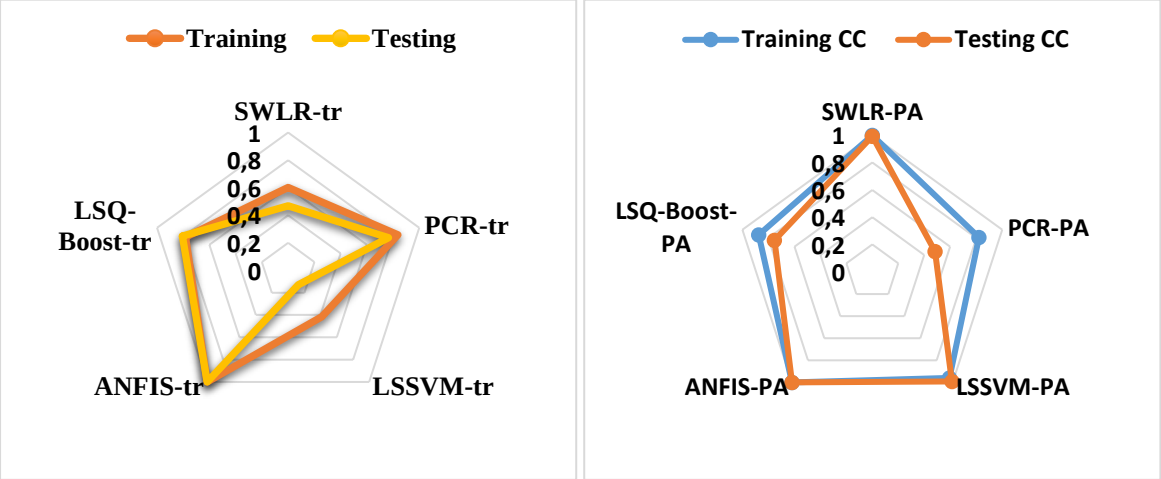


Table 6

Performance of the Ensemble machine learning techniques (SAE, WAE, NNE, and ANFIS-E)

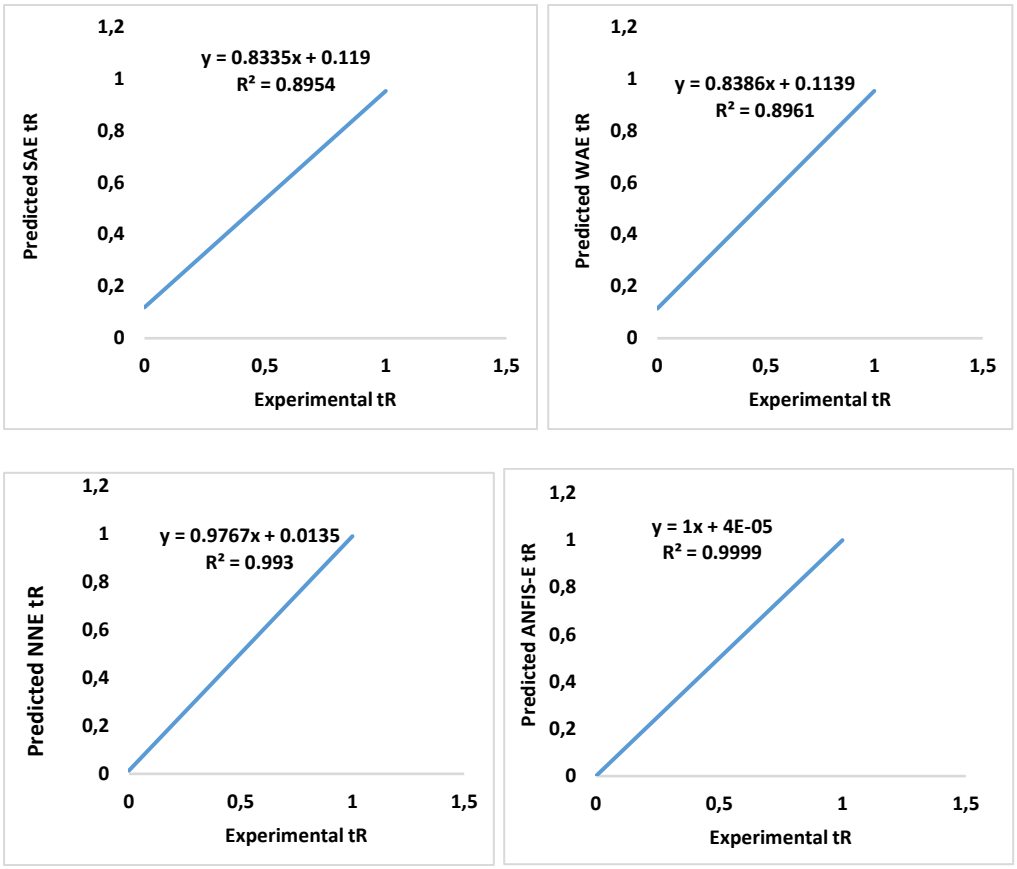
	Training				Testing			
	NSE	**CC**	**RMSE**	**MSE**	**NSE**	**CC**	**RMSE**	**MSE**
SAE-tr	0.842	0.917	0.087	0.009	0.843	0.918	0.002	0.000
WAE-tr	0.844	0.919	0.086	0.009	0.890	0.944	0.002	0.000
NNE-tr	0.989	0.995	0.025	0.001	0.986	0.993	0.001	0.000
ANFIS-E-tr	0.999	1.000	0.000	0.000	0.999	0.999	0.000	0.000
SAE-PA	0.998	0.999	0.010	0.000	0.987	0.993	0.025	0.001
WAE-PA	0.999	0.999	0.008	0.000	0.998	0.999	0.009	0.000
NNE-PA	0.999	1.000	0.015	0.000	0.999	1.000	0.005	0.000
ANFIS-E-PA	0.999	1.000	0.001	0.000	0.999	0.999	0.004	0.000

Performance of the Ensemble machine learning techniques

The analysis regarding the exploratory performance of the ensemble machine learning techniques for the prediction of tR can be pictured better using the scatter plots (Figure 35).

Figure 35

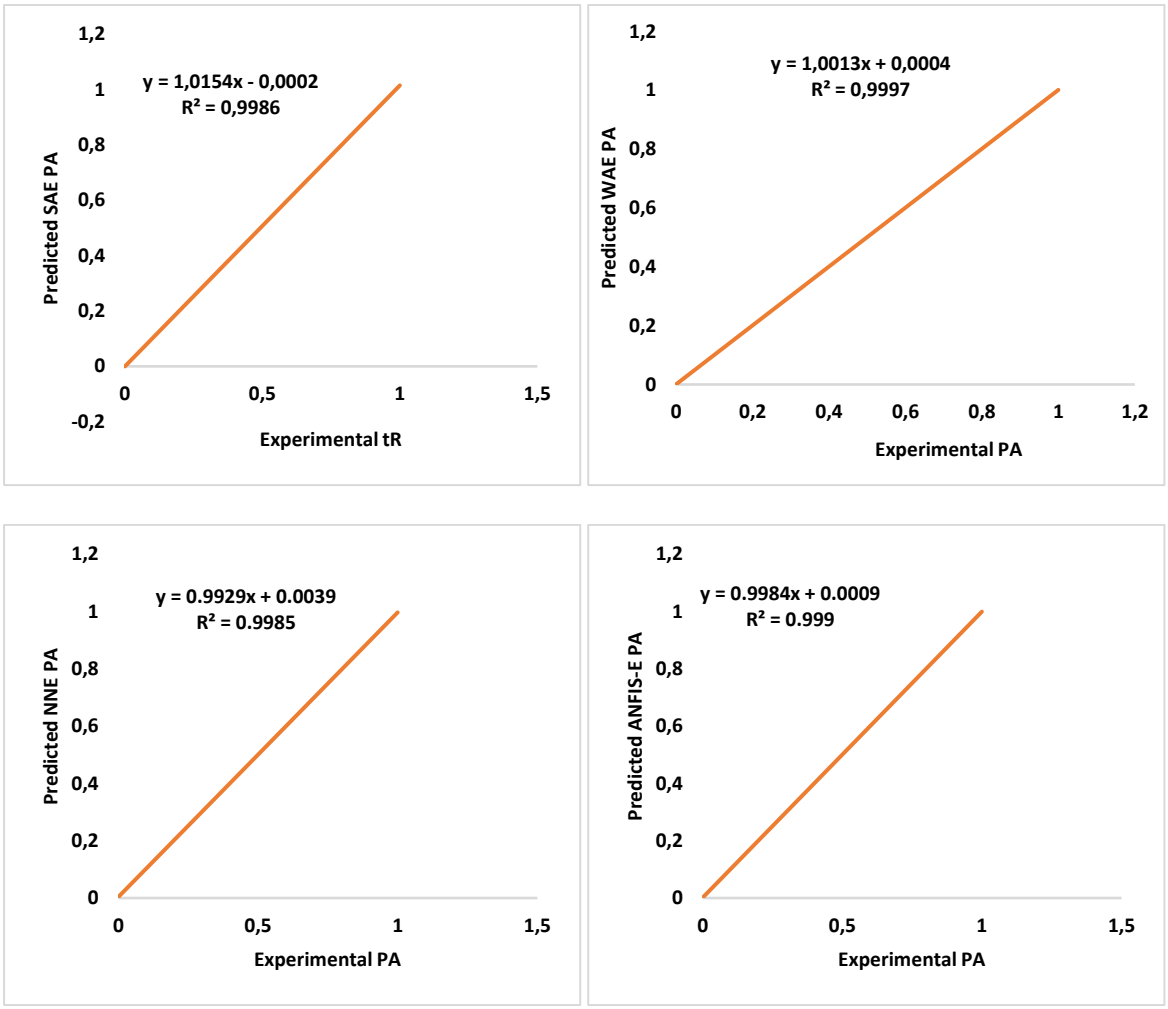
Scatter plots of the ensemble techniques for tR



More also, the performance simulation of the PA properties is shown using scatter plot in Figure 36.

Figure 36

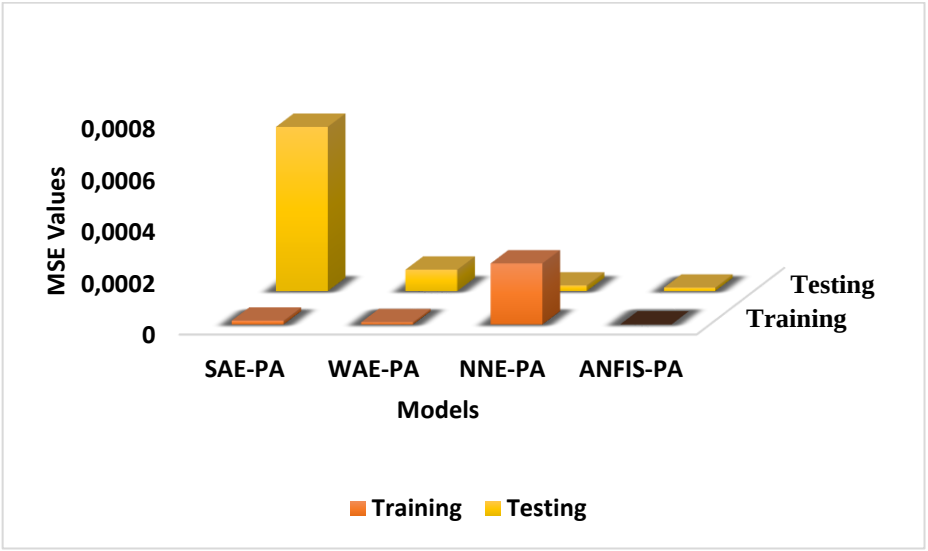
Scatter plots of the ensemble techniques for PA



The error performance of the ensemble machine learning techniques is equally shown in Figure 37. Based on Figure 37 it can be noted that all the ensemble techniques depicts strong performance efficiency in modelling the chromatographic behaviour of TQ.

Figure 37

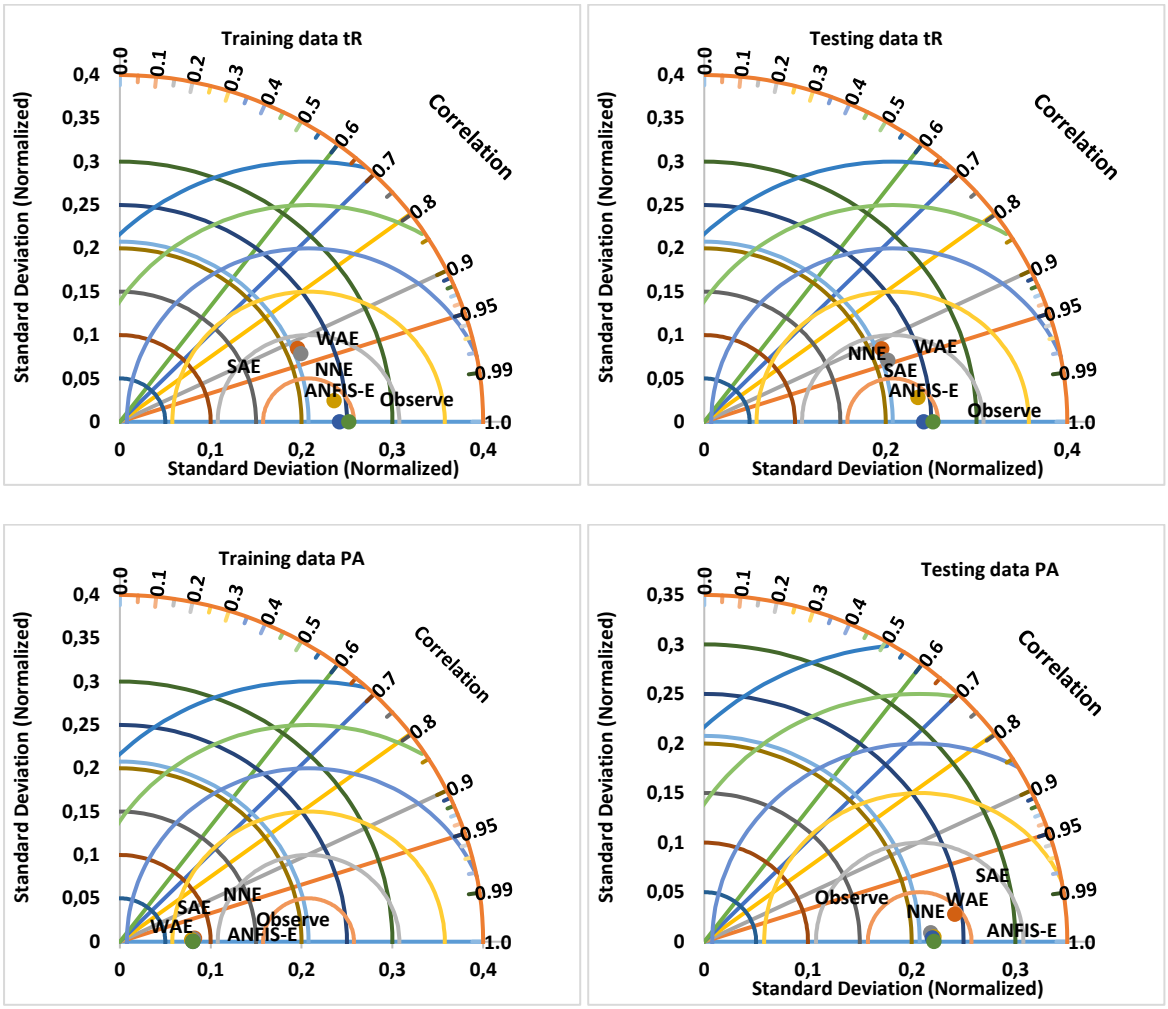
Error performance of the ensemble machine learning techniques inform of PA in both the training and testing stages



The performance skills of the ensemble machine learning technique was also shown through the use of a recent graphical representation called ‘Taylor diagram’. This diagram have been used in several areas of modelling such as computer science, computer vision and climate modelling owing to its significance. The diagram is used to depicts the goodness of fit in terms of CC as shown in this study (see Figure 38).

Figure 38

Taylor diagram indicating the performance of the Ensemble machine learning techniques for both the chromatographic behaviour of TQ in terms of PA and tR



Cytotoxicity results

The MTT assay is an in vitro quantitative colorimetric method applied to measure cellular metabolic activity as an indicator of cell viability, proliferation, and cytotoxicity (Butler & Nowak, 2004). Neutral red uptake assay allows measurement of lysosomal activity, quantifies cell viability; Viable cells can take up neutral red via active transport and take up the dye into their lysosomes, while non-viable cells cannot take up this chromophore (Governa et al., 1997).

Lactate dehydrogenase is a cytoplasmic enzyme found in nearly all cells. Due to damage to the plasma membrane (including apoptosis, necrosis, or other cellular death pathways), LDH can rise into the plasma (medium in cell culture).

The images of the plates of the MTT and Neutral Red experiments are given in Figure 40 The results of the same experiments are given in Table 7.

Table 7

MTT and Neutral Red Uptake Test IC_{50} (±SD) values of black cumin seed extracts

Sample name	MTT (µg/L)	Neutral red (µg/ml)
India	74.04 ± 12.03	172.63 ± 13.26
Syria	159.78 ± 6.93	538.87 ± 22.36
Iraq	395.21± 14.07	148.02 ± 6.47
S. Arabia	414.86 ± 5.27	525.96 ± 53.60
Turkey	499.67 ± 1.3	1211.35 ± 11.14

Figure 39

IC_{50} (±SD) values of percent cytotoxic activity when applied at IC_{50} concentrations calculated according to the MTT test

.

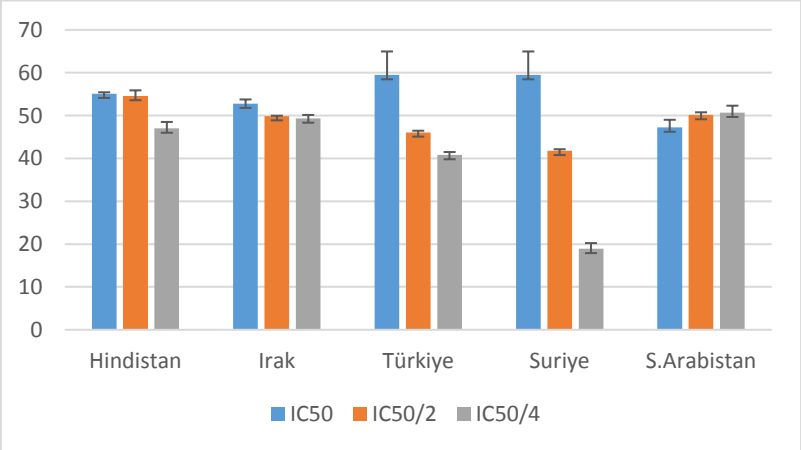
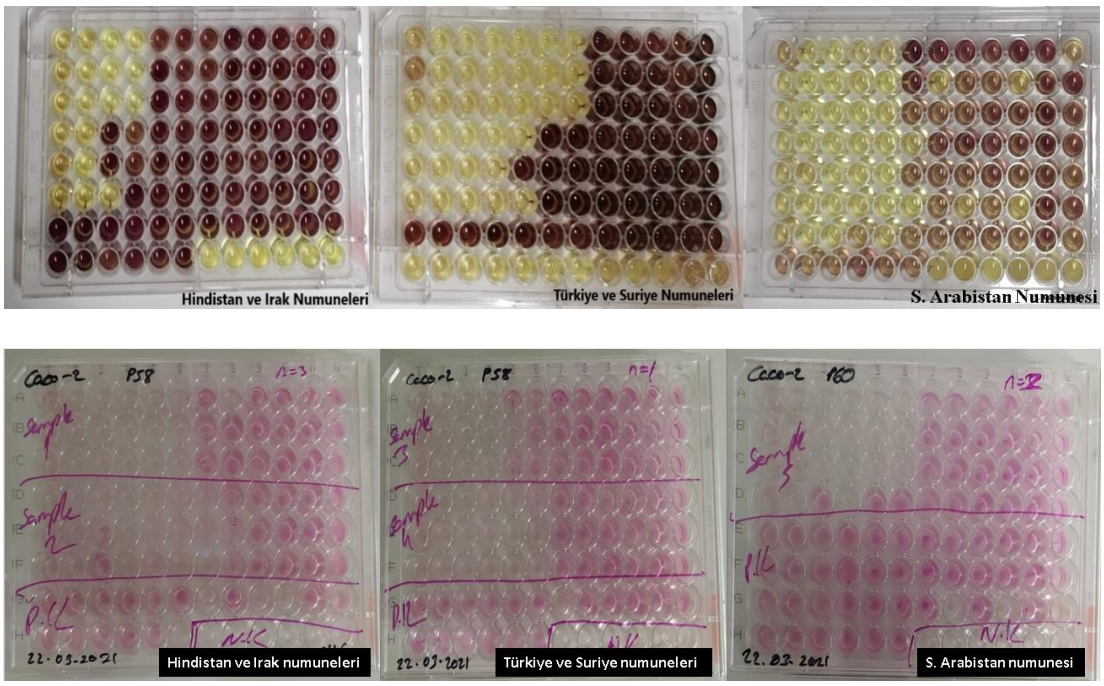


Figure 40

Images of the plates of the MTT and Neutral Red experiments



The findings of the LDH test are given in **Table 8.**

Table 8

LDH test (±SD) values

Nigella Extracts	**U/l**
India	101.46±0.05
Iraq	96.42±0.05
Turkey	70.29±0.01
Syria	72.99±0.24
S. Arabia	115.04±0.05

Figure 41

Appearance after 24 hours incubation following administration of Indian sample, positive (medium treated) and negative control (Triton X treated) at concentrations of 1635 ppm, 12.77 ppm to Caco-2 cells

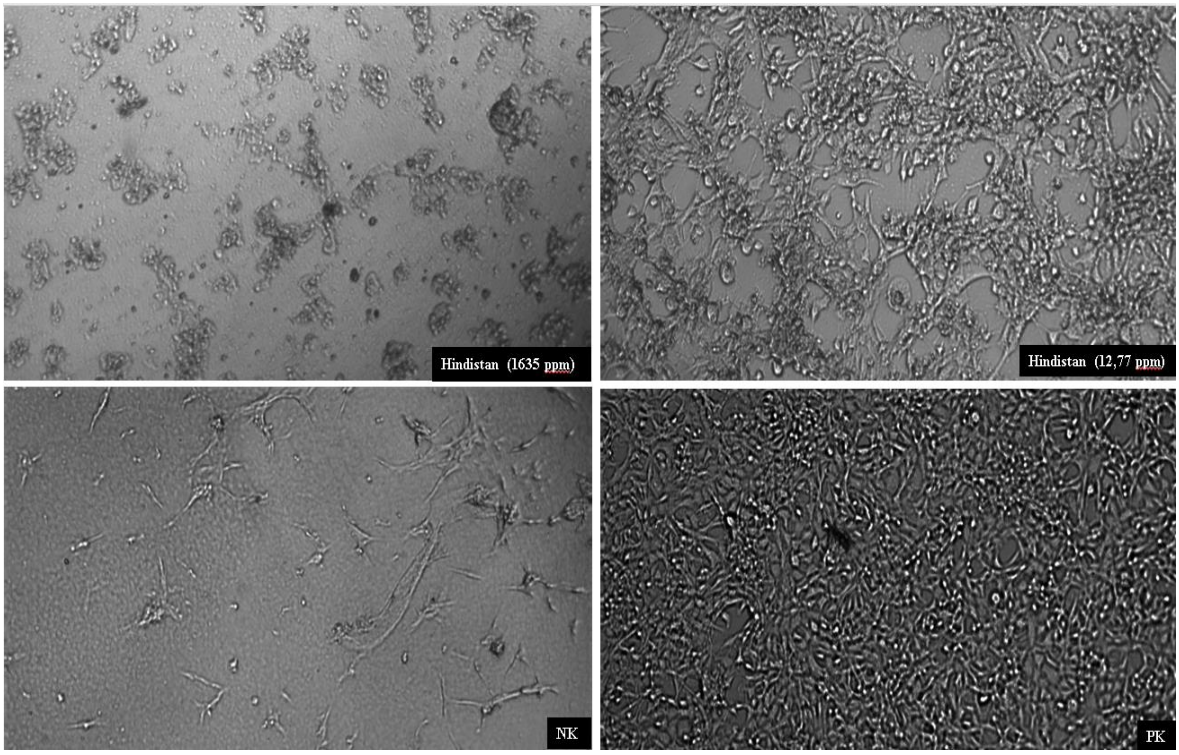


Figure 42

Syrian sample at concentrations of 7590 ppm, 118.59 ppm to Caco-2 cells; Appearance after 24 hours incubation following administration of 8410 ppm, 131.4 ppm Saudi Arabian sample.

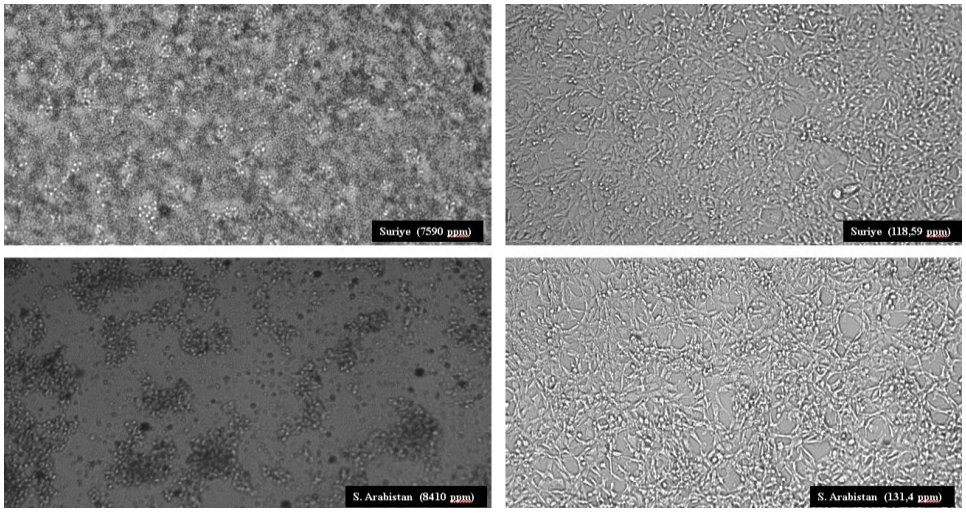
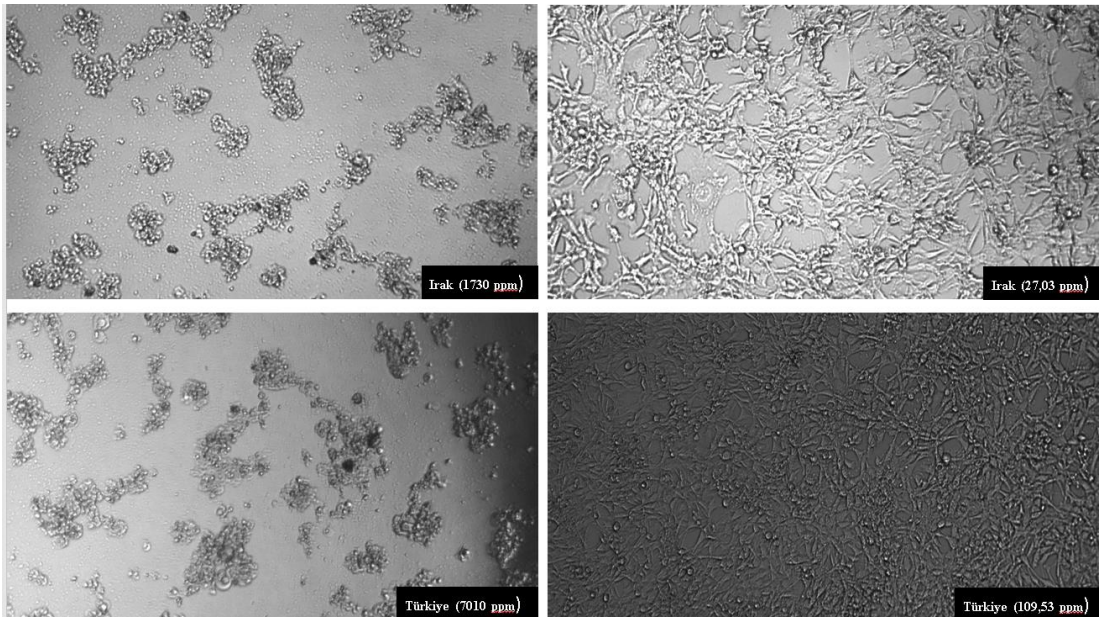


Figure 43

Iraq sample at concentrations of 1730 ppm, 27.03 ppm; after the application of 7010 ppm, 109.53 ppm Turkey sample, after 24 hours of incubation.



Malondialdehyde is a compound formed by lipid peroxidation. Lipid peroxidation is a known mechanism used as a marker of oxidative stress in cells and tissues of both plants and animals. The experiment was performed using the Cayman Chemical TBARS Assay Kit commercial kit.

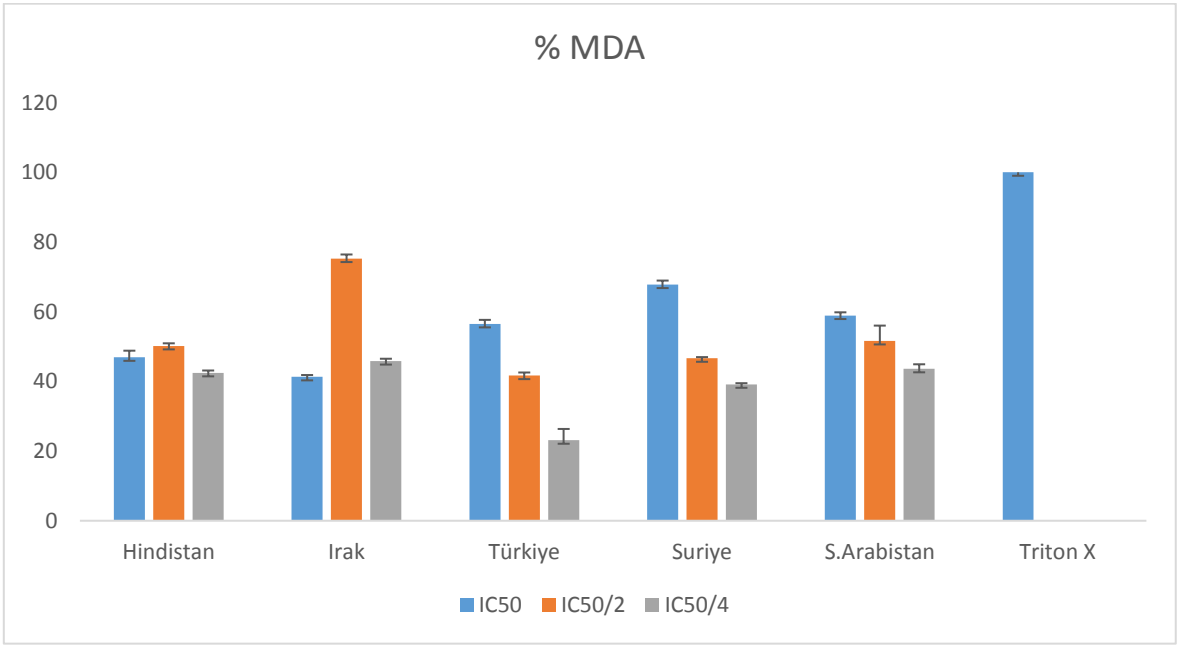
Table 9

Effect of extracts on MDA level

	India	**Iraq**	**Turkey**	**Syria**	**S. Arabia**
IC_{50}	9.27±0.38	9.60±0.12	8.70±0.18	8.04±0.14	8.56±0.13
$IC_{50}/2$	9.08±0.13	7.59±0.12	9.58±0.21	9.29±0.07	8.99±0.77
$IC_{50}/4$	9.53±0.15	9.33±0.14	10.68±1.50	9.73±0.08	9.46±0.28

Figure 44

Effect of extracts on MDA levels



The Crystal violet viability test is designed to work with adhered cells, while the Crystal violet stains the protein and DNA of adherent cells. According to the results of the crystal violet viability test, the % viability values are given in Table 10 and the staining appearances are given in Figure 45.

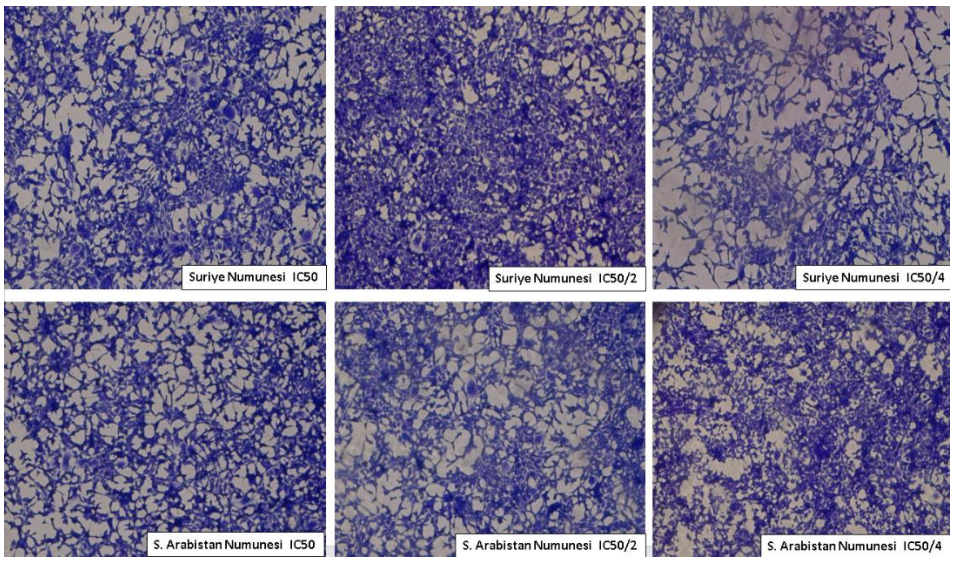
Table 10

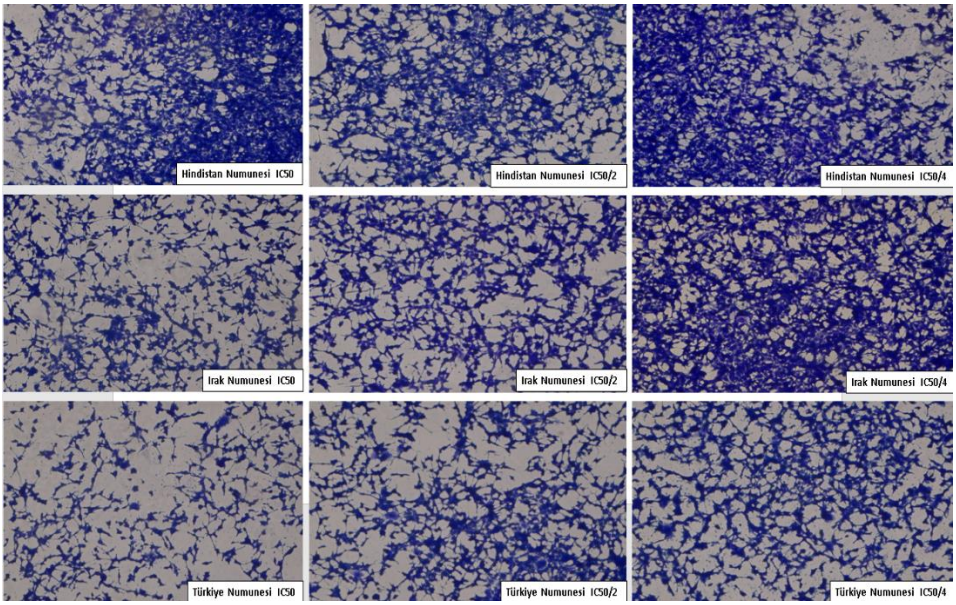
Crystal violet test percentage vitality values

Nigella Extracts	**percent vitality**
India	114.40±1.59
Iraq	38.45±0.22
Turkey	22.96±3.34
Syria	51.20±0.43
S. Arabia	20.65±1.41

Figure 45

Cell photos of black cumin seed extracts staining with crystal violet




Hindistan Numunesi IC50
Hindistan Numunesi IC50/2
Hindistan Numunesi IC50/4
Irak Numunesi IC50
Irak Numunesi IC50/2
Irak Numunesi IC50/4
Türkiye Numunesi IC50
Türkiye Numunesi IC50/2
Türkiye Numunesi IC50/4

CHAPTER V

Discussion

Based on Table 2, it can be observed that the calibration curve demonstrates higher linearity with determination co-efficient value (R^2) of 0.9965 (see Figure 23).

The detection limit (LOD) was equally determine using $3S_b/m$, in which S_b is defined as standard deviation of the intercept while m is the gradient of the linear equation was found to be 0.102. Moreover, the limit of quantitation (LOQ) was determined using $10S_b/m$ was found to be 0.309. The relative standard deviation (RSD) of the experiment was found to be 1.50.

Based on the seeds of black cumin obtained from diverse geographical regions; According to our research data, the highest amount of TQ was found in Iraq and Indian samples. Although the amount of TQ in all samples varied, TQ was detected in all samples; the amount of TQ was 0.031, 0.030, 0.022, 0.005 and 0.001% in the Iraq, India, Saudi Arabia, Syria and Turkey samples, respectively. The differences in TQ from black cumin seeds is attributed to the climate changes in the geographical regions where the seeds are grown, the soil quality, etc. Owing to differences in factors such as the ones presented in previous study of our team, the amount of TQ found in *Nigella sativa* seeds obtained from Ankara was found to be in the range of 0.010-0.376% (Isik, Kartal, & Erdem, 2017). The TQ content of the seeds of the black cumin plant cultivated in India and Kuwait was found to be in the range of 1039.85 mg/kg to 2940.43 mg/kg (Herlina, Aziz, Kurniawati, & Faridah, 2017). Gholamnezhad et al. (2015), in a study investigating the immunomodulatory and cytotoxic effects of black cumin seeds, found that the seeds contained 0.06% TQ. Although the amount of TQ in black cumin seeds calculated within the scope of this study complies with the TQ range determined in the literature, it can be said that the amount of TQ is in a very wide range. This wide variation for TQ in plants varies depending on genetic deviations, harvest time/season, and physiological conditions (Zribi, Omezzine, & Haouala, 2014). Therefore, determining the phytochemical content of the *Nigella sativa* plant to be used for medicinal purposes is important in terms of providing a standard effect and concentration of its major analyte TQ.

As earlier stated in chapter three, this work presented different chemometrics based models including the single models such as PCR, LSSVM, ANFIS, LSQ-Boost and SWLR as well as

the ensemble machine learning inform of SAE, WAE, NNE and ANFIS-E for the modelling of both the qualitative and quantitative properties of TQ. Statistical and correlation analysis were conducted prior to dwelling into the simulation step in order to know the nature and properties of the both dependent and independent variables. Table 4 demonstrates the statistical results of the variables indicating the mean, median, kurtosis and skewness among other statistical properties of the variables.

Table 5 demonstrates the quantitative performance efficiency of the single models PCR, LSSVM, ANFIS, LSQ-Boost and SWLR. Based on the comparative performance of these techniques, it can be seen that ANFIS model outperformed all the other single models PCR, LSSVM, LSQ-Boost and SWLR in modelling both tR and PA in the training and testing stages (see Table 4.4). Nourani et al. 2019 reported that the minimum Nash-Sutcliffe efficiency (NSE) needed for any model to be accepted should be 80% (In another word, the model should have a minimum of 0.8 R^2-value for it to be acceptable) (Nourani et al., 2019). Based on the performance table only ANFIS was able to simulate tR, which is attributed mainly due to its complex ability of modeling highly non-linear data, this is in line with (Khalid & Usman, 2021), (Asnake Metekia, Garba Usman, Hatice Ulusoy, Isah Abba, & Chirkena Bali, 2021), (Abba et al., 2021b), (Umar Muhammad Ghali, Alhosen, et al., 2020), (Umar Muhammad Ghali, Usman, et al., 2020), (USMAN, IŞIK, ABBA, & MERİÇLİ, 2020), (A. G. Usman, Işik, & Abba, 2021), (Abba, Usman, & IŞIK, 2020), (Abba, Pham, et al., 2020), (Ahmad, Usman, & Abba, 2021), (Abdullahi, Usman, Abba, & Abdullahi, 2020), (Abba et al., 2021a) and (Alsharksi, Danmaraya, Abdullahi, Ghali, & Usman, 2020) findings. Whereby, regarding the simulation of PA only SWLR, LSSVM and ANFIS were able to fulfill the minimum 80% requirement for a model to be accepted. Therefore, this showed a need to use other techniques such as ensemble machine learning to boost the performance ability of the single models.

Based on the comparative performance of the Ensemble machine learning techniques, it can be noted that all the four ensemble machine learning techniques are able to simulate the chromatographic properties of TQ inform of PA and tR with minimum and maximum NSE-values of 0.842 and 0.999 in the training phase and 0.918 and 0.999 in the testing phase respectively. The techniques equally showed lower error-values inform of RMSE and MSE as compared with the single models.

According to MTT test findings, the highest cytotoxic activity was found in the Indian sample with 74.04 µg/mL, while the lowest cytotoxic activity was found in the Turkish sample with 499.67. According to the Neutral Red test, the highest cytotoxic activity was found in the Iraq sample with 148 µg/mL, while the lowest cytotoxic activity was found in the Turkey (1211.35) sample, similar to the MTT test. The IC_{50} values of the Neutral Red test were found to be 2.34 times higher on average than the MTT test in all samples except the Iraq sample. This shows that plant extracts cause cell death via the mitochondrial pathway instead of the lysosomal pathway.

The LDH activity of Nigella extracts was found to be between 70.29-115.04. LDH activity showed a dose-dependent decrease except for the Saudi Arabian sample, but this decrease was not statistically significant ($p>0.05$). When applied at IC_{50} concentrations calculated according to the MTT test, the percent cytotoxic activity was found as 55.11, 52.79, 59.45, 59.45 and 47.21 for the samples of India, Iraq, Turkey, Syria and Saudi Arabia, respectively. When applied at the IC_{50} value, values close to 50% cytotoxic activity were also determined in the LDH test shows that in parallel with the mitochondrial pathway, the extracts also damaged the cell membrane. Application and control images of the extracts on the Caco-2 cell line are given in Figure 42 to 44. The crude extract cytotoxicity activity criteria for crude extracts considered active against cancer cells determined by the US National Cancer Institute (NCI) were determined as $IC_{50}<30$ µg/mL (Yurdakök & Baydan, 2013). The IC_{50} values of cytotoxic activities of methanolic extracts prepared from Nigella sativa seed collected from different geographical regions within the scope of the thesis were found to be between 47.21-59.45. Since the results were above 30 µg/ml, it was concluded that they did not have a significant activity on Caco-2 cells. In addition, Thymoquinone amounts of Nigella sativa seeds collected from different geographical regions were found to be between 0.001-0.030%. These differences can be attributed to different climate, soil characteristics and time of collection. There is no correlation between the amount of Thymoquinone and cytotoxic activity, indicating that other compounds in the seed are also responsible for the effect. In future studies, it is planned to investigate their cytotoxic activities by preparing extracts of different polarities, and to determine the substance(s) responsible for the effect by performing activity-directed fractionation studies.

Elevated MDA levels was noted in all samples treated with Nigella extract (Table 9), but this increase was dose-dependent in Turkey, Syria, and S. Arabia, while it was independent of dose

in samples of India and Iraq. The MDA level was found to be between 8.04 and 9.60 in μM/mL in the applications performed at IC_{50} dose (Figure 44).

According to the crystal violet vitality test, it was observed that the cells were stained more intensely at high concentration (IC_{50}) than at low concentration. However, in this technique, which has the feature of giving false positive results, the viability was 114.40% after the IC_{50} dose application in the Indian sample, while it varied between 20-51% in other applications. The presence of cytotoxic activity less than the IC_{50} value indicates that the samples may have been lifted prior to administration. The high value (114.40%) in the Indian sample also indicates that it may have stained other materials (the plant extract itself) other than living cells. In the project, the cytotoxic activity of the extracts was tested on the human epithelial colon cancer cell line (Caco-2), and according to the experiments on Caco-2 cells, changes in cell morphology, pycnotic nuclei, cell clusters, some cells were found to be lifted and floated at high concentrations.

It was determined that Nigella sativa seed extract inhibited the MDA-MB-231 human breast cancer cells proliferation at a dose of 2.5-5 μg/mL. In another study evaluating the effects of seed extract and seed oil extract on human lung cancer cell line, cell viability was found to be 0.25 by MTT method for seed extract; 0.5; It was found that 75%, 50% and 26% respectively at 1 mg/mLdoses. Another study showed that black seed oil has potential anti-proliferative and proapoptotic effects on human lymphocytes cells (Ciesielska-Figlon, Lisowska, Mikosik-Roczynska, & Witkowski, 2021).

It has been revealed in the literature that black cumin causes cell death in various human cells such as the lung, liver, colon and cell lines of the breast cancer (Attoub et al., 2013). In a study examining the in vitro cytotoxic activities of Nigella sativa seeds and the fixed oil obtained in A549 human lung cancer cell lines; extract and oil have been reported to significantly reduce the viability of A549 cells (Al-Sheddi et al., 2014). It was determined that the aqueous extract prepared from the seeds showed significant cytotoxic effect on oral cancer and precancerous leucoplakia cells in vitro.

CHAPTER VI

Conclusion and recommendation

Conclusion

The anticancer activity on human colon cell line of TQ from black cumin of different geographical regions was tested using different cytotoxity analysis was done in this theses. Prior to that the quantitative determine of each sample was done in order to determine the TQ content of each black cumin sample taken from different geographical regions. Different geographical factors such as rainfall, seasonal variations and soil plays a vital role in the total amount of TQ present in each sample as indicated in the literature. The results obtained implies that the highest amount of TQ was found in Iraq and Indian samples. Although the amount of TQ in all samples varied. TQ was detected in all samples; the amount of TQ was 0.031, 0.030, 0.022, 0.005 and 0.001% in the Iraq, India, Saudi Arabia, Syria and Turkey samples, respectively.

The amount of TQ affected the cytotoxic potential of the black cumin extract on Caco-2 cells. Indian black cumin was found to induce highest cytotoxic effect with highest TQ levels. This preliminary experiment provides evidence that the geographical differences were found to have an effect on the efficacy of the anticancer potency of black cumin.

More also, the study equally involved the application of both single models and ensemble machine learning approaches as one of the recent employed chemometrics methods for modelling chromatographic behaviour of different analytes. The results obtained from the chemometrics equally indicates the ability of these models in modelling both the qualitative and quantitative properties of TQ.

Recommendation

It is recommended that other cancer cell lines such as the breast and brain cancer cell lines should be used in determining the anticancer activity of TQ from black cumin of different geographical regions. More also, the microextraction methods such as switchable hydrophilicity solvents (SHS), dispersive liquid-liquid microextraction (DLLME) and deep eutectic solvents (DES) can be introduced in the subsequent work to preconcentrate and increase the sensitivity of the method. Furthermore, other metaheuristic approaches such Harris

Hawks' optimization algorithms (HHO) and particle swam optimizations (PSO) can be used in simulating the chromatographic properties of TQ.

References

Abba, S. I., Abdulkadir, R. A., Sammen, S. S., Usman, A. G., Meshram, S. G., Malik, A., & Shahid, S. (2021a). Comparative implementation between neuro-emotional genetic algorithm and novel ensemble computing techniques for modelling dissolved oxygen concentration. *Hydrological Sciences Journal*, *0*(0). https://doi.org/10.1080/02626667.2021.1937179

Abba, S. I., Abdulkadir, R. A., Sammen, S. S., Usman, A. G., Meshram, S. G., Malik, A., & Shahid, S. (2021b). Comparative implementation between neuro-emotional genetic algorithm and novel ensemble computing techniques for modelling dissolved oxygen concentration. *Hydrological Sciences Journal*, *66*(10), 1584–1596. https://doi.org/10.1080/02626667.2021.1937179

Abba, S. I., Hadi, S. J., Sammen, S. S., Salih, S. Q., Abdulkadir, R. A., Pham, Q. B., & Yaseen, Z. M. (2020). Evolutionary computational intelligence algorithm coupled with self-tuning predictive model for water quality index determination. *Journal of Hydrology*, *587*(March), 124974. https://doi.org/10.1016/j.jhydrol.2020.124974

Abba, S. I., Pham, Q. B., Usman, A. G., Linh, N. T. T., Aliyu, D. S., Nguyen, Q., & Bach, Q. V. (2020). Emerging evolutionary algorithm integrated with kernel principal component analysis for modeling the performance of a water treatment plant. *Journal of Water Process Engineering*, *33*(December 2019), 101081. https://doi.org/10.1016/j.jwpe.2019.101081

Abba, S. I., Usman, A. G., & IŞIK, S. (2020). Simulation for response surface in the HPLC optimization method development using artificial intelligence models: A data-driven approach. *Chemometrics and Intelligent Laboratory Systems*, *201*(April). https://doi.org/10.1016/j.chemolab.2020.104007

Abdelmeguid, N. E., Fakhoury, R., Kamal, S. M., & Al Wafai, R. J. (2010). Effects of Nigella sativa and thymoquinone on biochemical and subcellular changes in pancreatic β-cells of streptozotocin-induced diabetic rats. *Journal of Diabetes*, *2*(4), 256–266. https://doi.org/10.1111/j.1753-0407.2010.00091.x

Abdullahi, H. U., Usman, A. G., Abba, S. I., & Abdullahi, H. U. (2020). Modelling the Absorbance of a Bioactive Compound in HPLC Method using Artificial Neural Network and Multilinear Regression Methods. *Dutse Journal of Pure and Applied Sciences (DUJOPAS)*, *6*(2).

Adamson, G. E., Lazarus, S. A., Mitchell, A. E., Prior, R. L., Cao, G., Jacobs, P. H., … Schmitz, H. H. (1999). HPLC method for the quantification of procyanidins in cocoa and chocolate samples and correlation to total antioxidant capacity. *Journal of Agricultural and Food Chemistry*, *47*(10), 4184–4188. https://doi.org/10.1021/jf990317m

Ahmad, M. H., Usman, A. G., & Abba, S. I. (2021). Comparative performance of extreme learning machine and Hammerstein–Weiner models for modelling the intestinal hyper-motility and secretory inhibitory effects of methanolic leaf extract of Combretumhypopilinum Diels (Combretaceae). *In Silico Pharmacology*, *9*(1). https://doi.org/10.1007/s40203-021-00090-1

Al-Ali, A., Alkhawajah, A. A., Randhawa, M. A., & Shaikh, N. A. (2008). Oral and intraperitoneal LD50 of thymoquinone, an active principle of Nigella sativa, in mice and rats. *Journal of Ayub Medical College, Abbottabad : JAMC*, *20*(2), 25–27.

Al-Sheddi, E. S., Farshori, N. N., Al-Oqail, M. M., Musarrat, J., Al-Khedhairy, A. A., & Siddiqui, M. A. (2014). Cytotoxicity of Nigella Sativa seed oil and extract against human lung cancer cell line. *Asian Pacific Journal of Cancer Prevention*, *15*(2), 983–987. https://doi.org/10.7314/APJCP.2014.15.2.983

Alsharksi, A. N., Danmaraya, Y. A., Abdullahi, H. U., Ghali, U. M., & Usman, A. G. (2020). *Potential of Hybrid Adaptive Neuro Fuzzy Model in Simulating Clostridium Difficile Infection Status*. (1), 1–6. https://doi.org/10.35940/ijbsac.A0191.073120

Asnake Metekia, W., Garba Usman, A., Hatice Ulusoy, B., Isah Abba, S., & Chirkena Bali, K. (2021). Artificial intelligence-based approaches for modeling the effects of spirulina growth mediums on total phenolic compounds. *Saudi Journal of Biological Sciences*, (xxxx). https://doi.org/10.1016/j.sjbs.2021.09.055

Attoub, S., Sperandio, O., Raza, H., Arafat, K., Al-Salam, S., Al Sultan, M. A., … Adem, A. (2013). Thymoquinone as an anticancer agent: Evidence from inhibition of cancer cells viability and invasion in vitro and tumor growth in vivo. *Fundamental and Clinical Pharmacology*, *27*(5), 557–569. https://doi.org/10.1111/j.1472-8206.2012.01056.x

Badary, O. A., Taha, R. A., Gamal El-Din, A. M., & Abdel-Wahab, M. H. (2003). Thymoquinone is a potent superoxide anion scavenger. *Drug and Chemical Toxicology*, *26*(2), 87–98. https://doi.org/10.1081/DCT-120020404

Bashmail, H. A., Alamoudi, A. A., Noorwali, A., Hegazy, G. A., AJabnoor, G., Choudhry, H., & Al-Abd, A. M. (2018). Thymoquinone synergizes gemcitabine anti-breast cancer

activity via modulating its apoptotic and autophagic activities. *Scientific Reports*, *8*(1), 1–11. https://doi.org/10.1038/s41598-018-30046-z

Bermejo-Barrera, P., Moreda-Piñeiro, A., & Bermejo-Barrera, A. (2002). Sample pre-treatment methods for the trace elements determination in seafood products by atomic absorption spectrometry. *Talanta*, *57*(5), 969–984. https://doi.org/10.1016/S0039-9140(02)00139-X

Biswas, A. K., Rao, G. S., Kondaiah, N., Anjaneyulu, A. S. R., Mendiratta, S. K., Prasad, R., & Malik, J. K. (2007). A simple multi-residue method for determination of oxytetracycline, tetracycline and chlortetracycline in export buffalo meat by HPLC-photodiode array detector. *Journal of Food and Drug Analysis*, *15*(3), 278–284. https://doi.org/10.38212/2224-6614.2419

Buszewski, B., & Szultka, M. (2012). Past, Present, and Future of Solid Phase Extraction: A Review. *Critical Reviews in Analytical Chemistry*, *42*(3), 198–213. https://doi.org/10.1080/07373937.2011.645413

Butler, R., & Nowak, B. F. (2004). In vitro interactions between Neoparamoeba sp. and Atlantic salmon epithelial cells. *Journal of Fish Diseases*, *27*(6), 343–349. https://doi.org/10.1111/j.1365-2761.2004.00550.x

Calderilla, C., Maya, F., Leal, L. O., & Cerdà, V. (2018). Recent advances in flow-based automated solid-phase extraction. *TrAC - Trends in Analytical Chemistry*, *108*, 370–380. https://doi.org/10.1016/j.trac.2018.09.011

Celeste, M., Galvão, E., Rosa, B., Ferreira, E., Silva, R., & Caldas, C. (2019). Screening of Mangifera indica L . functional content using PCA and neural networks (ANN). *Food Chemistry*, *273*(December 2017), 115–123. https://doi.org/10.1016/j.foodchem.2018.01.129

Ciesielska-Figlon, K., Lisowska, K. A., Mikosik-Roczynska, A., & Witkowski, J. M. (2021). Nigella sativa oil inhibits proliferation and stimulates apoptosis of human lymphocytes in vitro. *Human Immunology*, *82*(8), 608–614. https://doi.org/10.1016/j.humimm.2021.04.010

Darakhshan, S., Bidmeshki Pour, A., Hosseinzadeh Colagar, A., & Sisakhtnezhad, S. (2015). Thymoquinone and its therapeutic potentials. *Pharmacological Research*, *95–96*, 138–158. https://doi.org/10.1016/j.phrs.2015.03.011

Das, K., Tiwari, R. K. S., & Shrivastava, D. K. (2010). Techniques for evaluation of medicinal

plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research*, *4*(2), 104–111. https://doi.org/10.5897/JMPR09.030

Deepak, K., Rupali, S., & Mahesh, K. (2016). An overview of major classes of phytochemicals: Their types and role in disease prevention. *Hislopia Journal*, *9*(1), 1–11.

Edris, A. (2009). Anti-Cancer Properties of Nigella spp. Essential Oils and their Major Constituents, Thymoquinone and β-Elemene. *Current Clinical Pharmacology*, *4*(1), 43–46. https://doi.org/10.2174/157488409787236137

Ehlert, S., Trojer, L., Vollmer, M., Van De Goor, T., & Tallarek, U. (2010). Performance of HPLC/MS microchips in isocratic and gradient elutionmodes. *Journal of Mass Spectrometry*, *45*(3), 313–320. https://doi.org/10.1002/jms.1719

Elahi, H., Reza, M., Masroorchehr, Nemati, S., Kamalinejad, M., Besharat, M., … Jalessi, M. (2014). *Historical Applications of Nigella sativa L . and Nazleh in Persian Medicine*. *3*(1).

Faraji, M., Yamini, Y., & Gholami, M. (2019). Recent Advances and Trends in Applications of Solid-Phase Extraction Techniques in Food and Environmental Analysis. In *Chromatographia* (Vol. 82). https://doi.org/10.1007/s10337-019-03726-9

Fotakis, G., & Timbrell, J. A. (2006). In vitro cytotoxicity assays: Comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. *Toxicology Letters*, *160*(2), 171–177. https://doi.org/10.1016/j.toxlet.2005.07.001

Frank, L. E., & Friedman, J. H. (1993). A statistical view of some chemometrics regression tools. *Technometrics*, *35*(2), 109–135. https://doi.org/10.1080/00401706.1993.10485033

Freund, Y.; Schapire, R.; Abe, N. A. (1999). A short introduction to boosting. *J. Jpn. Soc. Artif. Intell.*, (14), 771–780.

Ghali, U. M., Usman, A. G., Chellube, Z. M., Degm, M. A. A., Hoti, K., Umar, H., & Abba, S. I. (2020). Advanced chromatographic technique for performance simulation of anti-Alzheimer agent: an ensemble machine learning approach. *SN Applied Sciences*, *2*(11). https://doi.org/10.1007/s42452-020-03690-2

Ghali, Umar Muhammad, Alhosen, M., Degm, A., Alsharksi, A. N., Hoti, Q., & Usman, A. G. (2020). *Development Of Computational Intelligence Algorithms For Modelling The Performance Of Humanin And Its Derivatives In HPLC Optimization Method Development*. *9*(08), 110–117.

Ghali, Umar Muhammad, Usman, A. G., Alhosen, M., Degm, A., Alsharksi, A. N., Naibi, A.

M., & Abba, S. I. (2020). *Applications of Artificial Intelligence-Based Models and Multi-Linear Regression for the Prediction of Thyroid Stimulating Hormone Level in the Human Body*. *29*(4), 3690–3699.

Gheshlaghi, R., Scharer, J. M., Moo-Young, M., & Douglas, P. L. (2008). Application of statistical design for the optimization of amino acid separation by reverse-phase HPLC. *Analytical Biochemistry*, *383*(1), 93–102. https://doi.org/10.1016/j.ab.2008.07.032

Governa, M., Valentino, M., Amati, M., Visonà, I., Botta, G. C., Marcer, G., & Gemignani, C. (1997). Biological effects of contaminated silicon carbide particles from a workstation in a plant producing abrasives. *Toxicology in Vitro*, *11*(3), 201–207. https://doi.org/10.1016/S0887-2333(97)00018-0

Grosso, C., Figueiredo, A. C., Burillo, J., Mainar, A. M., Urieta, J. S., Barroso, J. G., … Palavra, A. M. F. (2009). Enrichment of the thymoquinone content in volatile oil from Satureja montana using supercritical fluid extraction. *Journal of Separation Science*, *32*(2), 328–334. https://doi.org/10.1002/jssc.200800490

Guler, T., Dalkiliç, B., Ertas, O. N., & Çiftçi, M. (2006). The effect of dietary black cumin seeds (Nigella sativa L.) on the performance of broilers. *Asian-Australasian Journal of Animal Sciences*, *19*(3), 425–430. https://doi.org/10.5713/ajas.2006.425

Hajhashemi, V., Ghannadi, A., & Jafarabadi, H. (2004). Black Cumin Seed Essential Oil, as a Potent Analgesic and Antiinflammatory Drug. *Phytotherapy Research*, *18*(3), 195–199. https://doi.org/10.1002/ptr.1390

Han, X., Gelein, R., Corson, N., Wade-Mercer, P., Jiang, J., Biswas, P., … Oberdörster, G. (2011). Validation of an LDH assay for assessing nanoparticle toxicity. *Toxicology*, *287*(1–3), 99–104. https://doi.org/10.1016/j.tox.2011.06.011

Hassan, M., Brown, R. D., Varma-O'Brien, S., & Rogers, D. (2006). Cheminformatics analysis and learning in a data pipelining environment. *Molecular Diversity*, *10*(3), 283–299. https://doi.org/10.1007/s11030-006-9041-5

Henri. (2018). 済無No Title No Title No Title. In *Angewandte Chemie International Edition, 6(11), 951–952.*

Herlina, Aziz, S. A., Kurniawati, A., & Faridah, D. N. (2017). Changes of Thymoquinone, Thymol, and Malondialdehyde Content of Black Cumin (Nigella sativa L.) in Response to Indonesia Tropical Altitude Variation. *HAYATI Journal of Biosciences*, *24*(3), 156–161. https://doi.org/10.1016/j.hjb.2017.08.004

Hoda, M., Hemaiswarya, S., & Doble, M. (2019). Role of Phenolic Phytochemicals in Diabetes Management. In *Role of Phenolic Phytochemicals in Diabetes Management*. https://doi.org/10.1007/978-981-13-8997-9

Huerta, M., Leiva, V., Liu, S., Rodríguez, M., & Villegas, D. (2019). On a partial least squares regression model for asymmetric data with a chemical application in mining. *Chemometrics and Intelligent Laboratory Systems*, *190*(April), 55–68. https://doi.org/10.1016/j.chemolab.2019.04.013

Isik, S., Kartal, M., & Erdem, S. A. (2017). Quantitative analysis of thymoquinone in Nigella Sativa L. (Black Cumin) seeds and commercial seed oils and seed oil capsules from Turkey. *Ankara Universitesi Eczacilik Fakultesi Dergisi*, *41*(1), 34–41. https://doi.org/10.1501/Eczfak_0000000593

Iwaniak, A., Minkiewicz, P., Darewicz, M., Protasiewicz, M., & Mogut, D. (2015). Chemometrics and cheminformatics in the analysis of biologically active peptides from food sources. *Journal of Functional Foods*, *16*, 334–351. https://doi.org/10.1016/j.jff.2015.04.038

Jang, M. H., Piao, X. L., Kim, J. M., Kwon, S. W., & Park, J. H. (2008). Inhibition of cholinesterase and amyloid-&bgr; aggregation by resveratrol oligomers from Vitis amurensis. *Phytotherapy Research*, *22*(4), 544–549. https://doi.org/10.1002/ptr

Kearns, M., & Valiant, L. (1994). Cryptographic Limitations on Learning Boolean Formulae and Finite Automata. *Journal of the ACM (JACM)*, *41*(1), 67–95. https://doi.org/10.1145/174644.174647

Khader, M., Eckl, P. M., Soepomo, J. P., Alam, P., Yusufoglu, H., Alam, A., … Kokoska, L. (2017). MENINGKATKAN AKTIVITAS FAGOSITOSIS MAKROFAG TIKUS BETINA GALUR SD (Sprague Dawley) YANG DIINDUKSI DMBA (7 , 12Dimetilbenz (α) antrasen) SECARA IN VITRO THE EFFECTS OF HEXANE EXTRACTS FROM BLACK CUMIN SEEDS TO THE PHAGOCYTIC ACTIVITY OF MACROPHAGES S. *Molecules*, *19*(3), 1–19. https://doi.org/10.1501/Eczfak

Khalid, G. M., & Usman, A. G. (2021). *Application of data-intelligence algorithms for modeling the compaction performance of new pharmaceutical excipients*.

Korany, M. A., Mahgoub, H., Fahmy, O. T., & Maher, H. M. (2012). Application of artificial neural networks for response surface modelling in HPLC method development. *Journal of Advanced Research*, *3*(1), 53–63. https://doi.org/10.1016/j.jare.2011.04.001

Kouidhi, B., Zmantar, T., Jrah, H., Souiden, Y., Chaieb, K., Mahdouani, K., & Bakhrouf, A. (2011). Antibacterial and resistance-modifying activities of thymoquinone against oral pathogens. *Annals of Clinical Microbiology and Antimicrobials*, *10*, 1–7. https://doi.org/10.1186/1476-0711-10-29

Kumar, K. (2017). Principal component analysis: Most favourite tool in chemometrics. *Resonance*, *22*(8), 747–759. https://doi.org/10.1007/s12045-017-0523-9

Kurmukov, A. G. (2013). Phytochemistry of medicinal plants. *Medicinal Plants of Central Asia: Uzbekistan and Kyrgyzstan*, *1*(6), 13–14. https://doi.org/10.1007/978-1-4614-3912-7_4

Langdon, S. P. (2004). Cancer Cell Culture Edited by. In *Methods*.

Larrosa, M., Tomás-Barberán, F. A., & Espín, J. C. (2006). The dietary hydrolysable tannin punicalagin releases ellagic acid that induces apoptosis in human colon adenocarcinoma Caco-2 cells by using the mitochondrial pathway. *Journal of Nutritional Biochemistry*, *17*(9), 611–625. https://doi.org/10.1016/j.jnutbio.2005.09.004

Lee, J. K., Han, W. S., Lee, J. S., & Yoon, C. N. (2017). A Novel Computational Method for Biomedical Binary Data Analysis: Development of a Thyroid Disease Index Using a Brute-Force Search with MLR Analysis. *Bulletin of the Korean Chemical Society*, *38*(12), 1392–1397. https://doi.org/10.1002/bkcs.11308

Lee, S. H., & Min, K. J. (2019). Phytochemicals. *Encyclopedia of Biomedical Gerontology*, pp. 35–47. https://doi.org/10.1016/B978-0-12-801238-3.62136-0

Lutterodt, H., Luther, M., Slavin, M., Yin, J. J., Parry, J., Gao, J. M., & Yu, L. L. (2010). Fatty acid profile, thymoquinone content, oxidative stability, and antioxidant properties of cold-pressed black cumin seed oils. *LWT - Food Science and Technology*, *43*(9), 1409–1413. https://doi.org/10.1016/j.lwt.2010.04.009

Ma, C., Chen, Y., Chen, J., Li, X., & Chen, Y. (2017). A Review on Annona squamosa L.: Phytochemicals and Biological Activities. *American Journal of Chinese Medicine*, *45*(5), 933–964. https://doi.org/10.1142/S0192415X17500501

Majdalawieh, A. F., Fayyad, M. W., & Nasrallah, G. K. (2017). Anti-cancer properties and mechanisms of action of thymoquinone, the major active ingredient of Nigella sativa. *Critical Reviews in Food Science and Nutrition*, *57*(18), 3911–3928. https://doi.org/10.1080/10408398.2016.1277971

Mansour, M. A., Ginawi, O. T., El-Hadiyah, T., El-Khatib, A. S., Al-Shabanah, O. A., & Al-

Sawaf, H. A. (2001). Effects of volatile oil constituents of Nigella sativa on carbon tetrachloride-induced hepatotoxicity in mice: Evidence for antioxidant effects of thymoquinone. *Research Communications in Molecular Pathology and Pharmacology*, *110*(3–4), 239–251.

Marrero-Ponce, Y., Barigye, S. J., Jorge-Rodríguez, M. E., & Tran-Thi-Thu, T. (2018). QSRR prediction of gas chromatography retention indices of essential oil components. *Chemical Papers*, *72*(1), 57–69. https://doi.org/10.1007/s11696-017-0257-x

Mashayekhi-Sardoo, H., Rezaee, R., & Karimi, G. (2020). An overview of in vivo toxicological profile of thymoquinone. *Toxin Reviews*, *39*(2), 115–122. https://doi.org/10.1080/15569543.2018.1514637

Mitra, I., Mukherjee, S., Reddy Venkata, P. B., Dasgupta, S., Jagadeesh Bose, C. K., Mukherjee, S., … Moi, S. C. (2016). Benzimidazole based Pt(II) complexes with better normal cell viability than cisplatin: Synthesis, substitution behavior, cytotoxicity, DNA binding and DFT study. *RSC Advances*, *6*(80), 76600–76613. https://doi.org/10.1039/c6ra17788c

Moghadamtousi, S. Z., Goh, B. H., Chan, C. K., Shabab, T., & Kadir, H. A. (2013). Biological activities and phytochemicals of Swietenia macrophylla king. *Molecules*, *18*(9), 10465–10483. https://doi.org/10.3390/molecules180910465

Moreda-Piñeiro, J., & Moreda-Piñeiro, A. (2015). Recent advances in combining microextraction techniques for sample pre-treatment. *TrAC - Trends in Analytical Chemistry*, *71*, 265–274. https://doi.org/10.1016/j.trac.2015.02.025

Nagesh, G. S. K., Ch, K., Ch, T., & Narayana, L. (2016). Development and Validation of the Chiral HPLC Method for Daclatasvir in Gradient Elution Mode on Amylose ‐ Based Immobilized Chiral Stationary Phase. *Chromatographia*, *79*(21), 1457–1467. https://doi.org/10.1007/s10337-016-3157-2

Nourani, V., Elkiran, G., & Abba, S. I. (2018a). Wastewater treatment plant performance analysis using artificial intelligence - An ensemble approach. *Water Science and Technology*, *78*(10), 2064–2076. https://doi.org/10.2166/wst.2018.477

Nourani, V., Elkiran, G., & Abba, S. I. (2018b). Wastewater treatment plant performance analysis using artificial intelligence - An ensemble approach. *Water Science and Technology*, *78*(10), 2064–2076. https://doi.org/10.2166/wst.2018.477

Nourani, V., Molajou, A., Uzelaltinbulat, S., & Sadikoglu, F. (2019). Emotional artificial

neural networks (EANNs) for multi-step ahead prediction of monthly precipitation; case study: northern Cyprus. *Theoretical and Applied Climatology*, *138*(3–4), 1419–1434. https://doi.org/10.1007/s00704-019-02904-x

Novotná, K., Havliš, J., & Havel, J. (2005). Optimisation of high performance liquid chromatography separation of neuroprotective peptides: Fractional experimental designs combined with artificial neural networks. *Journal of Chromatography A*, *1096*(1–2), 50–57. https://doi.org/10.1016/j.chroma.2005.06.048

Ostertag, F., Schmidt, C. M., Berensmeier, S., & Hinrichs, J. (2020). Development and validation of an RP-HPLC DAD method for the simultaneous quantification of minor and major whey proteins. *Food Chemistry*, (September), 128176. https://doi.org/10.1016/j.foodchem.2020.128176

Paramasivam, A., Raghunandhakumar, S., Priyadharsini, J. V., & Jayaraman, G. (2016). In vitro anti-neuroblastoma activity of thymoquinone against neuro-2a cells via cell-cycle arrest. *Asian Pacific Journal of Cancer Prevention*, *16*(18), 8313–8319. https://doi.org/10.7314/APJCP.2015.16.18.8313

Prakash, N., & Gareja, D. A. (2010). Cheminformatics. *Journal of Proteomics and Bioinformatics*, *3*(8), 249–252. https://doi.org/10.4172/jpb.1000147

Ramadan, Mohamed F., & Mörsel, J. T. (2002). Characterization of phospholipid composition of black cumin (Nigella sativa L.) seed oil. *Nahrung - Food*, *46*(4), 240–244. https://doi.org/10.1002/1521-3803(20020701)46:4<240::AID-FOOD240>3.0.CO;2-1

Ramadan, Mohamed Fawzy. (2007). Nutritional value, functional properties and nutraceutical applications of black cumin (Nigella sativa L.): An overview. *International Journal of Food Science and Technology*, *42*(10), 1208–1218. https://doi.org/10.1111/j.1365-2621.2006.01417.x

Ramadan, Mohamed Fawzy, & Mörsel, J. T. (2003). Analysis of glycolipids from black cumin (Nigella sativa L.), coriander (Coriandrum sativum L.) and niger (Guizotia abyssinica Cass.) oilseeds. *Food Chemistry*, *80*(2), 197–204. https://doi.org/10.1016/S0308-8146(02)00254-6

Repetto, G., del Peso, A., & Zurita, J. L. (2008). Neutral red uptake assay for the estimation of cell viability/ cytotoxicity. *Nature Protocols*, *3*(7), 1125–1131. https://doi.org/10.1038/nprot.2008.75

Ringnér, M. (2008). What is principal component analysis? *Nature Biotechnology*, *26*(3), 303–

304. https://doi.org/10.1038/nbt0308-303

Saito, K. (2013). Phytochemical genomics-a new trend. *Current Opinion in Plant Biology*, *16*(3), 373–380. https://doi.org/10.1016/j.pbi.2013.04.001

Sanikhani, H., & Kisi, O. (2012). River Flow Estimation and Forecasting by Using Two Different Adaptive Neuro-Fuzzy Approaches. *Water Resources Management*, *26*(6), 1715–1729. https://doi.org/10.1007/s11269-012-9982-7

Selin, A. G. U., & Abba, I. S. I. (2020). A Novel Multi‐model Data‐Driven Ensemble Technique for the Prediction of Retention Factor in HPLC Method Development. *Chromatographia*, (0123456789). https://doi.org/10.1007/s10337-020-03912-0

Shabana, A., El-Menyar, A., Asim, M., Al-Azzeh, H., & Al Thani, H. (2013). Cardiovascular benefits of black cumin (Nigella sativa). *Cardiovascular Toxicology*, *13*(1), 9–21. https://doi.org/10.1007/s12012-012-9181-z

Shojaeimehr, T., & Rahimpour, F. (2018). Retention time modeling of short-chain aliphatic acids in aqueous ion-exclusion chromatography systems under several conditions using computational intelligence methods (artificial neural network and adaptive neuro-fuzzy inference system). *Journal of Liquid Chromatography and Related Technologies*, *41*(12), 810–817. https://doi.org/10.1080/10826076.2018.1518846

Solgi, A., Pourhaghi, A., Bahmani, R., & Zarei, H. (2017). Improving SVR and ANFIS performance using wavelet transform and PCA algorithm for modeling and predicting biochemical oxygen demand (BOD). *Ecohydrology and Hydrobiology*, *17*(2), 164–175. https://doi.org/10.1016/j.ecohyd.2017.02.002

Sparkman, O. D. (2008). GC/MS: A practical user's guide, 2nd edition Marvin McMaster. *Journal of the American Society for Mass Spectrometry*, *19*(8), R1–R5. https://doi.org/10.1016/j.jasms.2008.05.001

Srinivasan, K. (2018). Cumin (Cuminum cyminum) and black cumin (Nigella sativa) seeds: Traditional uses, chemical constituents, and nutraceutical effects. *Food Quality and Safety*, *2*(1), 1–16. https://doi.org/10.1093/fqsafe/fyx031

Takruri, H. R. H., & Dameh, M. A. F. (1998). Study of the nutritional value of black cumin seeds (Nigella sativa L). *Journal of the Science of Food and Agriculture*, *76*(3), 404–410. https://doi.org/10.1002/(SICI)1097-0010(199803)76:3<404::AID-JSFA964>3.0.CO;2-L

Tavakkoli, A., Mahdian, V., Razavi, B. M., & Hosseinzadeh, H. (2017). Review on clinical trials of black seed (Nigella sativa) and its active constituent, thymoquinone. *Journal of*

Pharmacopuncture, *20*(3), 179–193. https://doi.org/10.3831/KPI.2017.20.021

Tessini, C., Mardones, C., von Baer, D., Vega, M., Herlitz, E., Saelzer, R., … Torres, O. (2010). Alternatives for sample pre-treatment and HPLC determination of Ochratoxin A in red wine using fluorescence detection. *Analytica Chimica Acta*, *660*(1–2), 119–126. https://doi.org/10.1016/j.aca.2009.11.011

Tierno, R., López, A., Riga, P., Arazuri, S., Jarén, C., Benedicto, L., & Ruiz de Galarreta, J. I. (2016). Phytochemicals determination and classification in purple and red fleshed potato tubers by analytical methods and near infrared spectroscopy. *Journal of the Science of Food and Agriculture*, *96*(6), 1888–1899. https://doi.org/10.1002/jsfa.7294

Tradit, A. J., Altern, C., Kurt, E., Dede, S., & Ragbetli, C. (2015). *THYMOQUINONE-TREATED RATS*. *12*, 68–72.

Usman, A. G., Işik, S., & Abba, S. I. (2021). Hybrid data-intelligence algorithms for the simulation of thymoquinone in HPLC method development. *Journal of the Iranian Chemical Society*, *18*(7), 1537–1549. https://doi.org/10.1007/s13738-020-02124-5

USMAN, A. G., IŞIK, S., ABBA, S. I., & MERİÇLİ, F. (2020). Artificial intelligence–based models for the qualitative and quantitative prediction of a phytochemical compound using HPLC method. *Turkish Journal of Chemistry*, *44*(5), 1339–1351. https://doi.org/10.3906/kim-2003-6

Usman, Abdullahi Garba, Ahmad, M. H., Danraka, R. N., & Abba, S. I. (2021). The effect of ethanolic leaves extract of Hymenodictyon floribundun on inflammatory biomarkers: a data-driven approach. *Bulletin of the National Research Centre*, *45*(1). https://doi.org/10.1186/s42269-021-00586-y

Usman, Abdullahi Garba, Işik, S., Abba, S. I., & Meriçli, F. (2021). Chemometrics-based models hyphenated with ensemble machine learning for retention time simulation of isoquercitrin in Coriander sativum L. using high-performance liquid chromatography. *Journal of Separation Science*, (December 2020), 1–7. https://doi.org/10.1002/jssc.202000890

Valerio, L. G., Arvidson, K. B., Busta, E., Minnier, B. L., Kruhlak, N. L., & Daniel Benz, R. (2010). Testing computational toxicology models with phytochemicals. *Molecular Nutrition and Food Research*, *54*(2), 186–194. https://doi.org/10.1002/mnfr.200900259

Van Meerloo, J., Kaspers, G. J. L., & Cloos, J. (2011). Cell sensitivity assays: The MTT assay. *Methods in Molecular Biology*, *731*(July 2015), 237–245. https://doi.org/10.1007/978-1-

61779-80-5_20

Wang, X., Gao, M., Zhang, Z., Gu, H., Liu, T., Yu, N., … Wang, H. (2018). Development of CO 2 -Mediated Switchable Hydrophilicity Solvent-Based Microextraction Combined with HPLC-UV for the Determination of Bisphenols in Foods and Drinks. *Food Analytical Methods*, *11*(8), 2093–2104. https://doi.org/10.1007/s12161-018-1187-0

Wangchuk, P., Keller, P. A., Pyne, S. G., Taweechotipatr, M., Tonsomboon, A., Rattanajak, R., & Kamchonwongpaisan, S. (2011). Evaluation of an ethnopharmacologically selected Bhutanese medicinal plants for their major classes of Phytochemicals and biological activities. *Journal of Ethnopharmacology*, *137*(1), 730–742. https://doi.org/10.1016/j.jep.2011.06.032

Wegner, J. K., Sterling, A., Guha, R., Bender, A., Faulon, J. L., Hastings, J., … Willighagen, E. (2012). Cheminformatics. *Communications of the ACM*, *55*(11), 65–75. https://doi.org/10.1145/2366316.2366334

Wei, J. N., Liu, Z. H., Zhao, Y. P., Zhao, L. L., Xue, T. K., & Lan, Q. K. (2019). Phytochemical and bioactive profile of Coriandrum sativum L. *Food Chemistry*, *286*(September 2018), 260–267. https://doi.org/10.1016/j.foodchem.2019.01.171

Woo, C. C., Kumar, A. P., Sethi, G., & Tan, K. H. B. (2012a). Thymoquinone: Potential cure for inflammatory disorders and cancer. *Biochemical Pharmacology*, *83*(4), 443–451. https://doi.org/10.1016/j.bcp.2011.09.029

Woo, C. C., Kumar, A. P., Sethi, G., & Tan, K. H. B. (2012b). Thymoquinone: Potential cure for inflammatory disorders and cancer. *Biochemical Pharmacology*, *83*(4), 443–451. https://doi.org/10.1016/j.bcp.2011.09.029

Wu, H., Li, G., Liu, S., Ji, Z., Zhang, Q., Hu, N., … You, J. (2015). Simultaneous Determination of Seven Biogenic Amines in Foodstuff Samples Using One-Step Fluorescence Labeling and Dispersive Liquid–Liquid Microextraction Followed by HPLC-FLD and Method Optimization Using Response Surface Methodology. *Food Analytical Methods*, *8*(3), 685–695. https://doi.org/10.1007/s12161-014-9943-2

Yang, J. L., Wang, R., & Shi, Y. P. (2011). Phytochemicals and biological activities of Ligularia species. *Natural Products and Bioprospecting*, *1*(1), 327–349. https://doi.org/10.1007/s13659-011-0003-y

Yang, J., Xu, G., Kong, H., Zheng, Y., Pang, T., & Yang, Q. (2002). Artificial neural network classification based on high-performance liquid chromatography of urinary and serum

nucleosides for the clinical diagnosis of cancer. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *780*(1), 27–33. https://doi.org/10.1016/S1570-0232(02)00408-7

Yaseen, Z. M., Ghareb, M. I., Ebtehaj, I., Bonakdari, H., Siddique, R., Heddam, S., … Deo, R. (2018). Rainfall Pattern Forecasting Using Novel Hybrid Intelligent Model Based ANFIS-FFA. *Water Resources Management*, *32*(1), 105–122. https://doi.org/10.1007/s11269-017-1797-0

Yaseen, Z. M., Ramal, M. M., Diop, L., Jaafar, O., Demir, V., & Kisi, O. (2018). Hybrid Adaptive Neuro-Fuzzy Models for Water Quality Index Estimation. *Water Resources Management*, *32*(7), 2227–2245. https://doi.org/10.1007/s11269-018-1915-7

Yi-ming, B. I., Guo-hai, C. H. U., Ji-zhong, W. U., Kai-long, Y., Jian, W. U., Fu, L., … Guo-jun, Z. (2015). Ensemble Partial Least Squares Algorithm Based on Variable Clustering for Quantitative Infrared Spectrometric Analysis. *Chinese Journal of Analytical Chemistry*, *43*(7), 1086–1091. https://doi.org/10.1016/S1872-2040(15)60842-8

Yurdakök, B., & Baydan, E. (2013). Cytotoxic effects of Eryngium kotschyi and Eryngium maritimum on Hep2, HepG2, Vero and U138 MG cell lines. *Pharmaceutical Biology*, *51*(12), 1579–1585. https://doi.org/10.3109/13880209.2013.803208

Zapata, M., Rodríguez, F., & Garrido, J. L. (2000). Separation of chlorophylls and carotenoids from marine phytoplankton: A new HPLC method using a reversed phase C8 column and pyridine-containing mobile phases. *Marine Ecology Progress Series*, *195*, 29–45. https://doi.org/10.3354/meps195029

Zhou, T., Wang, F., & Yang, Z. (2017). Comparative analysis of ANN and SVM models combined with wavelet preprocess for groundwater depth prediction. *Water (Switzerland)*, *9*(10). https://doi.org/10.3390/w9100781

Zou, N., Wei, X., Zong, Z., Li, X., Wang, Z., & Wang, X. (2019). A novel enzymatic biosensor for detection of intracellular hydrogen peroxide based on 1-aminopyrene and reduced graphene oxides. *Journal of Chemical Sciences*, *131*(4), 1–8. https://doi.org/10.1007/s12039-019-1604-y

Zribi, I., Omezzine, F., & Haouala, R. (2014). Variation in phytochemical constituents and allelopathic potential of Nigella sativa with developmental stages. *South African Journal of Botany*, *94*, 255–262. https://doi.org/10.1016/j.sajb.2014.07.009

Zwir-Ferenc, A., & Biziuk, M. (2006). Solid phase extraction technique - Trends, opportunities

and applications. *Polish Journal of Environmental Studies*, *15*(5), 677–690.

CURRICULUM VITAE

Personal information

Name:	**Abdullahi Garba Usman**
Nationality:	Nigeria
Gender:	Male
Birth:	1993
Tel:	+2347060680073/+905338690967
E-Mail:	abdullahigusman@gmail.com
Address:	NO. 153 Sallari Babbangiji, Kano-Nigeria

Education:

DEGREE	UNIVERSITY / DEPARTMENT	YEARS
Bachelor of Chemistry	Bayero University, Kano Faculty of Science, Department of Pure and industrial chemistry.	2011 – 2016
Master of Pharmaceutical chemistry	Near East University, Faculty of pharmacy, department of Pharmaceutical Chemistry	2016- 2018

Doctor of Philosophy	Near East University, Graduate School of Health Sciences, Department of analytical chemistry	2018-2022

Work Experience:

- Place: Fathurrahman Nursery and Primary school

Date: 2010
Post: Class Room Teacher
Responsibility: teaches, control, and monitor the movement of the Pupils.

- Place: Aminu Kano Teaching Hospital (Akth)

Date: 2014
Post: Student
Programs: student industrial workshop experience scheme (Siwes)
Responsibility: Learned how to produce certain drugs as part of the production team.

- Place: Amina Royal Academy

Date: 2016
Post: classroom teacher
Responsibility: teaches, control, and monitor the movement of the Students.

- Place: Near East University, Turkish Republic of Northern Cyprus

Date: 2017
Post: Research Assistant, Pharmaceutical chemistry department, faculty of pharmacy (Volunteer)
Responsibility: Guiding, teaching and assisting students in experiments and research

- Place: Near East University, Turkish Republic of Northern Cyprus

Date: 2018
Post: Research Assistant, Analytical chemistry department, faculty of pharmacy (Volunteer)
Responsibility: Guiding, teaching and assisting students in experiments and research

- Place: Bauchi State

Date: 2019
Post: National Youths service (NYSC)

Leadership positions with Date

- Secretary at Kano Youth progressive forum 2010
- Social welfare at Kano Day Science College 2010
- Founder/Director General at foundation for a greater tomorrow (FGT) 2015
- Member at Kano Youth coalition for Advocacy Development (Kaycad) 2015
- Member Board of Trustees at Kano Education Foundation (KEF) 2014
- Vice President African student Association (ASA) Near East University 2016
- Secretary Northern Nigerian Students in diaspora (Nnsd) Near East University 2017
- President Northern Nigerian Students in diaspora (Nnsd) Near East University 2018
- Founder and executive director of Arewa-Neu TV at Near East University 2019

TRAINING WORKSHOPS/SEMINARS;

- Strengthening advocacy and civic engagement 2.0 mind-set series organized by USAID at grand central hotel, Kano
- Fitila girls exhibition at institute of tourism and hospitality, Kano
- NGOs and corporate playing organized by kwandala foundation at centre for democratic research and training, Mambayya house, Bayero university kano
- Youth and Volunteerism organized by Kaycad at American corner Murtala Muhammad Library Kano
- Youth leading sustainability organized by Mufarka youth development initiative at American corner, Murtala Muhammad library
- Sensitisation workshop on youth engagement and religious tolerance in Kano organized by Nigeria stability and reconciliation program (NSRP)
- Public hearing on contributory health care management agency Bill 2016. The position of Kano state workers at kano state house of assembly
- Dividends of democracy as mechanism for ensuring peace and stability in Nigeria in commemoration of international peace day at Mambayya house

- The Network with participation of the ambassador of Turkey in TRNC organized by Cyprus International Students Association (Cisa) in collaboration with Yunus Emre institute Lefkosa.

Computer Skills and Competences:

- Matlab: Programming Language
- SPSS
- R Statistic language
- E-Views Software
- Excel

Languages:

- Mother language: Hausa
- English: Excellent
- Arabic: Beginner
- Turkish: Beginner

Publications:

A. Some of the Peer-review International Journals (Science citation index (SCI)/ Science citation index expanded/ Web of Science/Scopus)

1. Abdelgader Alamrouni, Fidan Aslanova, Sagiru Mati, Hamza Sabo, Maccido, **A. G Usman**, S I Abba (2022). Multi-Regional Modelling of Cumulative COVID-19 Cases Integrated with Environmental Forest Knowledge estimation: A Deep Learning Ensemble Approach. International Journal of Environmental Research and Public Health (**SCI-IF- 3.390**).
2. Metekia, W. A., **Usman, A. G.,** Ulusoy, B. H., Abba, S. I., & Bali, K. C. (2021). Artificial intelligence-based approaches for modeling the effects of spirulina growth mediums on total phenolic compounds. Saudi Journal of Biological Sciences (**SCI-IF- 4.219**).
3. **Usman, A. G.,** Işik, S., & Abba, S. I. Hybrid data-intelligence algorithms for the simulation of Thymoquinone in HPLC method development. Journal of the Iranian Chemical Society, 1-13. Jan. 2021 (**SCI-IF- 2.019**).
4. Abba, S. I., R. A. Abdulkadir, Saad Sh Sammen, **A. G. Usman**, Sarita Gajbhiye Meshram, Anurag Malik, and Shamsuddin Shahid. "Comparative implementation between neuro-emotional genetic algorithm and novel ensemble computing techniques for modelling dissolved oxygen concentration. Hydrological Sciences Journal. (2021) (**SCI-IF- 3.787**).

5. Khalid G.M, **Usman A.G**. Application of data-intelligence algorithms for modeling the compaction performance of new pharmaceutical excipients. Future Journal of Pharmaceutical Sciences. 2021 Dec; 7(1):1-1 **(E-SCI)**
6. Ahmad, M. H., **Usman A. G.,** & Abba, S. I. (2021). Comparative performance of extreme learning machine and Hammerstein–Weiner models for modelling the intestinal hyper-motility and secretory inhibitory effects of methanolic leaf extract of Combretumhypopilinum Diels (Combretaceae). In Silico Pharmacology, 9(1), 1-11 **(E-SCI)**
7. Abba, S. I., Pham, Q. B., **Usman, A. G.,** Linh, N. T. T., Aliyu, D. S., Nguyen, Q., and Bach, Q. V. (2020). ‘’Emerging evolutionary algorithm integrated with kernel principal component analysis for modeling the performance of a water treatment plant’’. *Journal of Water Process Engineering*, *33*, 101081 (**SCI-IF- 5.485**).
8. Abba, S. I**., Usman, A. G**., & Selin, I. Ş. I. K. (2020). ‘’Simulation for response surface in the HPLC optimization method development using artificial intelligence models: A data-driven approach. Chemometrics and Intelligent Laboratory Systems’’, 104007. (**SCI-IF- 3.491**).
9. **Usman, A.G.,** Işik, S. & Abba, S.I. **A** Novel Multi-Model Data-Driven Ensemble Technique for the Prediction of Retention Factor in HPLC Method Development. Chromatographia (2020). https://doi.org/10.1007/s10337-020-03912-0**.** (**SCI-IF- 2.044**).
10. **Usman AG**, IŞIK S, Abba SI, Mericli F. Artificial intelligence-based models for the qualitative and quantitative prediction of a phytochemical compound using HPLC method. Turkish Journal of Chemistry. 2020 Oct 26; 44(5):1339-51. (**SCI-IF- 1.239**).
11. **Usman, A.G**., Işik, S., Abba, S.I. and Meriçli, F., Chemometrics-based models hyphenated with ensemble machine learning for retention time simulation of Isoquercitrin in Coriander sativum L. using high performance liquid chromatography. Journal of Separation Science. 2020 (**SCI-IF- 3.645**).
12. Pham, Q. B., Abba, S. I., **Usman, A. G**., Linh, N. T. T., Gupta, V., Malik, A., ... and Tri, D. Q. (2019). ‘’Potential of Hybrid Data-Intelligence Algorithms for Multi-Station Modelling of Rainfall’’. Water Resources Management, 33(15), 5067-5087 **(SCI-IF- 3.868).**
13. Haruna, S. I., Salim Idris Malami, Musa Adamu, **A. G. Usman**, A. I. B. Farouk, Shaban Ismael Albrka Ali, and S. I. Abba. "Compressive Strength of Self-Compacting Concrete Modified with Rice Husk Ash and Calcium Carbide Waste Modeling: A Feasibility of Emerging Emotional Intelligent Model (EANN) Versus Traditional FFNN." Arabian Journal for Science and Engineering (2021): 1-16. **(SCI-IF- 2.53).**
14. Malami, Salim Idris, A. A. Musa, S. I. Haruna, U. U. Aliyu, **A. G. Usman**, M. I. Abdurrahman, Abba Bashir, and S. I. Abba. "Implementation of soft-computing models for prediction of flexural strength of pervious concrete hybridized with rice husk ash and calcium carbide waste." *Modeling Earth Systems and Environment* (2021): 1-15 **(E-SCI)**.

15. Ghali, U. M., **Usman, A. G.,** Chellube, Z. M., Degm, M. A. A., Hoti, K., Umar, H., & Abba, S. I. (2020). Advanced chromatographic technique for performance simulation of anti-Alzheimer agent: an ensemble machine learning approach. SN Applied Sciences, 2(11), 1-12. **(E-SCI)**
16. Ghali, U. M., **Usman, A. G**., Degm, M. A. A., Alsharksi, A. N., Naibi, A. M., & **Abba, S. I.** Applications of Artificial Intelligence-Based Models and Multi-Linear Regression for the Prediction of Thyroid Stimulating Hormone Level in the Human Body. *vol*, *29*, 3690-3699. **(Scopus)**.
17. **Abdullahi Garba Usman**; Mubarak Hussaini Ahmad; Rabi'u Nuhu Danraka; Sani Isah Abba "The effect of ethanolic leaves extract of Hymenodictyon floribundun on inflammatory biomarkers: A data-driven approach" (2021). Just accepted. Bulletin of the National Research Centre **(Scopus)**.
18. **Usman Abdullahi Garba**, Umar Muhammad Ghali, and Selin Işık. "Applications of miniaturized and portable near infrared (nir), fourier transform infrared (ft-ir) and raman spectrometers for the inspection and control of pharmaceutical products." (2020). **(Scopus)**.
19. Ghali, Umar Muhammad, Mohamed Alhosen Ali Degm, Ahmed Nouri Alsharksi, Qendresa Hoti, and **Abdullahi Garba Usman**. "Development of Computational Intelligence Algorithms For Modelling The Performance Of Humanin And Its Derivatives In HPLC Optimization Method Development. **(Scopus)**.

B. Publications in reputable journals

1. Abdullahi, H. U., **Usman, A. G**., & Abba, S. I. (2020). Modelling the Absorbance of a Bioactive Compound in HPLC Method using Artificial Neural Network and Multilinear Regression Methods. **DUJOPAS**
2. Abba, S. I., A. G. Usman, Y. A. Danmaraya, **A. G. Usman**, and H. U. Abdullahi. "Modeling of Water Treatment Plant Performance using Artificial Neural Network: Case Study Tamburawa Kano-Nigeria. **DUJOPAS**
3. Alsharksi, Ahmed Nouri, Y. A. Danmaraya, Hadiza Usman Abdullahi, Umar Muhammad Ghali, and **A. G. Usman**. "Potential of Hybrid Adaptive Neuro Fuzzy Model in Simulating Clostridium Difficile Infection Status."
4. **Usman, A. G.,** and A. M. Osama. "A Critical Review on the Applications of Metal Materials for Medical Implants.
5. Ammani, H. M., and **A. G. Usman**. "SYNTHESIS AND CHARCTERIZATION OF 2-BENZOXAZOLONE AND ITS DERIVATIVES." Asian Journal of Natural & Applied Sciences Vol 7 (2018): 2.
6. **Usman, Abdullahi Garba**. "STUDY ON SYNTHESIS AND CHARACTERIZATION OF SOME 2-BENZOXAZOLINONE DERIVATIVES."

7. **Usman, Abdullahi Garba**, H. M. Ammani, and Shamsu S. Bala. "Comparative Antioxidative and Cytotoxic Activity of Extracts of Moringa Oleifera and its Mistletoe."

C. Publications under review and their journals

1. Nonlinear hybrid data-driven intelligence model for simulation of manganese extraction using solid-phase tea waste (sorbent). Modelling Earth Systems and Environment **(E-Sci)**
2. Influence of chromatographic conditions on retention behavior of different psychoactive agents in high-pressure liquid chromatography: a machine learning based approach. Chemical Papers (SCI-IF- 2.097).
3. Anticancer Activity on Breast Cancer Cell Line of Coriandrum sativum L. from North Cyprus: A Data-Driven Technique. Journal of the Egyptian National Cancer **(E-Sci)**
4. Impact of serum uric acid as a predictor of diabetes: A Clinical and Ensemble Machine Learning Approach. Medical & Biological Engineering & Computing. (**SCI-IF-2.602)**
5. Simulation of liver functions enzymes as determinants of thyroidism: A novel Ensemble machine learning Approach. Egyptian Liver Journal **(E-Sci)**
6. Molecular Characterization of Hepatitis C Virus: A Clinical and Machine Learning-based Approach. Bulletin of the National Research Centre **(E-Sci)**
7. The effects of methanol leaves extract of Combretum hypopilinum Diels (Combretaceae) on hepatic enzymes: an artificial intelligence-based approach. Journal of Food Engineering (**SCI-IF-5.354)**
8. Implementation of hybridized Emerging Harris Hawks Optimization (HHO) for prediction of mycotoxins in food samples using chromatographic technique: A comparison of nature-inspired algorithms. Journal of the Egyptian Mathematical Society (ESCI)
9. APPLICATIONS OF NEW GENERATION SOLVENTS FOR EXTRACTION OF HERBAL PRODUCTS PRIOR TO ATOMIC AND MOLECULAR ANALYSIS. Ankara journal of pharmaceutical sciences **(Scopus)**.
10. State-of-art implementation of computing intelligent models for water demand modelling: A decade review and future direction. Journal of Water Process Engineering, 33, 101081 **(SCI-IF- 5.485).**

D. Published in National and International Conferences and Workshops

1. Second International Conference on ‘Environment: Survival and Sustainability, which was held on November 2019 at Near East University in Lefkoşa, Turkish Republic of Northern Cyprus. Natural Deep Eutectic solvents (NADES) as a novel method used in plants extraction for green and sustainable environment.
2. Second International Conference on ‘Environmental sustainability’, which was held on November 2019 at Near East University in Lefkoşa, Turkish Republic of Northern.

Determination of total hardness of water from six municipalities of north Cyprus tap water by complexometric titration.

3. First Virtual Annual Scientific Conference Held from 10th - 11th March 2021 THEME: Post COVID-19: Charting a Course for the Pharmaceutical Sciences and Health System at university of Lagos faculty of pharmacy. 'Comparative performance of extreme learning machine and Hammerstein–Weiner models for modelling the intestinal hyper-motility and secretory inhibitory effects of methanolic leaf extract of Combretumhypopilinum Diels (Combretaceae).
4. International Symposium on Pharmaceutical Sciences (ISOPS). Held July, 2021 at Ankara University Faculty of pharmacy. 'SPRAY DRYER OPTIMIZATION OF TEA (Camellia sinensis L.) EXTRACT FROM DUST CHAMBER RESIDUES AND OVEN FIBERS COUPLED WITH ARTIFICIAL INTELLIGENCE'.
5. International Symposium on Pharmaceutical Sciences (ISOPS). Held July, 2021 at Ankara University Faculty of pharmacy. 'DETERMINATION OF THYMOQUINONE FROM BLACK CUMIN USING HPLC TECHNIQUE: A CHEMOMETRICS BASED APPROACH'.
6. International Symposium on Pharmaceutical Sciences (ISOPS). Held July, 2021 at Ankara University Faculty of pharmacy. 'EFFECT OF GEOGRAPHICAL DIFFERENCES ON THYMOQUINONE CONTENT AND CYTOTOXICITY OF BLACK CUMIN SEEDS'
7. Fourth International Conference on 'Environment: which was held on November 2021 at Near East University in Lefkoşa, Turkish Republic of Northern Cyprus. A non-linear based neuro boosting sensitivity optimization of breast cancer classification towards environmental health sustainability.
8. Fourth International Conference on 'Environment:, which was held on November 2021 at Near East University in Lefkoşa, Turkish Republic of Northern Cyprus. Environmental sustainability based on Multi-step ahead forecasting using dynamic black-box optimization models for Covid-19 outbreak

E. Books and book chapters

1. **Abdullahi Garba Usman**. Synthesis and characterization of some 2-Benzoxazolone derivatives. Accepted and published by Lambert academic publishing (Lap). 2019. (ISBN 978-620-0-31838-1)

F. Online courses

1. Introduction to the Certified Peer Reviewer Course, conducted by researcher academy Elsevier, on Friday 27 September 2019
2. The origins of scientific publishing, conducted by researcher academy Elsevier, on Monday 23 September 2019
3. The journal publishing cycle, conducted by researcher academy Elsevier, on Friday 27 September 2019

G. Research Area RA) /Field of Interest (FI)

- Analytical Chemistry
- Pharmaceutical chemistry
- Medicinal chemistry
- High performance chromatography (HPLC)
- Instrumentation
- Anticancer activity
- Drug synthesis
- Standardization of phytopharmaceutical products
- Herbal products Analysis
- Water Analysis
- Organic synthesis
- Micro extraction methods
- New generation solvents
- Green chemistry
- Preconcentration
- Machine learning and artificial intelligence
- Mathematical modelling (Linear regression)

H. Others

1. Reviewer at International Peer-review Journals

a) Bulletin of the National Research Centre (Springer).
b) Indian Journal of Pharmaceutical Education and Research
c) Chemometrics and Intelligent Laboratory Systems (Elsevier)
d) Environmental Research (Elsevier)

2. Awards:

a) Kano State Scholarship 2016-2018 (25,000 USD)
b) Kano State Scholarship 2018-2022 (40,000 USD)

c) Near East University Scholarship 2016-2022

d) Honor award and certificate in Second International Conference on 'Environment: Survival and Sustainability, which was held on November 2019 at Near East University in Lefkoşa, Turkish Republic of Northern Cyprus.

e) Award of Excellence of Sardauna Award 2017 at Arewa Night organised by Northern Nigerian students Near East University (NNSD), Turkish republic of Northern Cyprus 20017

f) Most influential award of Sardauna Award 2017, at Arewa Night organised by Northern Nigerian students Near East University (NNSD), Turkish republic of Northern Cyprus 20017

Google Scholar: https://scholar.google.com/citations?user=8rj-AfUAAAAJ&hl=en

Research Gate: https://www.researchgate.net/profile/Abdullahi-Usman-7

References

1	**Assis. Prof. Dr. Selin Işık** Faculty of Pharmacy, Department of analytical chemistry. Near East University, TRNC. Email: selin_isikk@hotmail.com
2	**Assis. Prof. Dr. Sani Isah Abba** Baze university, Abuja Nigeria Email: saniisaabba86@gmail.com
3	**Prof. Mukhtar Atiku Kurawa** Vice Chancellor, Yusuf Maitama Sule university, Kano-Nigeria Email: makurawa.chm@buk.edu.ng
4	**Prof Junaidu Na'aliya** Senior Lecturer, Department of pure and industrial chemistry, Bayero University kano Email: jnaaliya@yahoo.com

ORİJİNALLİK RAPORU

%15	%11	%10	%
BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

BİRİNCİL KAYNAKLAR

1	Mohamed Fawzy Ramadan. "Nutritional value, functional properties and nutraceutical applications of black cumin (Nigella sativa L.): an overview", International Journal of Food Science & Technology, 10/2007 Yayın	%1
2	link.springer.com İnternet Kaynağı	%1
3	www.isops-ankara.org İnternet Kaynağı	%1
4	kipdf.com İnternet Kaynağı	%1
5	neu.edu.tr İnternet Kaynağı	%1
6	A. G. Usman, Selin Işik, S. I. Abba. "Hybrid data-intelligence algorithms for the simulation of thymoquinone in HPLC method development", Journal of the Iranian Chemical Society, 2021 Yayın	%1