Sudan University of Science and Technology

College of Graduate Studies

Antimicrobial Activity of *Trigonella foenum-graecum* (fenugreek) oil against β-lactamase producers *Escherichiacoli, Klebsella pneumoniae,and Pseudomonas aeruginosa* using Gas chromatograph/mass spectrometery and molecular docking

فعالية زيت الحلبة كمضاد للكائنات الدقيقه على مثبطات بيتا لاكتامايس الاشريكيه القولونيه، الكلبسيلة الرئوية،و الزائفة الزنجارية المعزولة باستخدام كروماتوغرافيا الغاز/قياس الطيف الكتلي والالتحام الجزئيي

A Dissertation Submitted in Partial Fulfillment for the Requirement of M.Sc. Degree in Medical Laboratory Science (Microbiology)

Submitted by

Duaa Tariq Izz-Aldin Othman

B.Sc in Medical laboratory Science, Sudan University for Science and Technology (2016)

Supervised by

Prof. Yousif Fadlalla Hamedelneel

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بسم الله الرحمن الرحيم

الاية

قال تعالى:

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (١) خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ (٢) اقْرَأْ وَرَبُّكَ الْأَكْرَمُ (٣) الَّذِي عَلَّمَ بِالْقَلَمِ (٤)عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ (٥)

صدق الله العظيم

سورة العلق الايات:(١-٥)

DEDICATION

I dedicate this thesis to

Martyrs of Sudanese Revolution

Rajaa Hassan my mother

And every strong independent women

ACKNOWLEDGEMENT

All thanks and praise to ALLAH the lord of all worlds for all givens rewards to me. With sincere thanks and gratefulness, I would like to acknowledg**e** my Supervisor **Prof. Yousif FadlAlla HamedAlneel** for this outstanding, knowledge encouragement, guidance, patience and constructive advice throughout this work.
I would like to express my gratitude to reham mirghani **.**

ABSTRACT

This a experimental study was conducted during the period from November,2019 to March,2020, aimed to testing the antibacterial activity**,** of *T.foenum-graecum (fenugreek) oil* using GC-MS and molecular docking *against β lactames producer Escherichia coli, Klebsiella pneumoniae,and Pseudomonas aeruginosa* . A total of forty five bacterial isolates were collected from from Almoaleem Hospital . These bacterial isolates were re identified by using different biochemical tests. the 45 bacterial isolates, 15(33.3%) were *Escherichia coli*,15(33.3%) were *Pseudomonas aeruginosa* and 15(33.3%)were *Klebsiella pneumoniae.* And they were tested for antibiotics sensitivity by using disc diffusion method, the antibiotics used were, Ciprofloxacin, Ceftazidime, Cefatoxime and Imipinem. The results showed that Ceftazidime had higher resistant rates (86.6%). Then test activity of *T.foenum-graecum (fenugreek) oil* against bacterial isolates by using cup plate agar diffusion method. The results showed that *T.foenum-graecum (fenugreek) oil* have antibacterial activity against *β lactames producer Escherichia coli, Klebsiella pneumoniae,and Pseudomonas aeruginosa* and reference isolate. The results revealed that *Escherichia coli* was high susceptible organism to *T.foenum-graecum (fenugreek) oil and Klebsiella pneumoniae* was low susceptible organism to oil.

Then *T.foenum-graecum (fenugreek) oil* was analyzed by Gas chromatography–mass spectrometry (GC/MS) to detect chemical component of *T.foenum-graecum (fenugreek) oil. The result showed 7different components.* Then bioinformatics tools was use to docking fenugreekine, linoleic acid, methyle ester and palmitic acid, methyle ester

with CTX-m15 enzyme. The result showed that the fenugreekine is the best one. *T.foenum-graecum (fenugreek) oil* showed high antimicrobial activity against *β lactames producer Escherichia coli*, *Klebsiella pneumoniae,and Pseudomonas aeruginosa* and reference isolate.

الخلاصة

دراسة اختبارية تم اجرائها في الفترة ما بين نوفمبر ٢٠١٩ حتى مارس ٢٠٢٠ ،هدفت الى اختبار نشاط زين نبات الحلبه كمضاد للكائنات الدقيقه، كروماتوغرافيا الغاز وقياس الطيف الكتلي والالتحام الجزئيي ضد العزل البكتيري الاحادي. اجمالي خمس واربعون عزلات بكتيرية تم جمعها من مستشفى المعلم الطبي ' هذه العزلات البكتيريه، ثم التعرف عليها وفرزها باستخدام اختبارات بيوكيميائية. من (٤٥) عزلة بكتيريه حوالي ١٥(٣٣,٣%) كانت الاشريكيه القولونيه،١٥(٣٣,٣%) الزائفة الزنجارية ،و١٥(٣٣,٣%)الكلبسيلة الرئوية.. بعد التعرف على هذه العزلات البكتيرية تم تعريضها للمضادات الحيوية باستخدام طريقة الانتشار الطبقي. المضادات الحيوية التي تم استخدامها احتوت على ' السيفتازيديم ' السبروفلوكساسين' الكوتراموكزازول و الايميبينيم . واظهرت النتيجة ان السيفتازيديم له المقاومة الاعلى بمعدل(٨٦,٦%)ومن ثم تم اختبار زيت نبات الحلبه ضدالعزلات البكتيرية باستخدام طريقة الانتشار بالاغار. اظهرت النتيجة ان زين نبات الحلبه يملك نشاط بكتيري ضد البكتيريا المسببة للامراض والبكتيريا المرجعية. كما اظهرت النتائج ان الاشريكيه القولونيه كانت الاكثر تاثرا زين نبات الحلبه و الكلبسيلة الرئوية.كانت الاقل تاثرا.

تحليل زيت الحلبة بواسطة جهاز كروماتوغرافيا الغاز وقياس الطيف الكتلي، للبحث عن مركبات كيميائية، ووجدنا ان زيت الحلبة يحتوي على سبعة مركبات مختلفة

باستحدام معدات المعلومات الحيوية لتحليل الالتحام الجزئي لكل من مركب الفينوقركين وحمض الليولنك وحمض البالمتك على مثبطات بيتا لاكتامايس كانت النتيجة ان مركب الفينوقركين هو الافضل على الاطلاق. واستخلصت هذه الدراسة الي ان زيت نبات الحلبه يحتوي على مضادات للكائنات الدقيقه المثبطة للبيتا لاكتميز الاشريكيه القولونيه، الكلبسيلة الرئوية،و الزائفة الزنجارية

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LIST OF ABBEVIATIONS

• **Abbreviation**	**Full name**
ADT	Auto Dock Tools
ESBL	Extended-spectrum ß-lactamase
FPEt	Fenugreek Seed Polyphenolic Extract
GC/MS	Gas Chromatography-Mass Spectrometry
GPr	Glutathione Peroxidase
GR	Glutathione Reductase
HPLC	high-performance liquid chromatography
KIA	Kilgler Iron Agar
LDH	High Density Lipprotein
LDL	Low Density Lipprotein
LPO	lipid peroxides
MHA	Muller Hinton Agar
NCCLS	National Culture Collection Laboratoties
SDF	Soluble Dietary Fiber
SOD	Superoxide Dismutase
TNF	Tumor Necrosis Factor
WHO	World Health Organization

CHAPTER I

INTRODUCTION

CHAPTER I

INTRODUCTION

1. 1.Introduction

According to the World Health Organization (WHO) more than 80% of the world population relies on traditional medicine for their primary health care(WHO, 2015). Herbal drugs have found wide spread use in many countries because they are easily, available, cheaper and safer than synthetics drug (Retnam and De Britto., 2007, Prusti *et al.*, 2008). Antimicrobial resistance is a major and increasing global problem. The increased consumption of antimicrobial agents and inappropriate use accelerates this phenomenon. Also the continuous migration of people plays an important role in acquisition and spread of multidrug resistant strains (Nerino *etal.*, 2013).The development of resistance in microorganisms to antibiotics and emergence of new infectious disease create urgent need to discover novel safe and effective antimicrobial compounds (Rojas *et al*. 2003). Several plants have ability to treat the multiple drug resistant strains (Carvalho and ferrreira., 2001).Fenugreek is an aromatic herb belonging to the family Fabaceae, is a legume and it has been used as a spice throughout the world to improve the sensory quality of foods. It is a medicinal plant that uses in disease some therapy. Modern research has recognized fenugreek as a valuable medicinal plant with potential for curing diseases and also as a source for preparing raw materials of pharmaceutical industry, like in steroidal hormones (Yasmeen and Shashikumar,2019).

1.2. Rationale

The appearance of multidrug resistant microorganism have paved the way to search for new antimicrobial drugs. Plants are good source of compounds which are of great significance in therapeutic treatment and help to cure the problem of multidrugs resistant organism (Mishra *et al,2016)*. Use of plant-derived medicinal compounds has been in practice since antiquity in many cultural systems including India, China, Egypt and Middle Eastern countries. In recent times, plant-derived medicinal compounds are being widely used and are suggested by doctors to be used in a number of ailments due to their minimal side effects and numerous positive effects on human health. Out of many such medicinal plants, *Trigonella foenum-graecumLinn* (Fabaceae) (fenugreek) has recently attracted the attention of scientists from across the globe. Fenugreek is native to Eastern Europe and parts of Asia but now widely cultivated almost all over the world for its leaves and seeds, which are commonly used as leafy vegetables and condiments (Yadav and Baquer.2014). medicinal plant could be a suitable alternative as they are effective, available, with affordable cost and minimal toxicity.

1.3. Objectives

1.3.1. General objective:

To study the antibacterial activity, using GC/MS and molecular docking of *Trigonella foenumgraecum* (fenugreek) oil against B-lactamase produce (*E.coli, K.pneumoniae, P.aeruginosa*).

1.3.2. Specific objectives

1.To tset antibacterial activity of commonly used antibiotics against B-lactamase *E.coli, K.pneumoniae, P.aeruginosa*.

2. To test antibacterial activity of *Trigonella foenumgraecum*(fenugreek**)** oil using cup-plate agar diffusion method against the B-lactamase *E.coli, K.pneumoniae, P.aeruginosa*

3. To test fenugreek oil chemical components using GC/MS.

4. To measure the affinity of fenugreekine, linoleic acid, methyl ester and palmitic acid, methyl ester to by computational analysis of inhibiter/substrate docking in the CTX-M15 active site

5. To compare account of the affinity of fenugreekine, linoleic acid, methyl ester and palmitic acid, methyl ester to that of cefotaxime.

CHAPTER II

LITERATUER REVIEW

CHAPTER II

2. LITERATUER REVIEW

2.1. Background:

Antimicrobial resistance is major and increasing global healthcare problem. Antimicrobial resistance increase due to the random use of available antimicrobial drug in treatment of infectious diseases (Abdulkahaleq, 2015). Due to their broad chemical diversity, medicinal plants are regarded as the basic building blocks for a significant number of highly effective pharmaceutical drugs, and are continuously considered the primary source for the discovery of new molecular components (Alwhibi and . Soliman, 2015).

2.2.1. *Trigonella foenumgraecum*(fenugreek):

Commonly called as Fenugreek is an aromatic herb belonging to the family Fabaceae, is a legume and it has been used as a spice throughout the world to improve the sensory quality of foods. It is a medicinal plant that uses in disease some therapy. The plant contains active constituents such as alkaloids, flavonoids, steroids, Saponins etc. Fenugreek is known to have antidiabetic, anticarcinogenic, hypocholesterolemic, antioxidant, and immunological activities. Modern research has recognized fenugreek as a valuable medicinal plant with potential for curing diseases and also as a source for preparing raw materials of pharmaceutical industry, like in steroidal hormones. Use of fenugreek has been found to be lethal against hazardous bacteria, specifically coli forms, *Pseudomonas* spp., *Shigella dysentiriae* and *Salmonella typhi*. These properties probably make fenugreek a valuable ingredient in food and pharmaceutical applications. (Yasmeen and Shashikumar,2019). *T. foenumgraecum* is reported to have antidiabetic, anti-fertility, anticancer, antimicrobial, anti-parasitic and

hypocholesterolaemic effects, lactation aid, gastric stimulant, for anorexia, galactogogue, hepatoprotective effect and anticancer. **(**Wani, and Kumar.2016**)** The seeds of the *T. foenum-graecum* herb possess toxic oils, volatile oils and alkaloids have been shown to be toxic to bacteria, parasites and fungi. The potential uses of in vitro propagated plants as sources for new drugs are still largely unexplored. Based on several investigative studies, a compound produced in an in vivo plant could be produced at the same or different levels or not produced at all in an in vitro grown plant. Considering the importance of the above said medicinal plant, the study was aimed to test the antimicrobial potentials of *Trigonella foenum-graecum* against enteric pathogens.(Selvaraj*et al,2015).*

2.2.2.Scientific classification of *Trigonella foenumgraecum* (fenugreek):

According to Integrated Taxonomic Information System (IT IS) classify to Domin: Eukaryota, Kingdom: Plantae, Subkingdom: Viridiplantae, Infrakigdom: Streptophyta, Superdivision: Embryophyta, Division: Tracheophyta, Subdivision: Spematophytina, Class: Magnoliopsida, Superorder: Rosnae ,Order: Fabales, Family: Fabaceae, Genus:*Trigonella*

Species: *Trigonella foenum graecum*

Preferred scientific name:

Trigonelle foenum graecum L.

Preferred common name:

Fenugreek

2.2.3. Chemical content of *Trigonella foenumgraecum* (fenugreek):

It contains a number of chemical constituents but three important chemical constituents of fenugreek are very important; i.e. 1) steroidalsapogenins; 2) galactomannans and 3) isoleucine.Three main constituents of fenugreek are saponins, flavonoids and alkaloids. Alkaloids and some other volatile compounds give the *T.foenumgraecum* the bitter taste and specific smell (Yasmeen and. Shashikumar,2019).

2.2.3.1. Alkaloids, saponin and flavonoids:

All these compounds are classified as biologically active as these have pharmacological effects on the human body when ingested. (Yasmeen and Shashikumar,2019).

2.2.3.2. Fiber

The fiber content of fenugreek seed extract plays a role in its ability to moderate metabolism of glucose in the digestive tract, and source of antioxidant, 100g of seeds give more than 65% of dietary fiber. Dietary fiber of fenugreek can exert short term beneficial effects by reducing energy intake and increasing satiety as per one of the study conducted on obese people.(Yasmeen and Shashikumar,2019).It was reported that fiber of the fenugreek binds to cancercausing toxins of the intestine and removes them; it also lowers the rate of glucoseabsorption and helps in controlling blood sugar level (Meghwal and Goswami, 2012).

2.2.3.3. Protein

Fenugreek endosperm is highly rich in protein such as globulin, albumin, histidine and lecithin. Seed of fenugreek has a high proportion of protein ranging from 20 to 30% as well as amino acid,

4-hydroxyisoleucine, which contains high potential for insulin stimulating activity (Isikli and Karababa, 2005).

2.2.3.4. Vitamins and Minerals

Fenugreek seed is a rich source of vitamins viz. vitamin A (3 ug/100g), B1 (0.43mg/100g), B2 (0.36 mg/100g), C (12-43 Mg/100g), nicotinic acid (1.1 Mg/100g) andniacin (6 mg/100g). Its leaves also contain vitamins, but on boiling, steaming or frying, 7–11% of them may be lost. Fenugreek seeds contain of potassium (603.0 mg/100g), magnesium (42.0 mg/100g), calcium (75.0 mg/100g), Zinc (2.4 mg/100g) and iron (25.8 mg/100g) (AlJasass and Al Jasser, 2012).

2.2.4.*Trigonella foenum-graecum* activity

2.2.4.1.Antibacterial and Antifungal activity

The seeds of this plant possess strong antibacterial activity (Palombo and Semple, 2001).*Trigonella* extracts showed effectiveness against Helicobacter pylori (O'Mahony *etal*., 2005; Randhir *etal*., 2004; Randhir& Shetty, 2007).*Trigonella* shown maximumpollens against *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* among other plants (Mercan *et al*.,2007). Aqueous extracts from various plant parts of fenugreek leaves and stems, roots, ground and non-ground seeds in petroleum ether, ethyl acetate and methanol fractions of the aerial parts and determine their antifungal potential against fungal strains including *Botrytis cinerea*, *Fusarium graminearum*, *Alternaria* sp., *Pythium aphanidermatum* and *Rhizoctonia solani*. They found that all parts of the fenugreek plant showed antifungal potential and the magnitude of effect depends upon fungal species and plant parts. They further identified that the methanol fraction has the main antifungal activity, which totally

inhibited the growth of *R. solani* and *Alternaria* sp. Fenugreek could be an important source of biologically active compounds useful for developing better and novel antifungal drugs (Haouala *et al*., 2008). Cloned *Trigonella*-derived cDNA of 225 bp cysteine-richdefensin named Tfgd1. The recombinant protein expressed in *E. coli* exhibited antifungal activity against the broad range of fungi, *R. solani* and the peanut leaf spot fungus (Olli and Kirti,2006)

2.2.4.2.Antioxidant activity

Trigonella seed has been shown to restore the altered activity of cellular antioxidant enzymes including superoxide dismutase (SOD), glutathione reductase (GR), catalase andglutathione peroxidase (GPx) in tissue such as heart, muscle and brain during diabetes (Baquer *et al.*, 2011). The antioxidant produce by germinated fenugreek seeds, which are considered to be more beneficial than dried seeds by providing essential amino acids (Yadav *et al*., 2014).

2.2.4.3.Antidiabetic activity

Controlling blood glucose is difficult and there is no medicine available to achieve this goal. Many investigators have indicated that crude as well as various extractions of *Trigonella* successfully decreased blood glucose levels in experimental animals as well as human diabetic patients. In type-2 diabetic rats daily oral administration of *Trigonella* seed-derived soluble dietary fiber (SDF) for 28 days decreased serum glucose, increased liver glycogen content and enhanced total antioxidant status; however, serum insulin and insulin secretion remained unaffected. In cultured 3T3-L1 adipocytes, glucose transport and insulin action increased by *Trigonella*. These studies suggest that antidiabetic effect of *T. foenum-graecum* seed derived SDF is mediated through inhibition of carbohydrate digestion and

absorption, and enhanced peripheral insulin action (Hannan *et al*., 2007). Also demonstrated ameliorative effects of *Trigonella* seed extract on painful peripheral neuropathy in rats. Fenugreek seed-derived fraction, named IND01, has been purified and standardized by high-performance liquid chromatography (HPLC) to a marker compound trigonelline. Daily oral administration of IND01 for 15 days restored motor nerve conduction velocity in rats with SNCI. The results from this study suggested a neuroprotectiverole of *Trigonella* in painful peripheral neuropathy commonly observed in diabetes (Morani *et al*., 2012).Administration of fenugreek seed polyphenolic extract (FPEt) improved insulin signaling and sensitivity and thereby promoted the cellularactions of insulin (Kannappan and Anuradha, 2009).

2.2.4.4.Antilipidemic activity

Low Density Lipprotein (LDL-cholesterol) and triglycerides, are controlled, it can markedly prevent many chronic inflammatory diseases that emanate from obesity related low-grade inflammation. Fenugreek showed lower serum TG and total cholesterol and hepatic lipid concentrations (Annida *et al*., 2004; Hannan *et al*., 2003; Raju and Bird, 2006).The pathophysiology of many diseases of aging brain involves lipid peroxides (LPO) derived from lipid membrane and cholesterol metabolism. Simultaneous supplementation of fenugreek seeds powder or extract for 5 months enhanced the levels of LPO in posterior brain, liver and plasma, along with lactate dehydrogenase (LDH) activities, whereas total cholesterol,TG and LDL-cholesterol levels reversed, suggesting anantiperoxidative role in the brain which may be attributed to its modulatory effect on plasma lipid metabolism (Belaı ¨d-Nouira *et al*., 2012).

2.2.4.5.Anticancer activity

Cancer is one of the leading causes of mortality in the world. *Trigonellafoenum-graecum*, has been recently demonstrated to possess anticarcinogenic potential.(Hibasami *et al*. 2003). Demonstrated that fenugreek-derived compound protodion displayed a growth inhibitory effect against HL-60 cells by inducing apoptotic changes. (Amin *et al* ,2005). Fenugreek extract showed growth inhibitory effects on breast, pancreatic and prostate cancer cell lines but primary prostate or immortalized prostate cells remained unaffected.(Shabbeer *et al*., 2009).

2.2.4.6.Anti-inflammatory activity

Inflammatory response is dangerous to tissue and body, have serious side effects such as gastric erosion and ulcers, exacerbation of asthma, kidney damage and in some cases myocardial infarction. In an in vitro model, a methanol extract of fenugreek seed inhibited the production of phorbol-12-myristate-13-acetate-induced inflammatory cytokines such as tumor necrosis factor (TNF)-α in cultured THP-1 cells (Kawabata *et al*., 2011). Ethanol extract of *Trigonella* significantly decreased paw edema and decreased levels of IL-1α , IL-1β, IL-2, IL-6 and TNF-α. The extract also significantly decreased the levels of LPO and increased the SOD and GSH levels in cartilage tissue (Suresh *et al*., 2012).*Trigonella* seed extract effects in analgesic and anti-inflammatory showedsignificant dose-dependent analgesic activity against chemically as well as thermally induced pain (Vyas *et al*., 2008). And showed significant analgesic and anti-inflammatory activity in the carrageenan-induced rat paw edema as compared to diclofenac sodium, That flavonoid components of fenugreek seeds in aqueous and acidified chloroform fractions could have anti-inflammatory effects as it significantly inhibited carrageenan-induced paw edema (Mandegary *et al*., 2012).

2.2.4.7 Hepatoprotective and nephroprotective activity

Hepatotoxicity and chronic liver injury due to variousreasons are the major metabolic disorders affecting individuals of all ages (Dhiman and Chawla, 2005).The digestion-stimulating effect of *Trigonella* may emanate from its hepatoprotective role. Showed that in human Chang liver cells EtOH treatment suppressed the Chang liver cells'growth, induced cytotoxicity, oxygen radical formation and mitochondrial dysfunction, and concentration of oxidized glutathione (GSSG), while decreased the GSH level ascompared with normal cells. Incubation of cells with a polyphenolic extract of fenugreek seeds along with EtOH significantly increased cell viability in a dose-dependent manner, reduced lactate dehydrogenase leakage, TBARS formation and normalized the GSH/GSSG ratio. These cytoprotective effects of FPEt are comparable with those of silymarin, a known hepatoprotective agent. (Kaviarasan *et al*.2006)

2.2.4.8Antigastric ulcer and anti-gallstone activity

It was showed antiulcer potential of fenugreek seeds. The effect of fenugreek seeds is comparable to omeprazole, Ethanol induced lipid peroxidation and subsequent mucosal injury is prevented by fenugreek seed extract presumably by enhancing antioxidant potential of the gastric mucosa (Pandian *et al*., 2002).

Fenugreek, onion and their combination reduced the incidence of cholesterol gallstones by 75, 27 and 76%, respectively, showing highest antilithogenic influence of fenugreek alone, and the presence of onion showed no augmentation to this effect. Consequently, the cholesterol/phospholipid ratio reduced significantly in serum, liver and bile. (Reddy and Srinivasan,2009; 2011a,b).Changes in the hepatic enzyme activities (3-hydroxy-3-methylglutaryl coenzyme A reductase, cholesterol-

7a-hydroxylase and cholesterol-27-hydroxylase) induced by HCD showed significant reversal by fenugreek (Reddy and Srinivasan, 2009). Further, increased accumulation of fat in the liver and inflammation of the gall bladder membrane produced by HCD decreased significantly by fenugreek as well as its combination with onion (Reddy & Srinivasan, 2011a).As cholesterol gallstones are known to be controlled by pro- and anti-crystallizing factors present in bile, examined the effect of dietary fenugreek on the composition of bile in rats fed for 10 weeks with a high cholesterol diet. Fenugreek supplementation of HCD decreased the cholesterol, total protein, glycoprotein, lipid peroxides and cholesterol saturation index in bile, and increased the bile flow rate, and cholesterol nucleation time.(Reddy and Srinivasan, 2011b).

2.2.4.9Other activities

Trigonella foenum-greacum Aids digestion :It purifies blood and helps in flushing out the harmful toxins. It helps in dissolving excess mucus, thereby making the digestive organ refreshed and clean. Also fenugreek seeds are useful in improving memory power too. Helps In Losing weight **:**The fiber in fenugreek fills the stomach, even when consumed in a little amount. by Soak a few fenugreek seeds in water and chew them in the morning, on an empty stomach. Antidote for skin problems :fenugreek seeds prove to be an excellent beauty product. They help prevent wrinkles, blackheads, pimples, dryness and rashes. Good for beauty and health :fenugreek helps attain hormonal balance in women and therefore, helps in enlargement of breasts. It helps increase the lactation in breast feeding women. Prevent hair loss :The Fenugreek seeds being high source of protein are very useful in hair fall ,so it helps in treating baldness, thinning of hair and hair fall. It also has Lecithin, a natural emollient which helps in strengthening and moisturisation of hair. It also keeps the dandruff away

and keeps the hair free of lices.(Moradikor *et al.2013)*. seed extracts 10% in hair tonic preparations shows the effect of significantly increasing hair growth rates (Juliana *et al*. 2019).

2.2.4.10.Potential side effects of *Trigonella*

Most of the side effects known today are the result of either user reported symptoms such as stomach upset, diarrhea, or bloating in animal studies (Muraki *et al*., 2011).Experts advise that some rather serious side effects including signs of low blood sugar such as nervousness, shakiness, fast heartbeat, sweating may occur. Although a very serious allergic reaction to this product is rare, minorrash itching/swelling, especially of the face, tongue, throat, severe dizziness, trouble breathing may occur in some patients. In theory, fenugreek may increase the risk of bleeding. There is some evidence that fenugreek may reduce potassium levels in the blood. Although it has not been widely studied in humans, fenugreek may alter the levels of thyroid hormones.

Consumption of fenugreek seeds during pregnancy has been associated with a range of congenital malformations ,including hydrocephalus, anencephaly and spina bifida. fenugreek may have deleterious toxic effects on reproductive performance and potential teratogenic effects in fetuses.(Khalki *et al*. 2010). Prenatal exposure of mice to a high dose of fenugreek seeds caused growth retardation and altered neurobehavioral performance in the post-weaning period, though the molecular reasons remain to be determined.(Khalki *et al*.,2012).

2.2.5.Molecular docking

Molecular docking has become an increasingly important tool for drug discovery, each year new target are being identified, structures of those target are being determined at an amazing rate, and our capability to

capture a quantitative picture of interaction between macromolecules and ligands is accelerating. To understand the design concepts of the various types of binding enzyme inhibitors, a basic knowledge of the binding forces between an enzyme's active site and its inhibitors is required. The forces involved in a substrate or an inhibitor binding to an enzyme's active site are, as with a drug binding to a receptor, the same forces that are experienced by all interacting organic molecules. These include ionic (electrostatic) interaction, ion-dipole and dipole-dipole interaction, hydrogen bonding, hydrophobic interaction, and van der Waals interaction. Molecular docking studies have been conducted usig software(ADT) which was used to calculate the average binding energy which is mechanical energy required to dissemble a whole into separate parts grid to account for receptor conformation result were analyzed by discovery studio software.

Auto Dock Tools (ADT) is program package of automated docking tools. It's designed to predict how small molecules, bind to receptor of known 3D structure. Besides generating binding energy in these docking studies.

2.2.6.Clinical Isolate

2.2.6.1.*Escherichia coli*

Is most common and important member of genus *Escherichia*. This organism is associated with variety of diseases. As pathogen illustrated by the fact of bacteria are: the most common gram-negative rods isolated from patient with sepsis (parrick *et al*.2009). The bacilli are arranged singly or in pairs. They are motile due to the presence of peritrichous flagella. Some strains of *E. coli* may be fimbriated. The fimbriae are of type I (hemagglutinating and mannose-sensitive). Some strains of *E. coli* isolated from extraintestinal infections possesses polysaccharide capsule. They do

not form any spores. *E. coli* is an aerobe and a facultative anaerobe. Ferments lactose, glucose, mannitol, maltose, and many other sugars with the production of acid and gas. They do not ferment sucrose. Somestrains of *E. coli* are late lactose or nonlactose fermenters(Parija, 2012).Treatmentof E. coli infection has been increasingly complicated by the emergence of resistance to most first-line antimicrobial agents (Sabate *et al*., 2008).Antibiotic resistance rates in *E. coli* are rapidly rising, especially with regard to flouroquinolones and third and fourth generation cephalosporins and extended-spectrum β-lactmas (Laupland *et al*., 2008 and Mesa *et al*., 2006).

2.2.6.2 *CTX-M15* ß-lactamase enzyme

Extended-spectrum ß-lactamase (ESBL)-producing *Enterobacteriaceae* have disseminated worldwide and become a major concern for clinicians because of their limited treatment options in common infections (Paterson and Bonomo, 2005; Pitout and Laupland, 2008; Mathers *et al*., 2015; Tal Jasper *et al*., 2015). In the last decade, *CTX-M*-type ESBLs have replaced TEM- and SHV-type ones (Livermore *et al*., 2007), becoming dominant in clinical *Enterobacteriaceae* isolates. Among the CTX-M-type ESBLs, *CTX-M-15* is one of the most common *CTX-M*-type among *Escherichia coli* isolates.

2.2.6.3.*Klebsiella pneumoniae*

Member of genus *Kelbsiella* have a predominant capsule that is responsible for the mucoid appearance of the isolated colonies and the enhanced virulence of the oraganism invivo. Pneumonia caused by *Klebsiella* species frequently involves the necrotic destruction of alveolar spaces, formation of cavities, and the production of blood-tinged sputum. These bacteria can

also cause wound, soft tissue, and urinary tract infection (Parrick *et al*.2009).

It is Gram-negative, short and straight rods, They are non motile and nonsporing. They are arranged singly or in pairs. Freshly isolated strains show a well-definedpolysaccharide capsule. They produce lactose-fermenting red colonies on MacConkey agar (Parija, 2012). Before 2003 most ESBLs reported in *Klebsiella* spp were mutant TEM and SHV penicillinase. They occurred mainly in specialist units and were often hospital acquired (Livermore, 2007). *K. pneumoniae* is the most frequently encountered carbapenemase -producing *Enterobacteriacae* (Won *et al*., 2011).

2.2.6.4.*Pseudomonas aeruginosa*

It is Gram negative rod, obligate aerobe, non-sporing and motile, some strains are capsulated. It is usually recognize by pigment production including pyocyanin a blue-green pigment and pyoverdin a yellow-green fluorescent pigment. *P.aeruginosa* can be found in the intestinal tract, water, soil and sewage. It frequently found in moist environments in hospital and able to grow in some eye drops, saline and aqueous solution. Many infections with *P. aeruginosa* are opportunistic hospital acquired and often difficult to eradicate. *P. aeruginosa* cause Skin infections, Septicemia, urinary tract infection, respiratory tract infection and eye infection (Cheesbrough, 2006). Its intrinsic resistant mechanism considered a problem (Nicolle, 2005). It has become increasingly clear that resistant development in *P. aeruginosa* is multifactorial, with mutations in genes encoding protein, efflux pumps, benicillin-binding protein and chromosomal β-lactmase, all contributing to resistance to β- lactams, carbapenems, aminoglycosides and fluoroquinolones (Ozer *et al*.,2009).

CHAPTER III

MATERIAL AND METHOD

CHAPTER III

3. MATERIAL AND METHOD

3.1. Study design

This is experimental study.

3. 2. Study area

The study was conducted in Khartoum State and Sudan University of Science and Technology, college of Medical Laboratory Science.

3. 3. Study duration

This study was conducted from November 2019 to March 2020.

3. 4. Study subject

Different clinical isolate (*E.coli, K.pneumoniae, and P.aeruginosa)*.

3.5. Inclusion criteria

β-lactmase producer (*E.coli, K.pneumoniae, and P.aeruginosa)* were included in this study

3.6. Exclusion criteria

Non β-lactmase producer was excluded **.**

3. 7. Sample size

Forty five clinical isolate (n =45) were collected randomly from patients during study duration in Khartoum State

3.8. Ethical consideration

Permission to carry out the study was taken from scientific research committe the College of Medical Laboratory Sciences, Sudan University of Science and Technology.

All the participate were informed for the purpose of the study before collection of the specimens and consent was taken from them.

3.9. Reidentification of the clinical isolates

The clinical isolates were identified in the laboratory by standard microbiology procedures including the following steps:

3. 9. 1. Colonial morphology

Colonial morphology was used as first identification steps focusing on colony size, color, edge and fermentation of lactose in MacConkey agar solid media.

3.9. 2. Gram stain

Fixed and dried smears were prepared from growth. The smear was stained with firstly crystal violet stain for 30-60 seconds, washed with water followed by Lugol‟s iodine for 30-60 sec, washed again then decolorized rapidly by alcohol, washed immediately with water and covered with Safranin for 2 min then washed and dried to examined microscopically by oil immersion lens (X100) to detected Gram reaction and arrangement of bacteria (Cheesbrough, 2006).

3. 9. 3. Biochemical tests

Biochemical tests including Kliglar Iron Agar test (KIA), indole, urease, citrate, motility test. The principle of the tests started with indole test, the tested organism was cultured in medium contain tryptophan; indole production is detected by kovac‟s reagent which contain 4-dimethylaminobenzaldehyde. This reacts with indole to give red color compound (Cheesbrough, 2006). Then urease test was done, the tested organism was cultured in medium contains urea and indicator phenol red. If there is change in color from yellow to pink color was recorded (Cheesbrough, 2006), then citrate test, the tested organism was cultured in a medium contain sodium citrate, ammonia salt and indicator bromo-thymol blue showing turbidity. The change in color of the indicator from green to blue was considered as positive (Cheesbrough, 2006). The motility test also done that depend on the properties of bacteria structure which contain flagella or no Then Kligler Iron Agar test (KIA) was used for detection of H2S,

gases production and sugar fermentation (lactose), to help in identification of *Enterobacteriasae* (Cheesbrough, 2006).

3. 9. 4. Preservation and storage of isolated organisms

Isolated organisms were kept in nutrient agar slope at 4° C for further identification and susceptibility tests. Glycerol (20 ml) with peptone water (80 ml) (20% v/v) was used for long preservation of the isolates at -20° C (Cody, 2008).

3. 10. Collection of plant material

Trigonella foenum-graecum were collected from spice dealer in sudan 2019.

3. 11. Preparation of *Trigonella foenum-graecum* (fenugreek) oil

The *Trigonella foenum-graecum* (fenugreek) seed oil obtained by Soxhlet extraction using petroleum ether as a solvent in Medicinal and Aromatic Plants and Traditional Medicine Research Institute Sudan.

3. 12. Cup plate method

The agar well diffusion method was done on Muller Hinton Agar (MHA) medium for the assay of the antimicrobial activity of *Trigonella foenum-graecum* (fenugreek) oil against the isolated pathogens, 3 colonies with the same characteristics were emulsified in 1 ml normal saline and adjusted to 0.5 McFarland turbidity standard. A sterile cotton swab was inserted into the bacterial suspension, rotated and then pressed against wall of the test tube to expel any excess fluid. The swab was then streaked on the surface of MHA plate. To ensure a uniform, confluent growth, the swab was streaked three times over the entire plate surface (Cheesbrough, 2006). A sterile cork borer was then used to make wells (6mm diameter) on MHA medium

3. 13. Quality control

3. 13. 1. Control of culture media

The performance of culture media was controlled by testing each patch with control strains *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 to check the quality of the media.

3. 13. 2. Control susceptibility testing method

The quality control strain ***E. coli*** ATCC25922were used as described by NCCLs document M7-A7 (NCCLS, 2000) to assess the antimicrobials disks efficiency. The control strains were brought from National Public Health Laboratories in Khartoum.

3. 13.3. Batch quality control

Each batch of susceptibility test was tested within reference strains to determine if zone diameter obtained with in the expected range or not and to check the quality of biochemical tests.

3.14.Gas Chromatography/Mass Spectrometery(GC/MS)

3.14.1.Sample preparation:

Two ml of the sample was mixed thoroughly with 7ml of alcoholic sodium hydroxide (Naoh) that was prepared by dissolving 2g in100ml methanol. Seven ml from alcoholic sulfuric acid (1ml H_2SO_4 to 100ml methanol) was then added. The mixture was then shaked for 5 min .the content of the test tube was left to stand overnight. 1ml of super saturated sodium chloride (NaCl) was then added and the contents being shaken. 2ml of normal hexane was added and the contents were shaked thoroughly for3 min. then the n-hexane layer (the upper layer of the test tube) was taken using disposable syringe. 5µ the n-hexane extract was diluted with 5 ml of diethyl ether. Then the mixture was filtered through syringe filter 0.45µm and

dried with 1g of anhydrous sodium sulphate as drying agent and 1µl of diluted sample was injected in the GC.MS instrument.

3.14.2.Method of analysis (GC/MS condition):

The qualitative and quantitative analysis of the sample was carried out by using GC/MS technique model (GC/MS-QP2010-Ultra) from Japans ,Simaduz Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25mm×0.25µm).the sample was injected by using split mode, helium as carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60℃ with rate 10℃/min to300℃ as final temperature degree with 5 min hold time, the injection port temperature was 300℃, the ion source temperature was200C and the interface temperature was 250℃, the sample was analyzed by using scan mode in the range of m/z 40-500℃harges to ratio and the total run time was 29 min. identification of components for the sample was achieved by comparing their retention index and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology (NIST).

3.15. Molecular Docking

3.15.1. Preparation of Ligand file

The structure of the ligand (fenugreekine) was download from pubchem (http://www.ncbi.nIm.nih.gov/pubchemcompound)

3.15.2 Preparation of Macromolecule file

The CTX-M15 enzyme was taken from the protein data bank (PDB) in (pdb) format. This is a special format for protein structure that are obtained by X-ray crystallography studies.

3.15.3 Docking Process

Docking of Fenugreekine (PubChem CID 444170) and CTX-M-15 (PDB ID 4HBU) was performed with Glide in the Schrodinger 2018 suite. The ligand and protein crystal structure were prepared by ligprep and protein preparation tool in Maestro. The Glide SP and XP modes, as integrated in Maestro were subsequently used to dock the compound library into the NXL104 binding site. The avibactam (NXL104) of CTX-M-15 was chosen to create the docking grid. All the other settings were default. The top scored binding poses were chosen as proposed binding modes.

3. 16. Statistical Analysis

Data was computed and analyzed by using Statistical Package for Social Sciences computer software version 20 to check frequency and mean.

Chapter IV

Results

Chapter IV

4. Results

4.1Clinical isolates frequency

In this study 45 clinical isolates were collected as follows; 15(33.3%) *Escherichai coli*, 15(33.3%) *Pseudomonas aeruginosa*, 15(33.3%) *Klebsiella pneumonia (Fig.1)*

4.2. Susceptibility of Clinical isolate to selected antibiotics

The result of 4 antibiotics were show sensitive and resistant to tested isolate in table 4.1

Table 4.1.Antimicrobial Susceptibility Testing Results of tested isolate

Antibiotics / Organisms	Imipenem		Ciprofloxacin		Cefotaxime		Ceftazidime	
	S%	R%	S%	R%	S%	R%	S%	R%
E.coli N=15	80% 12	20% 3	46.7% 7	53.3% 8	53.3% 8	46.6% 7	20% 3	80% 12
K.pneumoniae N=15	53.3% 8	46.6% 7	46.7% 7	53.3% 8	80% 12	20% 3	6.6% 1	93.3% 14
P.aeruginosa N=15	53.3% 8	46.6% 7	66.7% 10	33.3% 5	33.3% 5	66.7% 10	13.3% 2	86.6% 13

S: Sensitive, R: Resistant.

4.3. Susceptibility testing against standard bacteria to *Trigonella foenum-graecum* (fenugreek) oil

Antimicrobial activity of *Trigonella foenum-graecum* (fenugreek) oil screened against reference strains (*E.coli* ATCC25922) (table4.2)

Table4.2. Antibacterial activity of *Trigonella foenum-graecum* (fenugreek) oil Against standard isolates

Standard isolates	Mean of inhibitory zones in mm
E.coli ATCC25922	25

4.4. Susceptibility testing against β-lactmase producer isolates to *Trigonella foenum-graecum* (fenugreek) oil was shown in table4.3

Table4.3.Antibacterial activity of *Trigonella foenum-graecum* (fenugreek) oil against different isolates

Bacterial isolates	Mean of inhibitory zones in mm
E.coli	15.8
K.pneumoniae	15.1
P.aeruginosa	15.3

4.5. Gas chromatography results

GC-MS Chromatography of *Trigonella foenum graecum* (fenugreek) oil clearly showed 7 peaks indicating the presence of 7 phytochemical compounds, the information about 7 compounds are mention in table 5and all of them showed antibacterial activity.

Table4.5: GC/MS result:

Peak	R.Time	Area	Area%	Name
1	16.065	2526641	34.81	Hexadecanoic acid, methyl ester
2	17.067	24521	0.34	Heptacosanoic acid, methyl ester
3	17.706	1100825	15.17	9,12,Octadecadienoic acid (Z,Z)-, methyl ester
4	17.753	2411093	33.22	9, Octadecenoic acid (Z)-, methyl ester
5	17.974	953495	13.14	Methyl stearate
6	19.725	178993	2.47	Eicosanoic acid, methyl ester
7	21.343	62673	0.86	Docosanoic acid, methyl ester
		7258241	100.00	

4.7 Molecular Docking result

Inhibition of CTX-M enzyme by fenugreekine

The docking score was found to be -7.119ΔG kcal/mol. Result of docking showed that the predominant interaction between fenugreekine and CTX-M enzyme were through hydrogen bound interaction and hydrophobic interaction; three H-bound between 44-hydroxyl group and side chain of PRO268, between 47-oxygen and backbone of ASN104, between 41-hydroxyl group and SER130. And hydrophobic interaction between TYR105 and C-49, C-45 AND C-57.(Fig10-11)

Inhibition of CTX-M enzyme by linoleic acid, methyl ester

The docking score was found to be -0.188 ΔG Kcal/mol. Result of docking showed that the predominant interaction between linoleic acid, methyl ester and CTX-M enzyme were through hydrogen bound interaction and hydrophobic interaction; The (2-O) carbonyl formed bi-dentate hydrogen bond with ASN 105 and ASN 132. In addition, the terminal ethyl group formed hydrophobic interaction with TRY 129 and TRY 105.C-7 also formed hydrophobic interaction with ASN 105.(Fig12-13)

Inhibition of CTX-M enzyme by Palmitic acid, methyl ester :

The docking score was found to be 0.697 ΔG **Kcal/mol**. Result of docking showed that the predominant interaction between Palmitic acid, methyl ester and CTX-M enzyme were through hydrogen bound interaction and hydrophobic interaction; The (O) carbonyl formed bi-dentate hydrogen bond with ASN 104 and ASN 132. In addition, the aliphatic chain formed hydrophobic interaction with TRY 129, PRO 107, ILE 108 and TRY 105.(Fig14-15)

Chapter V

DISSCUSSION, CONCLUSION and RECOMMENDATION

Chapter V

5. DISSCUSSION, CONCLUSION and RECOMMENDATION

5.1. Discussion

Natural products are important sources for drug development. The amounts of bioactive natural products in natural medicines are always fairly low. Medicinal plant is the process of their fight against diseases over thousands of years (Zhang *et al.,2018.* Mishra *et al.*.2016. Yadav et al..2014. *S. Irshad et al*.2011.).

Due to multidrug resistance to antibodies and there side effect, the study showed that there is a need to screen local medicinal plants with possible antibacterial properties to find novel alternatives (Ahmed *et al.,* 2000).

In this study antimicrobial sensitivity result showed resistance to different tested antibiotics drugs 86.6% Ceftazidime, followed by 46.6% for Ciprofloxacin and 44.4% for Cefotaxime and 37% for Imipenem. increasing rate of resistance of this antibiotics is due to overuse and misuse of these medication (Gould and Bal, 2013; Viwanathan, 2014 and Michael *et al.,* 2014).

In this study *Trigonella foenum-graecum* oil was found effective against selected β-lactamase clinical isolate (*E.coli* with inhibition zone (13-21mm), *K.pneumoniae* with inhibition zone(13-20mm), and *P.aeruginosa* with inhibition zone (12-21mm). this result was in agreement with that of (Abdul Kahaleq *et al*,2015**)**was found that Zone of inhibition of fenugreek essential oil after 48 hrs against *P. aeruginosa* range from 12 to 22 mm of concentrated essential oil (100%)**.**and in agreement with Nahar, *et al* (2016) in that essential oil showed the strongest antibacterial effect against

K. pneumonia (15 ± 0) and disagreement in zone og inhibition in *E. coli* (8 ± 0) . while (Selvaraj *et al.2015)*reported that *E. coli* shows highest predominance to fenugreek extract, An earlier study on the antibacterial activity of fenugreek seed extracts revealed its antibacterial potential against *E. coli, B. cereus, L. acidophilus* and *Pneumococcus*, with the choloform extract demonstrating high antibacterial activity. (Chalghoumi,2016) said that An inhibition zone diameter (IZ) above 7 mm was considered as a positive result.

Furthermore phytochemical compounds of *Trigonella foenum graecum* (fenugreek) oil was determined by gas chromatography showed 7 compounds, all of them contain antibacterial constituents. There is study on fenugreek phytochemical compounds conducted by Nahar, *et al*. (2016) that showed 14 different compounds with different with this study and this compounds may be due variation in environmental, genetic factors and climates and varieties.

Various phytochemical; fenugreekine, linoleic acid, methyl ester and Palmitic acid, methyl ester were screened for their β-lactamase inhibitory activities by molecular docking studies. The docking result revealed that all ligand-protein complexes were stabilized by hydrogen bound. The glide score of different phytochemical revealed that the β-lactamase had strong affinity for fenugreekine.

The fenugreekine showed glide score -7.1 when compared with that of cefotaxime 6.5(Adam,2017).

5.2. Conclusion:

Trigonella foenum graecum possesses high antibacterial activities against β-lactamase producer (*E.coli*, *P.aeruginosa, K.pneumoniae,*), and standard isolates (E.coli ATCC25922). *E.coli*, and *P.aeruginosa* was more susceptible isolate to *Trigonella fenum graecum* oil with inhibition zone (21mm). all compound of *Trigonella fenum graecum* oil have antibacterial activity. Molecular docking showed that fenugreekine is best than linoleic acid, methyl ester and Palmitic acid, methyl ester in inhibitory of CTX-M15 enzyme.

5.3. Recommendations:

- Further studies in *Trigonella fenum graecum* should be done by using large sample size.
- Further studies in *Trigonella fenum graecum* from different locations and different parts of plant.
- Examine *Trigonella fenum graecum* on different isolates and use different methods and different solvents for extraction process.
- Determination of Minimum Inhibitory Concentration by using tube dilution method.
- Pharmacological and toxicological studies should be carried out to assess their therapeutic efficiency and potential for commercial utilizations

Reference

Reference

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APPENDICES

APPENDICES

Appendix 1: Compound information of *Trigonella fenum graecum* oil analysis by Gas chromatography

1. **Hexadecanoic acid, methyl ester**

Formula: $C_{17}H_{34}O_2$ CAS:112-39-0 Mol Weight: 270 RetIndex: 1878

ConmpName : Hexadecanoic acid, methyl ester **$$** Palmitic acid, methyl ester$$ n-Hexadecanoic acid methyl ester $$ Metholene 2216$$ Methyl Hexadecanoate

2. **Heptacosanoic acid, methyle ester**

Formula:$C_{28}H_{56}O_2$ CAS:55682-91-2 Mol Weight::424 RetIndex:2972

ConmpName : Heptacosanoic acid, methyle ester$$ Methyle Heptacosanoate

3. **9,12-Octadecadienoic acid(Z,Z)-, methyl ester**

Formula:$C_{19}H_{34}O_2$ CAS:112-63-0 Mol Weight:294 RetIndex:2093

ConmpName : 9,12-Octadecadienoic acid(Z,Z)-, methyl ester $$Linoleic acid, methyl ester $$ Methyl cis,cis-9,12-octadecadienoate $$ Methyl linoleate

4. **9-Octadecenoic acid (Z)-, methyl ester**

Formula:$C_{19}H_{36}O_2$ CAS:112-62-9 Mol Weight:296 RetIndex:2085

ConmpName : 9-Octadecenoic acid (Z)-, methyl ester $$ oleic acid, methyl ester $$Emery Oleic acid ester2301 $$Methyl cis-9-Octadecenoate $$ Methyl Oleate

5. **Methyl stearate**

Formula:$C_{19}H_{38}O_2$ CAS:112-61-8 Mol Weight:298 RetIndex:2077

ConmpName : Methyl stearate $$ Octadecanoic acid, methyl ester $$ Stearic acid, methyl ester $$ n-Octadecanoic acid methyl ester $$ Kemester 9718

6. **Eicosanoic acid, methyl ester**

Formula:$C_{21}H_{42}O_2$ CAS:1120-28-1 Mol Weight:326 RetIndex:2276

ConmpName: Eicosanoic acid, methyl ester $$ Methyl arachisate $$ methyl Eicosanoate $$ arachidic acid methyl ester $$ Kemester 2050

7. **Docosanoic acid, methyl ester**

Formula:$C_{23}H_{46}O_2$ CAS:929-77-1 Mol Weight:354 RetIndex:2475

ConmpName: Docosanoic acid, methyl ester $$ Behenic acid, methyl ester $$ Methyl Behenate $$Methyl Docosanoate $$ n- Docosanoic acid, methyle ester

The result of gas chromatography and mass spectrometric in figure

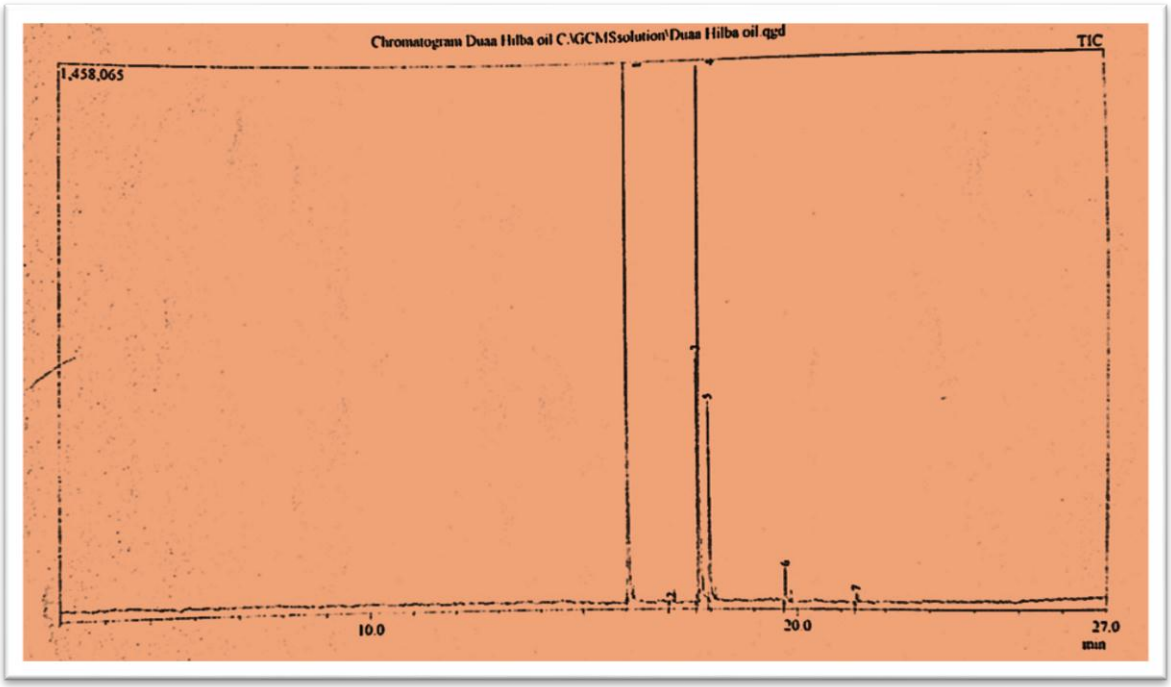

Figure1: chromatograme fenugreek oil by GC/MS

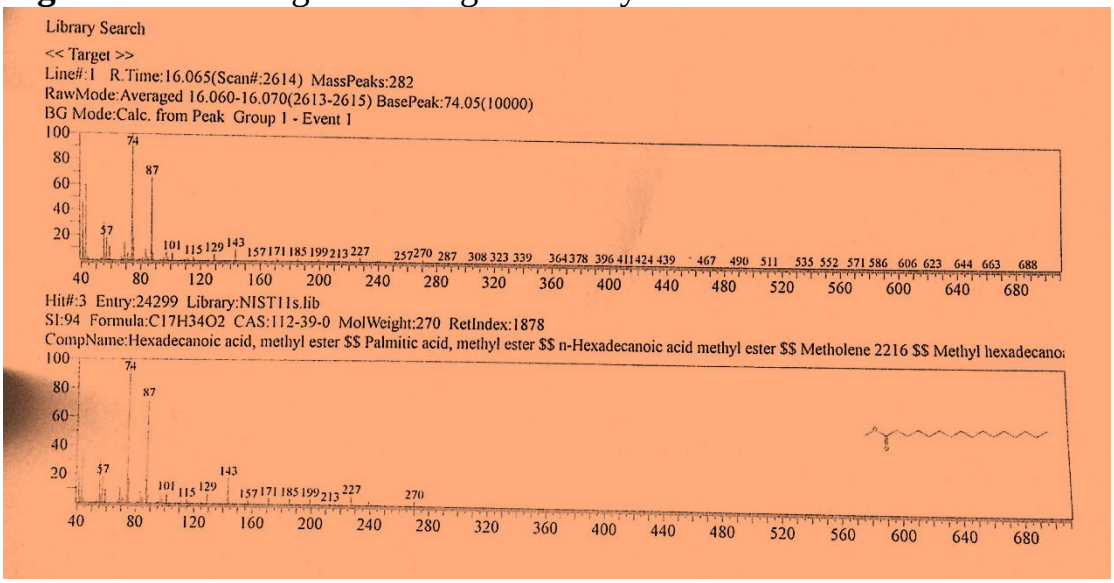

Figure2: comparison between mass peak1 and hit in GC/MS

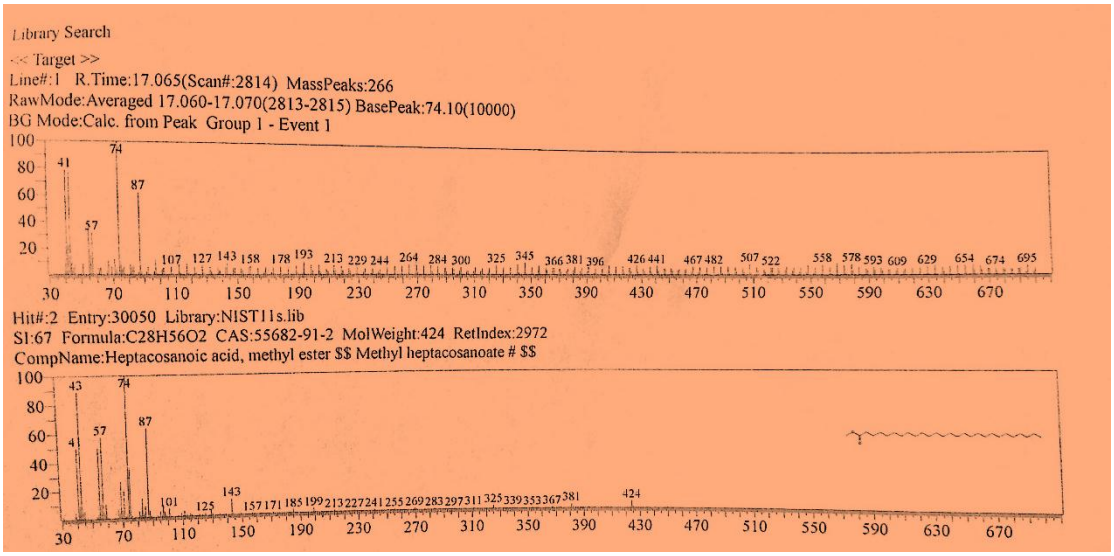

Figure3: comparison between mass peak2 and hit in GC/MS

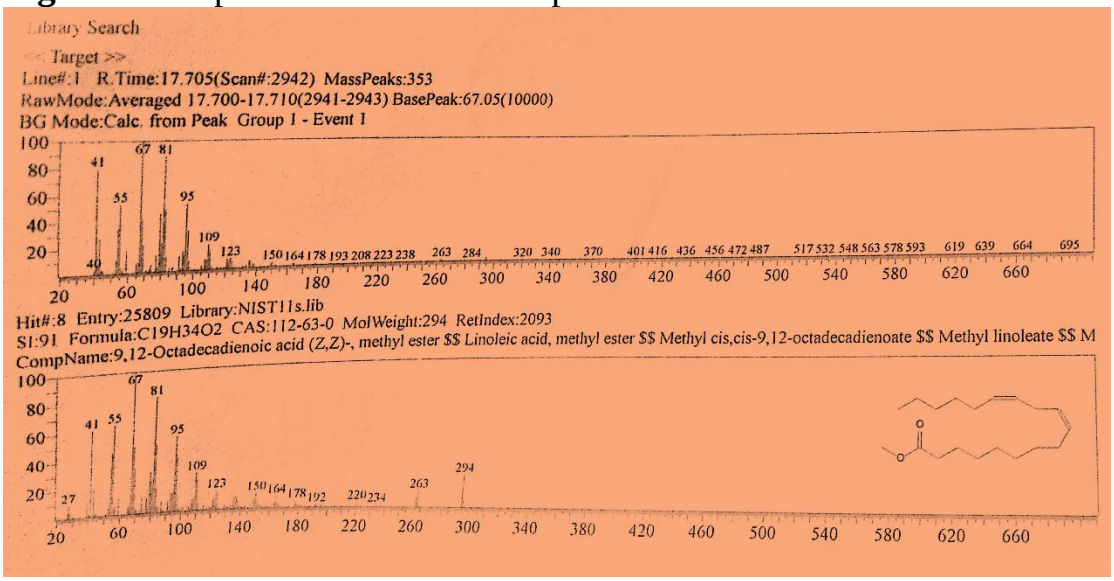

Figure4: comparison between mass peak3 and hit in GC/MS

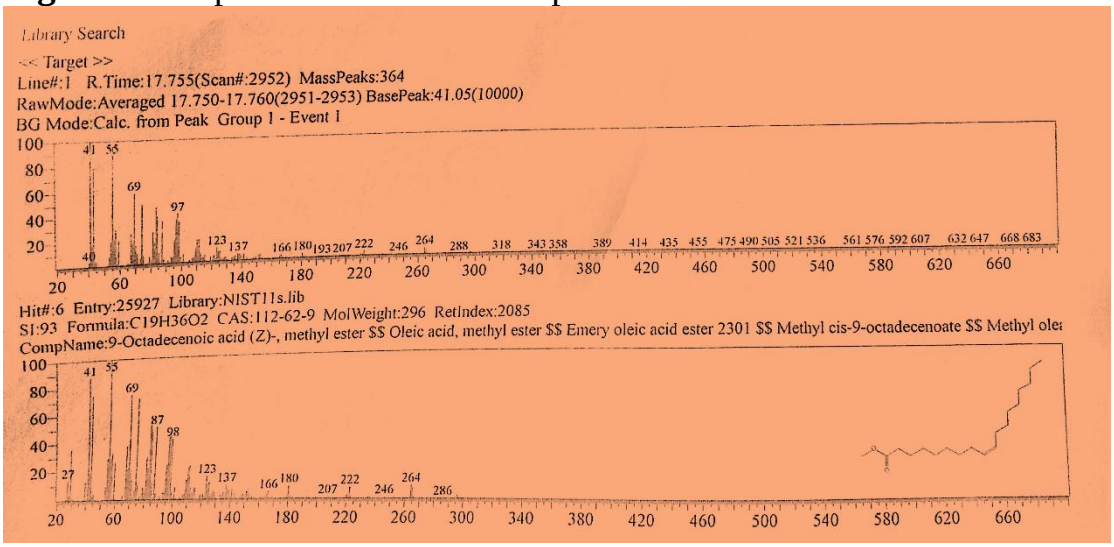

Figure5: comparison between mass peak4 and hit in GC/MS

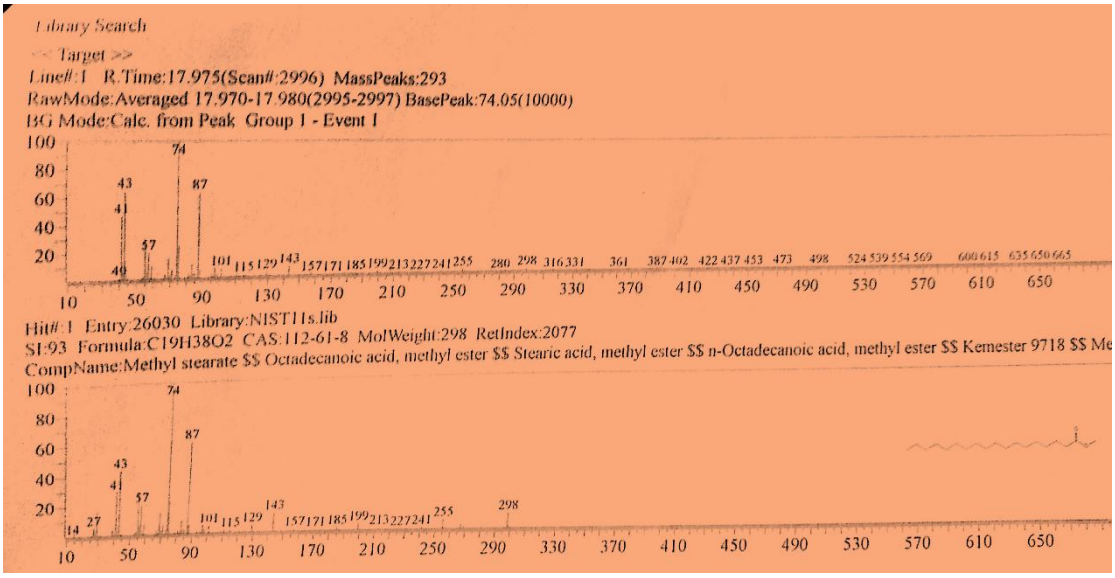

Figure6: comparison between mass peak5 and hit in GC/MS

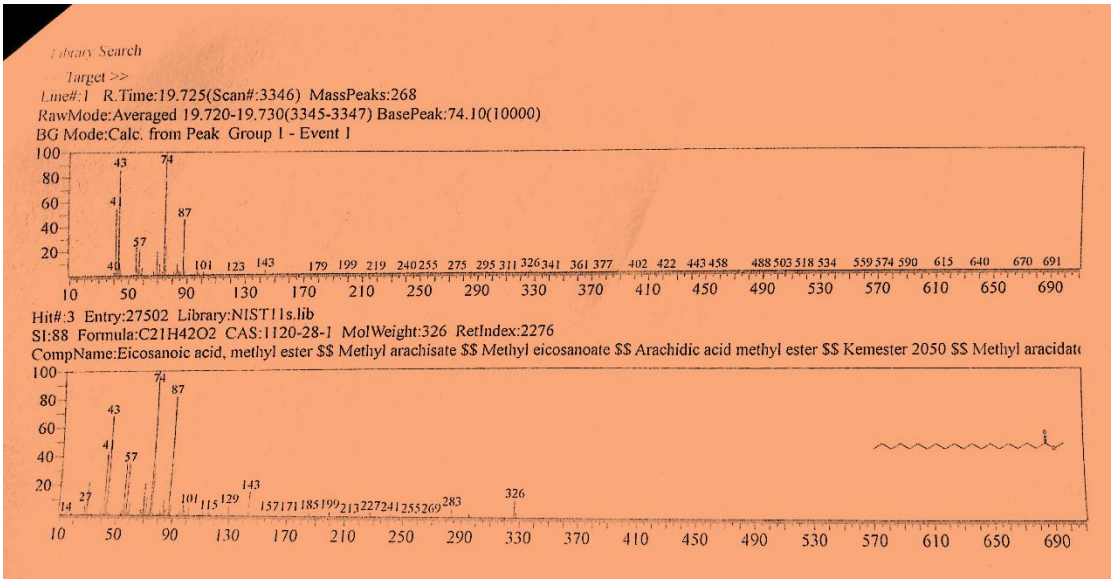

Figure4.7: comparison between mass peak6 and hit in GC/MS

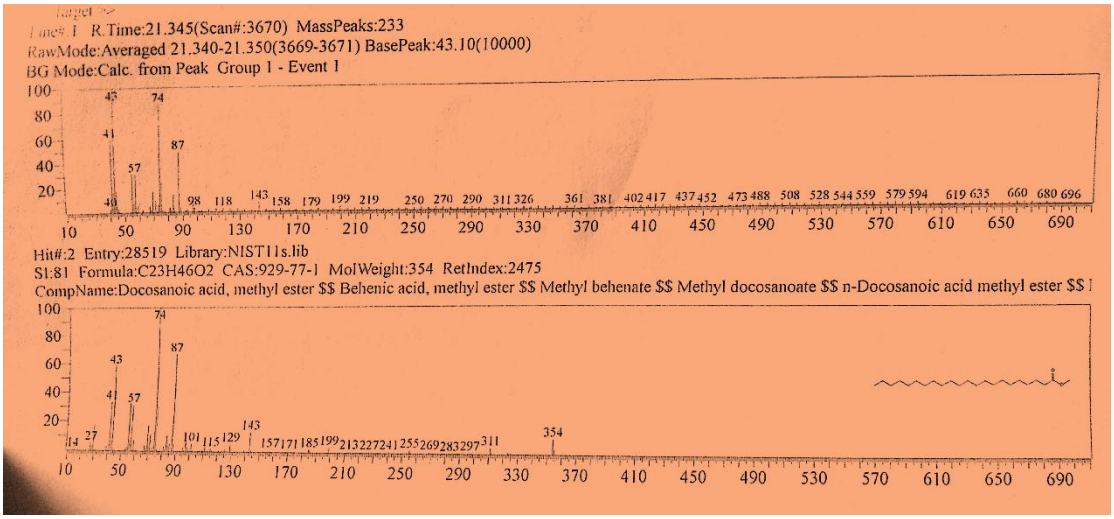

Figure4.8: comparison between mass peak7 and hit in GC/MS

The result of molecular docking in figure below:

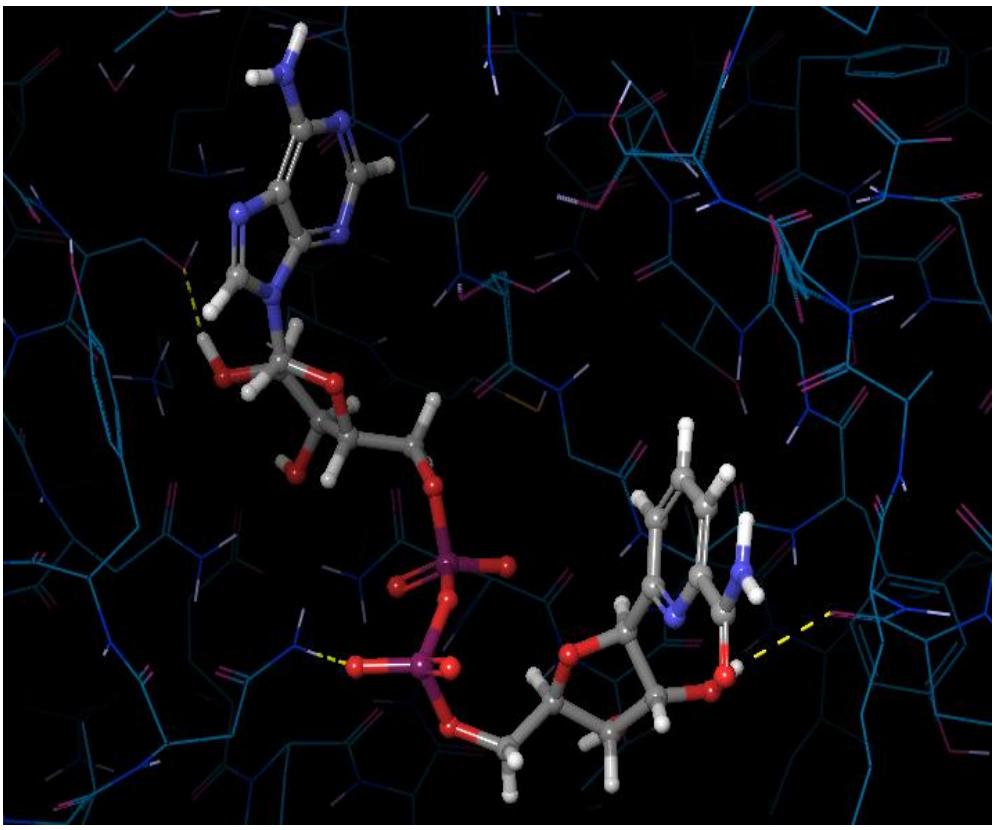

Figure10: interaction between fenugreekine and inhibitor site of CTX-M enzyme by autodock plotted by Maestro software.

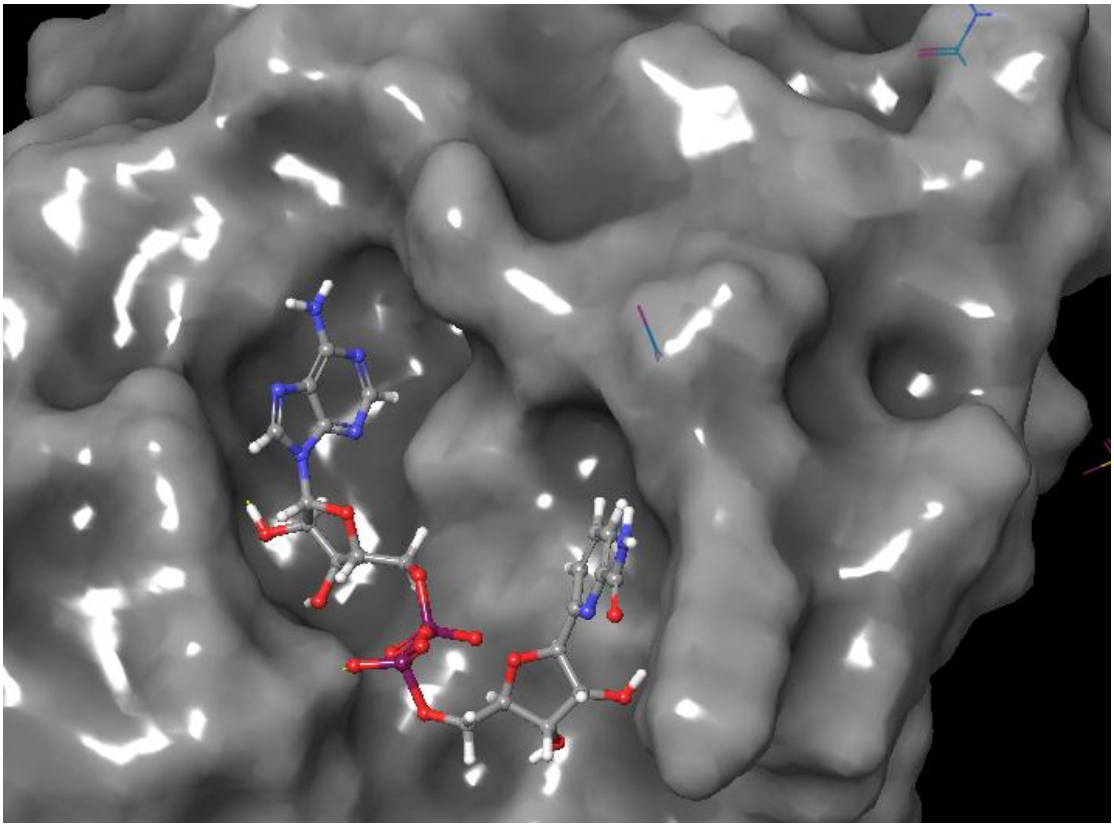

Figure11: inside frame shows the orientation of fenugreekine inside the inhibitor site as predicted by Discovery Studio Visualize

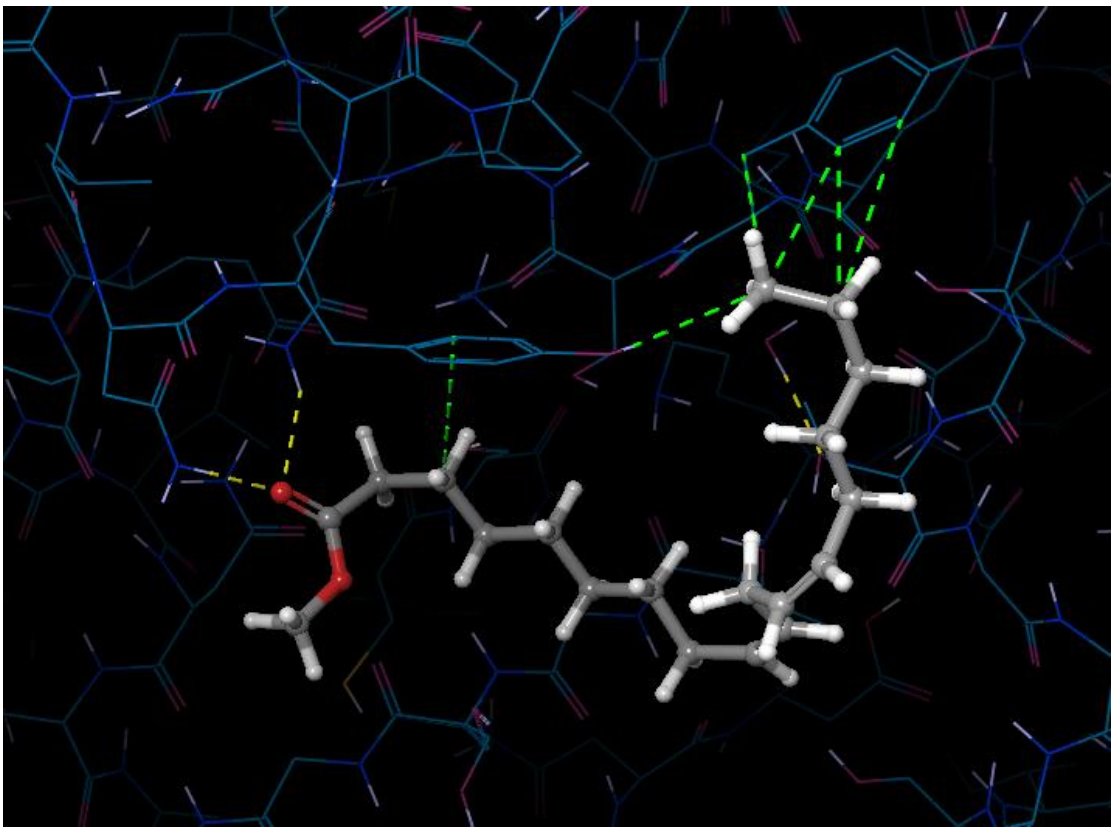

Figure12: interaction between linoleic acid, methyl ester and inhibitor site of CTX-M enzyme by autodock plotted by Maestro software.

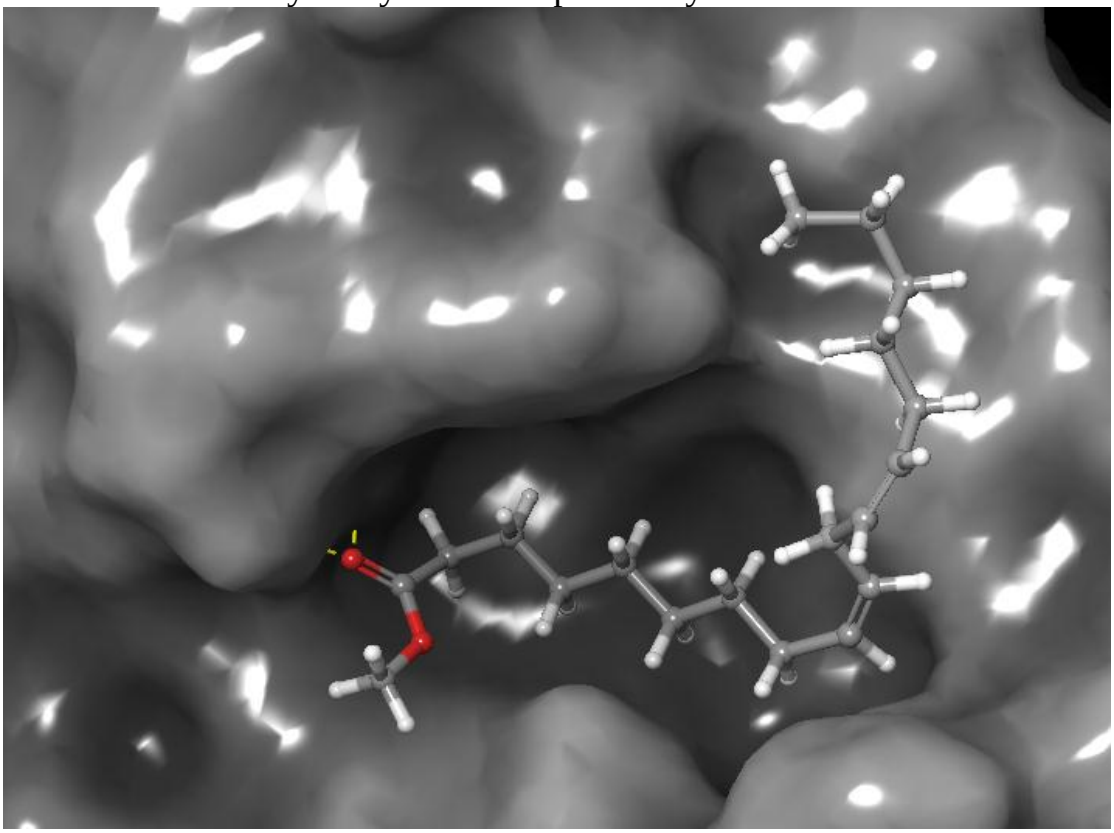

Figure13: inside frame shows the orientation of linoleic acid, methyl ester inside the inhibitor site as predicted by Discovery Studio Visualize

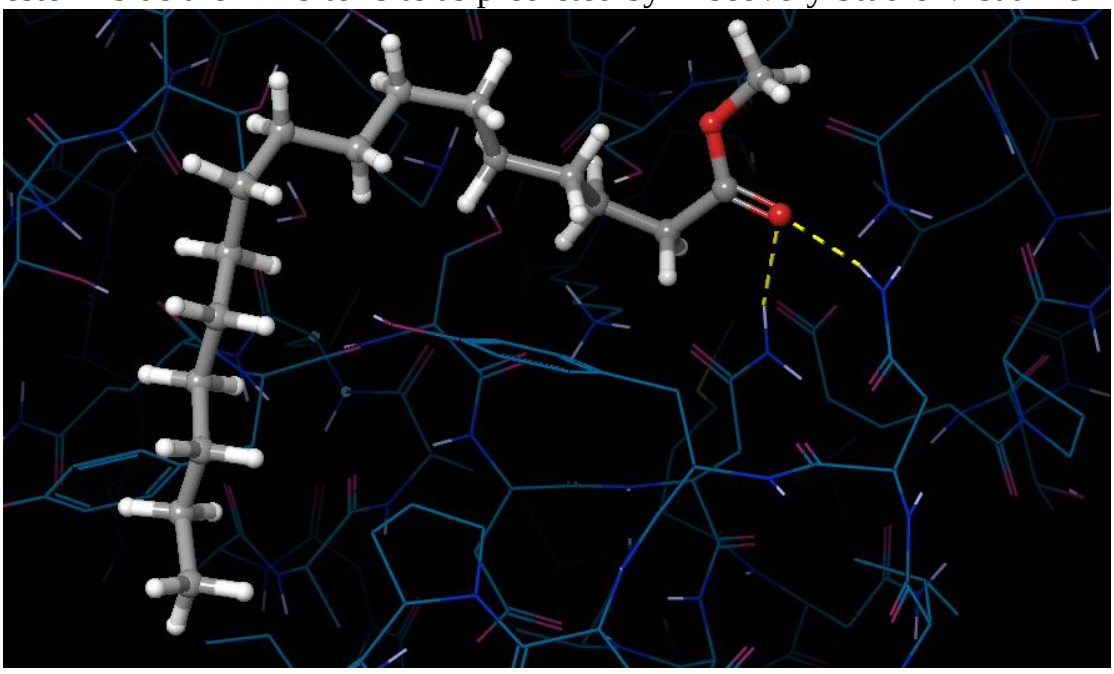

Figure14: interaction between Palmitic acid, methyl ester and inhibitor site of CTX-M enzyme by autodock plotted by Maestro software.

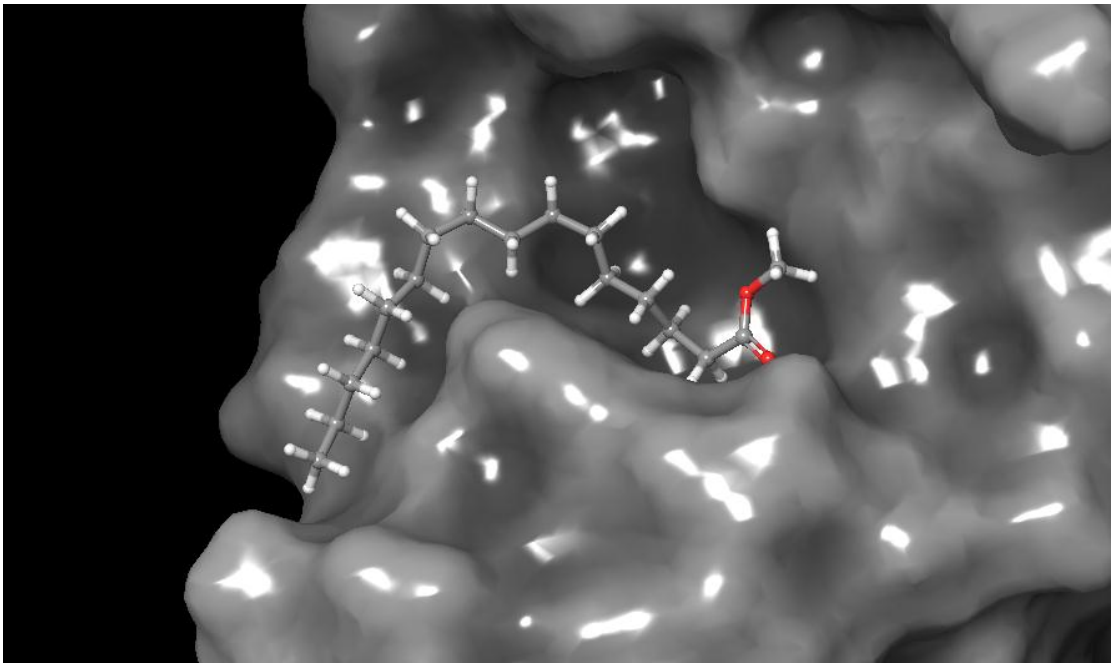

Figure15: inside frame shows the orientation of Palmitic acid, methyl ester inside the inhibitor site as predicted by Discovery Studio Visualize