Sudan University of Science and Technology
College of Graduate Studies

Extraction, and Physicochemical Characterization of Solanum dubium Seed Oil.

الإستخلاص والتوصيف الفيزيوكيميائي لزيت بذرة الجبيّن

A Thesis Submitted in Partial Fulfillment for the Requirements of the Degree of M.Sc in Chemistry

By

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إستهلال

قال تعالي:

﴿ رَبَنا لا تزَغ قُلُوبَنا بعدَ إِذْ هَدَيْتَنا وَ هَبْ لَنا مِنّ لَدُنكَ رَحْمةً إِنَّكَ أَنْتَ الوَهَّاب ﴾

سورة آل عمران

الأية(8)

صدق الله العظيم

Dedication

This study is dedicated to

My Parents,

Brothers,

and Sisters.

Acknowledgments

Praise to Allah Almighty for giving me the strength and health to complete this work.

I would like to express my thanks and deep respect to all those who helped me in this work especially my supervisor **Dr. Mohammed Suliman Ali Eltoum** for his encouragement and kindness.

Also I would like to thanks Dr. Raja Satti Mohammed, AlNeelain University, for technical support.

Thanks are extend to University of Nyala for financial support.

To my friends and colleagues for moral support.

Abstract

The aim of this study is to investigate the quantity of *Solanum dubium* seeds oil and study some physio-chemical properties of the extracted oil.

The results show the oil yield was (50%), fatty acid composition was (75%), phytochemical screening of the seed powder show higher Alkaloids contents (+ + +), moderate Flavinoid composition **(**+ +), low tannin content (+) and free of Saponine, Cumarin, Steroids, and Anthraquinones.

Investigation of the chemical properties show the Saponification value (131.835), Ester value (130.713), acid value was (1.122), mg/g and no Peroxides.

The study of antimicrobial sensibility shows that Candida(16.5mm), Bacillus (15.5mm), Pseudomonas, Staphylococcus (15mm), and E. coli unaffected.

GC-MS of the oil show that it is contain 9-12Octadacenoic acid (54.17%), Hexadecanoic acid (17.8)%, 9-Octadecenoic acid (15.82%), Methyl stearic acid (7.49%) as the major fatty acids (98%).

المستخلص

الهدف من هذه الدراسة هو التعرف على كمية الزيت المستخلص من بذور نبات الجبيّن ودراسة بعض خواصه الكيميائية والفيزيائية .

النتائج أوضحت أن نسبة الزيت كانت (50%) ، وتركيب الأحماض الدهنية كانت (75%)، دراسة التركيب الكيميائي لمسحوق البذور أوضحت نسبة عالية من الكلويدات (+++)، نسبة متوسطة من تركيب الفلافونويد (++)، محتوى أقل من التانين والترايتربين (+)، وخالية من المواد الصابونية، الكيومارين، استرويدات وانثراكوينون.

دراسة الخواص الكيميائية أوضحت أن قيمة التصبن (131.835)، قيمة الاستر (130.713) ، قيمة الحموضة كانت (1.122) ملجم/جم وخالية من البيروكسيد.

ودراسة اختبار الحساسية الميكروبية أوضحت أن فطر كانديدا (16.5 ملم)، باسليس (15.5 ملم)، بزيدوموناس، استافيلو كوكس (15 ملم) ولم تتأثر اسكريشاكولاي.

دراسة كروموتوغرافية الغاز أوضحت أن محتوى الزيت من حمض**9-12** اوكتاديكادينويك (54.17%)، حمض هكساديكانويك (17.8%)، حمض **9-** اوكتاديكينويك (15.82%)، وحمض ميثيل استياريك (7.49%). كأحماض دهنية أساسية (98%).

Table of Contents

List of Tables

List of Figures

Chapter One

Introduction and Literature Review

Introduction and Literature Review:

1-1 Introduction:

Solanum dobium one of the most popular plant known and wildly spread worldwide in the eastern Europe and western Asia. Small *S.dubium* is found in east and west of Sudan.

S.dubium plant grow at fall.the Gubbainis a widly used in industry of the cheese it contains milk-clotting Enzyme (Renen) as coagulating agent of fresh milk (Osman1987) .

S.dubium plant contains green fruits having white seeds during the primary stage of growing and rapidly maturate to orange fruites with black seeds .the second species of the gubbain is biger in size similar to peace of citrus (lemon)in the same properties .gubbain fruites solution during the inatial stage of growth, with white or black seeds on of the importants reagent for the preparation of chesse in the pastures of the most area in Sudan such as Darfur, Senar and Kordofan (Tuckey,1967).

The milk became frozen when we add certain amount of newness fruits solution were added to fresh milk at the room temperature milk is easily coagulate (similar to ice-cream) the different is only cheese is cool.

During the milk-clotting the Gubbain enzyme (Renen) separates milk proteins from the milk water and resulting two layers (upper is water and lower is cheese).

The lower layer (cheese) deemed a good food .also capable to drying as way of milk keeping for long period. The dry milk produced by this way naturally pure but pasteurization milk properties are lost.

Gubbain fruit has bitter taste and including small strong thistles on these steams and leafs this deemed defending mediums .there for the Gubbain one of most aroma and medical plant known in the world.

The aroma and taste refer to the present of some organic compounds according to phytochemical screening study results of the Gubbain (Anwar2007).

The *Solanum dubium* plant constituted important plant in the pharmaceutical industry for production of antibiotic (Sana Mukhtar 2012).

Dry Black *Solanum dubium* seeds is source of Renen enzyme and oil (Anwar2007).

1-2 Scientific classification of *S. Dubium*:

Kingdom	**Plantae**
(Un ranked)	**Angiosperms**
(Un ranked)	**Asterids**
Order	**Solanales**
Family	**Solauaceae**
Genus	**Solanum**
Species	**S.dubium**

1-3 Literature review

1-3-1 History of cheese

In ancient times, in Eastern Europe and Western Asia, the practice of carrying milk in bags made of animal's stomach probably resulted in the first cheeses being made more or less by accident. The Romans first described cheese making in detail, and the Roman legion was instrumental in spreading the art of cheese making throughout Europe. In Roman times, an enzyme preparation from goat, lamb or even hare stomachs was mixed with sheep or goat's milk (cow's milk was not produced on a large scale before the thirteenth century). The curds separated from the whey were salted and stored for later on consumption (Madden, 1995).

O'Conner (1993) reported that the origin of cheese making is lost in unrecorded history. There is evidence to suggest that cheese was made as far back as 7000 BC. There are numerous references to cheese making in the Bible while the writings of Homer and Aristotle indicate that cheese was made from the cow milk, sheep, mare and asses. Around 300 AD trade in cheese between countries especially on sea routes became so great that the Roman emperor Diocletain had to fix maximum prices for the cheese.

By the nineteenth century, some farms were selling rennet extracts in small quantities for the convenience of domestic cheese manufacture. In 1874, a Danish chemist Christin Hansen founded laboratory in Copenhagen and started the first industrial production of calf rennet extract. This was obtained from the stomachs of unweaned calves that were slaughtered for veal production and not specifically to obtain the

enzyme. World production of rennet now exceeds 25 million litres per year (Madden, 1995).

1-3-2 Sudanese white cheese:

Osman (1987) reported that (white cheese) is a traditionally fermented, pickled type of cheese (Gibna: cheese, Bayda: colloquial Sudanese Arabic word for white). It is considered that the art was introduced to Sudan through the Greek immigrants.

Sudanese white cheese (Gibna Bayda) is the most common cheese in Sudan. It has strong odour and taste. It is made from raw or pasteurized whole milk, skimed milk or reconstituted milk, depending on natural lactic acid bacteria and no starter is used (Khalid and El Owni 1991).

In Sudan cheese processing is a major preservation method of surplus milk in rural areas especially during rainy season when plenty of milk is available (Osman 1987). El Owni and Hamid (2007) stated that the most popular type of cheese produced in Sudan is the white cheese locally known as Gibna Bayda. It is generally consumed fresh or matured for a period of several months. It's made from full fat raw milk; high concentrations of sodium chloride are adde before renneting (Osman 1987).

Raheem added that (white cheese) is made in Sudan. It is similar to Domiati cheese made in Egypt. Starter is not used, and the storage life of the cheese may be more than one year. The procedure for making this cheese includes heating of the fresh milk to 350C followed by salt addition to give 7-10% solution in milk. Rennet or rennet extract is added to obtain a firm coagulum which develops in four to six hours. The coagulum is transferred thereafter to wooden moulds lined with cheese cloth muslin and the whey is allowed to drain overnight. The curd is cut

into 10cm cubes preserved in the whey in tins or other suitable air tight containers and sealed.

1-3-3 Mechanisms of milk coagulation:

The main purpose of coagulant in cheese making is the conversion of liquid milk to gel that can be catalyzed by different proteases (Green, 1984). There are two main phases in the mechanism of milk clotting: the primary or enzymatic phase secondary coagulation phase (Dalgleish, 1982; Payens, 1993).

The coagulation of milk is the result of two processes: the attack on the k-casein of the casein micelles by the proteolytic enzymes contained in rennet and the clotting of the micelles which have been destabilized by this enzymatic attack. Milk-clotting enzymes split bovine k-casein. The rate of the enzymes reaction has been shown to increase linearly with the enzyme concentration in agreement with a first-order reaction (Castle and Wheeloek, 1972; Dalgleish, 1979). The aggregation phase occurs by random, diffusion-controlled Smoluchowski mechanism (Dalgleish et al., 1981; Green, 1984), the rate of micellar aggregation being independent of their size and not affected by doubling rennet concentration (Dalgleish et al., 1981). Intermicellar linkages which appear on electron micrographs during micellar aggregation become stronger with time bringing the micelles into contact and, eventually, micelles fuse together (Green and Morant, 1981; McMahon and Brown, 1984).

Several theories have been proposed by different workers on the coagulation of milk by protease enzyme to explain this mechanism. Since 1930, Linderstrom-Lang and Holter developed a theory that casein complex of milk owe its stability to the presence of a component that acts as a stabilizer. Rennet action start by degrading this component

specifically and the modified complex flocculates in a secondary phase (Eck, 1987).

1-3-4 Rennet and rennet substitutes:

Rennet is a natural complex of enzymes produced in mammalian stomach to digest the mother's milk. Rennet contains a proteolytic enzyme protease that coagulates the milk, causing it to separate into a slid mass and liquid whey. The active enzyme in rennet is called chumosin but there are also other important enzymes in it, such as pepsin or lipase (Anwar 2003) Rennet is one of the best known coagulants used widely by cheese makers for many varieties of cheese. Crude rennet extract may be obtained from the fourth stomach (abomasum) of goat kids or calves when they are about four weeks old. Male goat kids or calves that have been fed on milk and that are not required for breeding are usually used (O'Conner, 1993) also the study enzyme is similar to that enzyme which found in child stomach, called renen enzyme which case milkclotting by clotting in the stomach.

In the 1960's the food and Agriculture Organization of the United Nations predicted a severe shortage of calf rennet. It was anticipated that an increased demand for meat would lead to more calves being reared to maturity so that less rennet would be available. Also a growing number of people following vegetarian's diets do not eat cheese made with calf rennet. As a result, over the last thirty years several substitutes for calf rennet have been made available, allowing the supply of enzyme to keep pace with cheese production and providing alternative sources for vegetarians. Today there are two major sources of chymosin for coagulating milk: from animals (veal calves, adult cows and pigs) and from different kinds of plants to new chymosins derived from genetically modified microbes. In the latter case, copies of the genes responsible for

chymosin production are isolated from calf stomach cells and incorporated into the genetic material of yeast cells. These can be grown on an industrial scale and the chymosin isolated (Madden, 1995).

The most common rennet substitutes include bovine, porcine and to a lesser extent, chicken pepsins and microbial proteases from *Rhizomucor miehei, R. pusillus* and *Cryphonectria parasitica* (Fox and McSweeney, 1997; Fox et al., 2000). The proteolytic activities of chymosin and porcine pepsin were compared on buffalo, cow and goat whole casein by Awad et al. (1998) and it was reported that both enzymes attacked using caseins in the same region as calf rennet.

Microbial rennet was more proteolytic than calf rennet and exhibited specific proteolytic action on k- casein similar to that obtained with calf rennet. Other casein fractions were degraded continuously and non specific, but p-casein was the fraction most susceptible to hydrolysis (Melachouris and Tuckey, 1967). However, Joseph et al. (1993) stated that cheese made with chymosin and *Micro miehei* proteases were similar in functional characteristic in general.

Fungal enzyme from *Penicillium funiculosum* E-NRC629 rennet substitute was used as milk clotting enzyme in the manufacture of Edam cheese from cow's milk. Obtained Results show that, the reakdown of protein content, and total volatile fatty acids in Edam cheese made with fungal enzyme or its mixture with rennet nzyme than in control cheese throughout the ripening period (Degheidi, 1996).

1-3-5 Plant proteases:

Plant proteases have been investigated as milk coagulants, but only a small number of aspartic proteinases from plant origin have been isolated and partially characterized (Tavaria et al., 1997; Sousa, 1998). A unique

feature shared by most of these plant proteinases is an extra segment of about 100 amino acid residues which bears no sequence similarity with proteinases of mammalian or microbial origins (Faro et al., 1995).

Many aspartic and other proteinases are obtained from plants and some of them have been studied as coagulants, i.e., proteinases from *Benincasa cerifera* (Gupta and Eskin, 1977), *Calotropis procera*

(Ibiama and Griffiths, 1987; Mohamed and O'Connor, 1996), *Dieffenbachia maculate* (Padmanabhan et al., 1993), fruit parts of *Solanum dubium* (Yousif et al., 1996), *Centaurea calcitrapa* (Tavaria et al., 1997) and flowers of Cynara cardunculus (Barbosa, 1983; Sousa, 1993 and Sousa, 1998). Although most plant coagulant preparations were reported to have an excessively low ratio of milk clotting to proteolytic activity, which results in bitter peptides in ripened cheese, or to an excessively low clotting power that gives rise to low cheese yields. The difficulties experienced with these preparations result mainly from the unique composition of the plant extracts, which contain a complex cocktail of enzymes whose activity is difficult to control.

Miniature (20 g) Cheddar-type cheeses were manufactured using blends

Cheeses manufactured with coagulant blends containing *C. cardunculus* proteinases exhibited higher levels of pH 4.6-soluble nitrogen than cheese made using chymosin as coagulant. The extent of breakdown of usi-casein, as measured by urea-polyacrylamide gel electrophoresis (urea-PAGE), was greater in cheeses made using coagulant preparations containing *C. carclunculus* proteinases as a constituent than in cheese made using 100% chymosin as coagulant. Different reverse-phase high-performance liquid chromatography (RP- I-IPLC) peptide profiles of the ethanol-soluble and -insoluble fractions were obtained for cheeses made

using either *C. cardunculus* proteinases or chymosin as a coagulant. Principal component analysis and hierarchical cluster analysis of RP-HPLC data confirmed that the inclusion of even small proportions (25%) of *C. cardunculus* proteinases with chymosin in the coagulant blend greatly altered the pattern and extent of proteolysis in miniature Cheddar-type cheeses (O'Mahony et al., 2003).

O'Conner (1993) reported that juice extracts from fruits and plants have long been used as milk coagulants. These include extracts from papaya (papain), pineapple (bromelin), castor oil seeds (ricin) and the latex of the fig tree and the plant *Calotropis procera* which grows abundantly in many parts of Africa. These extracts are suitable for softer crude cheese which is consumed within a few days. The extracts are not suitable for hard cheese with long maturing periods on account of their excessive proteolytic activity which leads to bitter flavours in the ripened cheese.

1-3-6 *Solanum dubium* plant in Sudan:

Solanum dubium Fresen is an indigenous plant in nofthern and central Sudan. It is a woody herb; stem is solid erect, green in colour and about 30 cm in height. The stem and its branches bear numerous sharp spines, white in colour about l- 3 mm in length and about I mm in thickness near the base. The leaves are alternate, long petiole, imple, ovate, acuminate or obtuse at the apex, pale green in colour. The petiole is 2- 6.5 cm long and 1- 3 mm in diameter covered with sharp whitish spines. The lamina bears spines only on the midrib and ain veins. The main root is about 5 mm in thickness and 15 cm in length. It bears numerous, verb thin rootlets brown in colour. The inflorescence is composed of 2- 8 pedicellate flowers arranged in *moncheasial scorpoid cyme*. The flower is a hermaphrodite, actinomorphic with a yellow center core- like structure formed of the persistent of 5 united green sepals, and bears numerous

sharp spines. The corolla is violet in colour, rotate, of five petals united at the base with distance of 4 mm forming a tube which is terminating with 5 oval lanceolate lobes and a very short filament. Anthers open by two apical pores. The fruits are grouped in clusters with exile alternately bent to bring all clusters to one side of the stem or the branch. It's a berry globular in shape being 1cm in diameter with smooth lustrous surface. Unripened fruits are green and almost enclosed in spiny calyx, while, the ripened fruits are yellow. The seeds are dark brown in colour. The taste is minutely pitted (Andrews, 1956; Salih, 1979).

According to Yousif et al. (1996) (*Solanum dubium*), a major problem for many farmers in Sudan, is a noxious weed belonging to the plant that flourishes during the rainy season (typically starting in June- August in Sudan) and usually bears fruits about January with green fruits which become yellow when fully ripened. Fruits are usually dry on the stem; their thorny surface causes them to adhere to grazing animals and facilitates seed dissemination. Animals do not eat *Solanum dubium* because of its bitter taste and thorny leaves.

1-3-7 Chemical composition of *Solanum dubillm*:

Solanum dubium fruit contains 45.10% fiber, 20.15% carbohydrate, 16.63% crude proteins, 5.60% ether extract, 5.90% moisture and 6.0% ash (Habbani, 1992). X-ray fluorescence analysis showed the presence of potassium, calcium, iron, manganese, sulphur, phosphorous, copper and bromine as the most abundant elements in *Solanum dubium* fruits. *Solanum dubium* is readily soluble at low salt concentrations functionality was determined as nitrogen and at the extremes of pH values (Habbani, 1992).

According to Salih (1979) *Solanum* plants containing steroidal substances are of considerable medicinal and economic importance due to the

suitability of these substances as starting materials for the synthesis of steroidal drugs such as corticosteroids and sex hormones. Over 100 species of the genus *Solanum* have been reported to contain steroidal compounds, and the *solasodine*, the most suitable starting material for the synthesis of steroidal drugs, has been isolated from at least 60 species of *Solanum*.

Thin layer chromatography and gas liquid chromatography revealed the presence of six amino acids in *Solanum*, three of which being present in the hydrolysate were identified as phenylalanine, valine, and alanine (Osman, 1996).

Sulieman et al. (1988) studied the aqueous extract of the whole ruit, fruit coat and seed of *Solanum incanum* Lim for their milk coagulating properties. Phytochemical examination indicated that the active principle is glycoside and the whole extract was necessa1Y for coagulation.

1-3-8 Effect of incubation temperature on milk-clotting activity:

Recently Osman (2001) reported that Solanum rennet has a maximum activity at 600C. Osman (1996) showed that the enzyme extract from *Solanum dubium* fruits has a maximum activity at a temperature between 45-50C, a gradual decrease in activity was observed as the temperature increased reaching its minimum activity at 650C.

Habbani (1992) reported that the maximum activity of *Solanum dubium* extract was shown at 31 C. Similar results were found by Mohamed and Habbani (1996) who pointed that the "Gubbain" extract activity was increased up to 380C and started to decline thereafter.

Sidrach et al. (2005) found that the milk clotting protease from *Cynara scolymus* express maximum activity at 700C.

Vieira De So and Barbosa (1972) showed that cardo clotting enzyme is stable at high temperature and shows an increasing clotting activity up to 700C, above this temperature the activity falls and above 750C completely disappears. Bodansky (1924) studied milk-clotting enzyme of *Solanum elaeagnifolium*, and he found that the enzyme has a higher optimum temperature (80- 850C) and resists heat better than animal rennet. Similar results were reported by Melachouris and Tuckey (1967) that the maximum activity of microbial rennet isolated from a culture of *Bacillus cereus* was obtained between 75-800C. Milk clotting enzyme extracted from Kesinai (Streblus asper Lour) leaf exhibited maximum activity at 650C. Results were also reported by Aworh and Nakai (2006) that the enzyme from Sodom apple (*Calotropis procera*) leaves was more active at 650C than 350C.

Pascaline and Daniel (2006) found that *Mucor miehei* and *Mucor pusillus* protease are much more stable at 530C for 100 minutes, while bovine pepsin and the *Endothia parasitica* proteases are rapidly inactivated at 530C in 100 minutes. Extracellular aspartate protease from *Rizopus oryzae* was purified 91 times with 26% recovery using ammonium sulphate fraction, ion- exchange and size- exclusion chromatographic techniques, which acts optimally at 600C and was more stable in temperature range of 30- 450C (Kumer et al., 2005). Similar results were also reported by D' Ambrosio et al. (2003) who found that proteolysis and milk clotting activity in extract obtained from the *crustacens Munida* has optimal temperature at 55-60c.

Raposo and Domingos (2008) showed that, the optimum temperature for proteolytic activity of aspartic proteinases (APS) from *Centaurea calcitrapa* plant cell suspensions was 520C. The enzymes remained fully active when exposed for 6 hours at 40C and 250C. For all other

temperatures, after I hour of incubation, activity decreased. At 37 oc activity decreased 40%, and at 520C decreased 60%. After 6 hours of incubation at 705C, purified Aps extract lost almost all of its activity. However, the purified enzyme from goat (Capra hircus) was stable up to 550C with maximum activity at 30C (Kumar et al., 2006). Campos et al. (1990) pointed that the proteolytic activity of the crude extract from wild thistle (*Cynara cardunculus*) was found to be 3 TC.

1-3-9 Effect of pH on the activity of *Solanum dubium* extract:

Osman (2001) reported maximum activity of *Solanum dubium* extract was observed at pH5.5 and the enzyme activity decreased with increasing pH value. Similar results were obtained by Habbani (1992) who reported that the activity of *Solanum dubium* rennet decreased with increasing pH value and the maximum activity was at pH 4.6 and 4.5. Kumer et al. (2005) showed that the purified enzyme from *Rizopus oryzae* is an acid protease with optimum pH of 5.5 and etained 96% of residual activity between pH 5.5 and 7.5. However, Thunell et al. (1979) found that the milk clotting activity from *Mucor miehei* protease to be destroyed at 79.50C, 76.60C and 73.90C above pH.

1-3-10 Effect of pH on milk clotting activity:

The maximum enzyme activity from Bacillus sphaericus was at a wide range of pH 5.7- 7.5 (El-Bendary et al., 2007). However, Melachouris and Tuckey (1967) demonstrated that the milk clotting activity of microbial rennet isolated from a culture of Bacillus cereus was found to be less sensitive to pH changes of the substrate than calf rennet.

Aworh and Nakai (2006) reported that the milk clotting activity using enzyme from Sodom apple leaves increased with pH at 650C with enzyme being almost twice as active at pH 6.4 as at pH 5.4- 5.7.

Similar results were obtained by Tavares et al. (1997) in their findings that Tuna protease was less sensitive to losses of activity than rennet at pH values above 6.4. Both enzymes became unstable beyond pH 7 and completely lost their activities at pH 8. While D'Ambrosio et al. (2003) found the optimal value of pH of the total proteolytic activity of the *crustaceans Munida* to be in the ranges of pH 6.6-7.5. Raposo and Domingos (2008) studied the optimum pH of purified plant aspartic proteinases from *C.calcitrapa* cell suspensions and they detected maximum activity at pH 5.1. Kumar et al. (2006).

Found that the milk clotting activity of a purified enzyme extracted from abdominal tissue of goat kid decreased steadily as pH increased and indicated maximum activity at pH 5.5. The proteolytic activity of the crude extract from wild thistle (*Cynara cardunculus*) was found to be at pH 5.7 (Campos et al., 1990). However, extract from kesinai (Streblus asper Lour) leaf showed maximum enzyme activities at pH 7.2 (Ishak et al., 2006). Milk clotting protease from *Cynara scolymus* expressed maximum activity at pH 5 (Sidrach et al., 2005).

Heimgartner et al. (1989) studied three proteases (Cynara l, 2 and 3) purified from dried flowers of *Cynara cardunculus*. All three enzymes expressed maximum milk-clotting activity at pH 5.1. Nouani ei al. (2009) reported that optimum activity falls within the range of the pH

acids, 5 for the fig tree (*Ficus carica*) enzyme and 5.5 for the artichoke protease (*Cynara scolymus*).

1-3-11 Effect of calcium chloride concentration on *Solanum dubium* extract activity:

Calcium is an important factor in cheese making, its effect on the activity of *Solanum dubium* extract showed an increase with increasing

concentration (Habbani, 1992 and Mohamed and Habbani, 1996). Chazarra et al. (2007) found that the rennet strength of artichoke (Cynara scolymus) flowers extract increased with increasing concentration of calcium.

Ishak et al. (2006) showed that the presence of calcium chloride up to 6 mM decreased the milk clotting time of extract obtained from kesinai (*Streblus asper* Lour) leaf. Study of a milk clotting activity from *Bacillus sphaericus* was done by El-Bendary et al. (2007), and the results from their study indicated that the milk clotting activity of the purified enzyme was stimulated with increasing calcium chloride concentration up to 0.25%.

1-3-12 Toxicity of *Solanum dubium*:

Although some species of *Solanum* are highly toxic and contain the steroid alkaloid, solanidine, glycoside, solanine, and a variety of other glycoalkaloids, the toxicity of *Solanum dubium* seed was studied by feeding rats the extract enzyme as well as white cheese made with enzyme. The results showed that *Solanum dubium* seed extract and Solanum cheese did not significantly affect the total protein and minerals of serum of all fed groups, and no remarkable gross or histopathogical alteration were detected in the liver or kidney of all experiment and control group (Osman, 2001).

1-3-13 Salting and the role of salt in cheese:

Salt has three major functions in cheese: it acts as a preservative, contributes directly to flavour, and is a source of dietary sodium. Together with the desired pH, water activity and redox potential, salt assists in cheese preservation by minimizing spoilage and preventing the growth of pathogens. The dietary intake of sodium in the modern western

diet is generally excessive, being two to three times the level recommended for desirable physiological function (2.4 g Na, or 6 g NaCl per day). However, cheese generally makes a relatively small contribution to dietary sodium intake except if high quantities of high-salt cheeses such as Domiati and Feta are consumed (Guinee, 2004). Sodium chloride influences cheese ripening principally through its effect on water activity, control of varios enzyme activities in cheese, synerisis of the curd and physical changes in proteins which influence cheese texture and solubility (Fox et al., 1995).

In addition to these functions, salt level has a major effect on cheese composition, microbial growth, enzymatic activities and biochemical changes, such as glycolysis, proteolysis, lipolysis and para-casein hydration, which occur during ripening. Consequently, the salt level markedly influences cheese flavour and aroma, rheology and texture properties, cooking performance and, hence, overall quality. Many factors affect salt uptake and distribution in cheese and precise control of these factors is a vital part of cheese making process to insure consistent, optimum quality (Guinee, 2004).

Cheese is salted by adding sodium chloride directily to milk and packed in tin (Khalid and El Owni 1991). In Sudanese white cheese softer, creamier and less bitter, and was rated significantly higher in overall liking by both trained and consumer panels.

1-3-14 Whey of cheese:

Whey is the liquid that remains after most of the fat and the protein in the milk are removed during cheese making process. Whey contains valuable nutrients, i.e. whey protein, carbohydrate and minerals. The whey from cheese making vary according to the type of cheese made and, therefore, the content of protein, salt and lactose also vary. As whey contains about

half of the total solids in the original milk it should not be thrown away as waste but should be used as animal feed or for human nutrition (O'Conner, 1993; Butylina, 2007).

Whey from milk coagulated with vegetable rennet had 10.4% total solids, 2.54% fat and 0.33% total N. High levels of total Nitrogen in whey from milk coagulated with vegetable rennet may be ascribed mainly to the strong proteolytic activity of the coagulant with formation of soluble N which resulted in heavy fat and total solids in whey than from milk coagulated with vegetable rennet. This break down of casein network resulted in heavy fat and total solids losses in whey from milk coagulated with vegetable rennet (Nufiez et al., 1991).

Barbosa et al. (1976) reported 1.14% total proteins for whey from vegetable rennet and 0.86% for whey from animal rennet in Camembert cheese making. Significant increases of protein in whey from vegetable rennet were also obtained for Grana, Provolone and Bel Paese cheeses (Barbosa et al., 1981). Higher solids, protein and fat contents were found in whey from cheddar cheese made with Bacillus

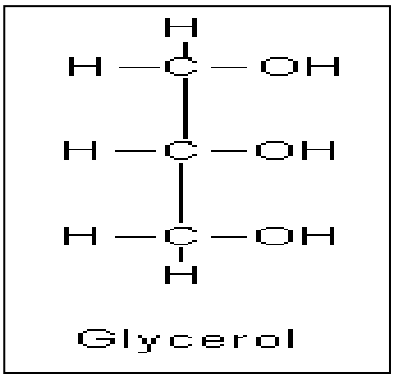
subtilis proteinases as coagulant enzymes (Puhan and Irvine, 1973)

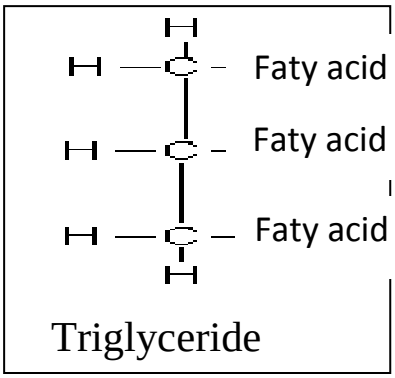
which lowered cheese yield by 10% compared with that made with

calf rennet.

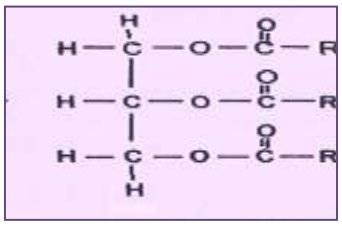
1-3-15 The basic chemistry oils and fat:

Basically oils and fat are mixture of triglycerides when three fatty acid are combined with one glycerol molecule .

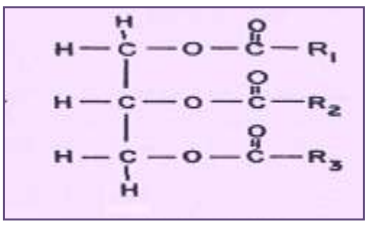




The alkyle groups(R)in the fatty are indentical in simple triglyceride but in the mixed tri glyceride two or three different alkyle groups(R)is present in the molecules.

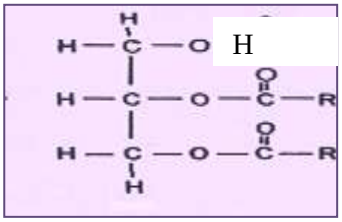


Simple triglycerole

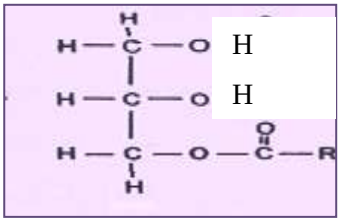


mixed triglyceroide

In only two fatty acid are attached to specific glycerol molecule called di glyceride but when one fatty acid is attached called mono glycerid.



Diglyceride



Monoglyceride

In this case three different fatty acid are connected to glycerol.

There are numerous different fatty acid and which ones one r esterifies to glycerol largely determine the properties of the fats .including whether they are solid or liquid at room temperature.

Short chain fatty acid gives softer fat or lower melting points than do long chain fatty acid.

1-4 Aims and objectives:

This research aims are to investigate some physiochemical properties of *solanum dubium* seeds collected from Darelnaeem area (Areed) used to prepare cheese as alternative source of animal rennet. The objectives are to study:-

1.To extract specific of the oil from the seeds of *Solanum dubium*.

2.To characterize *Solanum dubium* oil.

3.To study phytochemical screening of seed powder.

4.To study *Solanum dubium* extract sensory evaluation and microbiological quality on the cultures and GC-MS of oil.

Chapter Two
Materials and Methods

Chapter Two

Materials and Methods

2-1 Materials:

The plant material used in this study was collected from Darelnaeem area (Areed) Alfasher. The *Solanum dubium* fruits Fig (2.1) were collected at one stage of maturity in December when the fruits were yellow with black and completely dry seeds Fig (2.2).

The *Solanum dubium* fruits were carefully hand cleaned to obtain black seeds

2-1-1 Sample preparation:

Solanum dubium seeds was powdered using electric grinding. Fig (2.3)

2-2 Methods of extraction *Solanum dubium* seeds oil using soxhlet method:

Extraction was carried out according to method described by Sukhdev *et. al.* (2008):

The plant sample was coarsely powdered using mortar and pestle. sample was successively extracted with n-Hexane using soxhlet extractor apparatus. Extraction carried out for four hours till the colour of solvents at the last siphoning time returned colorless. Solvents were evaporated under reduced pressure using rotary evaporator apparatus. Finally oil extract was allowed to air dry in glass container till complete dryness and the yield percentage was calculated as follows:

$$\text{Yield percentage} = \frac{\text{volume of extract obtained}}{\text{weight of plant sample}} \text{ X100}$$

Fig(2.1): *Solanum dubium* fruits.

Fig (2.2): *Solanum dubium* seeds.

Fig (2.3): *Solanum dubium* seeds powder.

Fig(2.4) :Soxhlet procedure.

Fig(2.5) : *Solanum dubium* oil.

2-3 Phytochemical screening:

Phytochemical screening for the active constituents was carried out on methanolic extract using the methods described by (Martinez and Valencia (1999), Sofowora (1993), Harborne (1984) and Wall et al (1952)) with many few modifications.

2-3-1Test for tannins:

Procedure**:**

0.5 g of the extract washed times with petroleum ether, dissolved in 10ml hot saline solution and divided in two tests tubes. To one tube 2-3 drops of ferric chloride were added and to the other one 2-3 drops of gelatin salts reagent were added. The occurrence of a blackish blue colour in the first test tube and turbidity in the second one denotes the presence of tannins.

2-3-2 Test for sterols and triterpens:

Procedure:

0.5g of the extract was washed three times with petroleum ether and dissolved in 10ml of chloroform. To 5ml of the solution, 0.5ml acetic anhydride was added and then 3drops of conc. Sulphuric acid at the bottom of the test tube. At the contact zone of the two liquids The gradual appearance of green, blue pink to purple color was taken as an evidence of the presence of sterols (green to blue) and or triterpenses (pink to purple) in the sample.

2-3-3 Test for Alkaloids:

Procedure:

0.5g of the extract was heated with 5ml of 2N Hcl in water bath and stirred for about 10minutes, cooled filtered and divided into two test tubes. To one test tube few drops of Mayer's reagent were added while to the other tube few drops of Valser's reagent were added. A slight turbidity or heavy precipitate in either of the tow test tubes was tanked as presumptive evidence for the presence of alkaloids.

2-3-4 Tests for Flavonoids:

Procedure:

0.5g of the extract was washed three times with petroleum ether, dissolved in 30ml of 80% ethanol. The filtrate was used for following tests: -

A- to 3ml of the filtrate in a test tube 1ml of 1% potassium hydroxide solution in methanol was added. Appearance of a yellow color indicated the presence of Flavonoids. Flavones or chalcone.

B- to 2ml of the filtrate 0.5ml of 10% lead acetate was added. Appearance of creamy turbidity was taken as an evidence of flavonoids .

2-3-5 Test for Saponins:

Procedure:

0.3g of the extract was placed in a clean test tube. 10ml of distilled water were added, the tube were stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of foam, which persisted for least an hour, was taken as evidence for presence of saponins.

2-3-6 Test for Cumarins:

Procedure:

0.2g of the extract dissolved in 10ml distilled water in test tube and filter paper attached to the test tube to be saturated with the vapor after a spot of 0.5N KoH was placed. Then the filter paper was inspected under UV light, the presence of coumrins was indicated if the spot have found to be adsorbed the UV light.

2-3-7 Test for Anthraquinone glycoside:

Procedure:

0.5g of the extract was boiled with 10ml of 0.5N KoH containing 1ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10ml of benzene. 5ml of the benzene solution was shacken with 3ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of anthraquinones was indicated if the alkaline layer was found to have assumed pink or red color.

2-4 Chemical properties:

2-4-1 The acid value:

Is the number that expresses, in milligrams the quantity of potassium hydroxide required to neutralise the free acids present in 1g of the substance

Procedure:

10.00g of *Gubbain* powder were dissolved in 50ml of a mixture of equal volumes of *ethanol (96%)* and *light petroleum*, previously neutralised with *0.1M potassium hydroxide*, unless otherwise specified, using 0.5ml of *phenolphthalein solution* as indicator. The mixture is titrated with *0.1M potassium hydroxide* until the pink colour persists for at least 15min (*n* ml of titrant).

$$Av = \frac{5.610n}{m}$$

Where:

N: is the volume of titrant

M: is the weight of the oil

2-4-2 The saponification value:

Procedure:

Is the number of miligrams of potassium hydroxide required to neutralise the free acids and to saponify the esters in 1g of the substance.

3.5-4g of *potassium hydroxide* were dissolved in 20ml of *water* and add sufficient ethanol *(96%)* was added to produce 100ml. Allow to stand overnight and pour off the clear liquid was poured off.

Weigh 2g of the substance into a 200ml flask, add 25.0ml of the ethanolic solution of potassium hydroxide and boil under a reflux condenser for 1hour, rotating the contents frequently. While the solution is still hot, titrate the excess of alkali with 0.5M *hydrochloric acid*, using 1ml of *phenolphthalein solution* as indicator. Repeat the operation without the substance being examined (blank test) Saponification value is calculated from the following equation:

$$\text{Saponification value} = \frac{\mathbf{28.05\ (V2-V1)}}{\mathbf{m}}$$

V1 : is the volume of titrant used in oil titration

V2 : is the volume of the titrant used in blank titration

M : is the weight of the oil

2-4-3 The peroxide value:

Procedure:

5.00g of *Gubbain* powder were placed in a 250ml conical flask fitted with a ground-glass stopper. Add 30ml of a mixture of 2volumes of *chloroform* and 3volumes of *glacial acetic acid* were added. The mixture was shaken to dissolve the substance and add 0.5ml of *saturated potassium iodide solution* were added. The mixture were shaken for exactly 1min 30ml of water were added and titrated with *0.01M sodium thiosulphate*, the titrant was slowly added with continuous vigorous shaking, until the yellow colour is almost discharged. 5ml of *starch solution* were added and continue the titration content, shaking vigorously, until the colour is discharged ($n_1$ml of *0.01M sodium thiosulphate*). A blank test was conducted under the same conditions (n_2 ml of *0.01 M sodium thiosulphate*). Peroxide value was calculated as follows:

$$\text{Peroxide value} = \frac{10\ (V2-V1)}{m}$$

V1 : is the volume of titrant used in oil titration

V2 : is the volume of the titrant used in blank titration

M : is the weight of the oil

2-4-4 Ester value:

Ester value was calculated as follows:

Ester value = Saponification value – Acid value

2-5 Test microorganisms:

2-5-1 Bacterial microorganisms:

Bacillus subtilis	NCTC 8236 (Gram + ve bacteria)
Staphylococcus aureus	ATCC 25923(Gram +ve Bacteria)
Escherichia coli	***ATCC 25922(Gram -ve bacteria)***
Pseudomonas aeruginosa	ATCC 27853 (Gram -ve bacteria)

2-5-2 Fungal microorganisms:

Candida albicans	ATCC7596

2-5-3 Methods:

2-5-3-1 Preparation of the test organisms:

2-5-3-1-1 Preparation of bacterial suspensions:

One ml aliquots of 24hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37ºC for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce suspension containing about 10^8-10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4°C till used.

The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra, 1938). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37°C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the

stock suspension, expressed as the number of colony forming units per ml suspension.

Fresh stock suspension was prepared each time. All the above experimental conditions were maintained constant so that suspensions with very close viable counts are obtained.

2-5-3-1-2 Preparation of fungal suspension:

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25°C for 4days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used.

2-5-3-2 Testing of antibacterial susceptibility:

2-5-3-2-1 Disc diffusion method:

The paper disc diffusion method was used to screen the antibacterial activity of plant extract (oil) and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20µl of a solution of each plant extract(oil).The inoculated plates were incubated at 37°C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

2-6 GC-MS of oil:

2-6-1 Instrument Information:

Name :GC.MS

Detector :Mass spectrometer

Model :GC.MS-QP2010 Ultra

Company :Shimadzu

Country :Japan

Colum :Rtx-

5Ms…Lengh(30m)…Diameter(0.25mm)…Thickness(0.25µl).

Carrier :Helium

Serial Number :020525101565SA

2-6-2 Sample preparation (Methylation):

2ml from the sample were taken in to test tube and then 7ml from alcoholic NaOH were added by dissolve 2g sodium hydroxide in 100ml methanol 7ml of alcoholic H_2SO_4 1% prepared by mixing 1ml Conc. H_2SO_4+99 ml methanol were added to the mixture.

The mixture was shaken by vortex for 3minutes the contents is left to overnight 2ml supersaturated NaCl are added with 2ml normal hexane and shaken for three minutes, the hexane layer was collected. 5µL from hexane collected are diluted with 5ml diethyl ether. 1g from sodium sulphate as drying agent was added.

The mixture was Filtered through syringe filter 0.45µm.

the filtrate transfered directly to the GC-MS vial.

finally 1µL Injected directly to the GC-MS.

2-6-3 Method of analysis and GC-MS Conditions:

The qualitative and quantitative analysis of the sample was carried out using GM/MS technique model (GC/MS-QP2010-Ultra) from Japan Simadzu Company, serial number 020525101565SA and capillary

column (Rtx-5ms-30m×0.25 mm×0.25µm).The sample was injected using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60C with rate 10C/min to 300C as final temperature degree with 3minutes hold time, the injection port temperature was 300C, the ion source temperature was 200C and the interface temperature was 250C.The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 26minutes .Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology (NIST)., results were recorded

Chapter Three

Results and Discussion

Chapter Three
Results and Discussion

3-1 Results of the preparation of the extract (oil):

Name of plant	Weight of dried seeds powder in gm	volume of oil in ml	Yield%
Gubbain	30	15	50%

Yield percentage of oil extract = $\frac{\boldsymbol{volume\ of\ extract}}{\boldsymbol{weight\ of\ plant\ sample}} \times 100$,

$$= \frac{15}{30}\ \% = 50\%\text{ml/gm}$$

The yield is 50%, this result agreement with result from a big *solanum dubium* oil (Fatima, 2013).

In this study oil was extract from *solanum dubium* seeds and subjected to different physiochemical tests the extract oil was liquid at room temperature and yellow colour with palatable flavor.

3-2 Results of phytochemical screening:

Table (3.1): show phytochemical screening

Results: Sample	Saponin	Cumarin	Alkaloids	Flavonoids	Tannins	Steroids	Triterpens	Anthraquinone glycoside
Gubain seeds	-	-	+++	++	+	-	+	-

Key: + Trace, ++ Moderate, +++ High, - Negative

Table (3.1) shows the results of Phytochemical screening of the sample which showed the presence of Saponin and Cumarin and Steroids and Anthraquinoneglycoside is ze**ro.**

But the results showed presence of small trace of Tannins and Triterpens while showed moderate of Flavonoids and sample is rich (high) of Alkaloids.

3.3 Results of Chemical properties of oil

Table (3.2): comparative of quality parameters for three types of oils [Sudanese standard and meteorology organization (SSMO)]

Parameters	Results mg/g		
	Gubbain oil	Moringa oil	Sesame oil
Acid value(Av)	1.122	0.77982	1.342
Saponification(Sv)	131.835	189.03865	129.16
Peroxide value(Pv)	zero	zero	4.951
Ester value (Ev)	130.713	188.2588	91.893

Table (3.2) showed that Saponification and ester values are almost similar. In comparison of some properties of Gubbain oil and some food oils such as Moringa and Sesame oil, Table (3.2). It's clear that from the results of the chemical properties of three previous oils there are quantitative relationships.

3-4 Results of antimicrobial activity:

Antimicrobial activity = $\frac{con1+con2}{2}$

Table (3.3): Results of antimicrobial activity

Microbial species	Con1(100)	Con2(100)	Antimicrobial activity
E.coli	-	-	-
Pseudomonas aeruginosa	16	14	15
Staphylococcus aureus	14	16	15
Bacillus subtilis	15	16	15.5
Candida albicans	17	16	16.5

The results showed that the oil has no antimicrobial sensitivity toward E.coli and activity of Pseudomonas is equal to the activity of the Staphyl-coccus (15) but in the case of candida albicans is (16.5) and Bacillus is (15.5)mm.

Fig(3.1) : Activity of E.coli

Fig(3.2): Activity of Pseudomonas aeruginosa

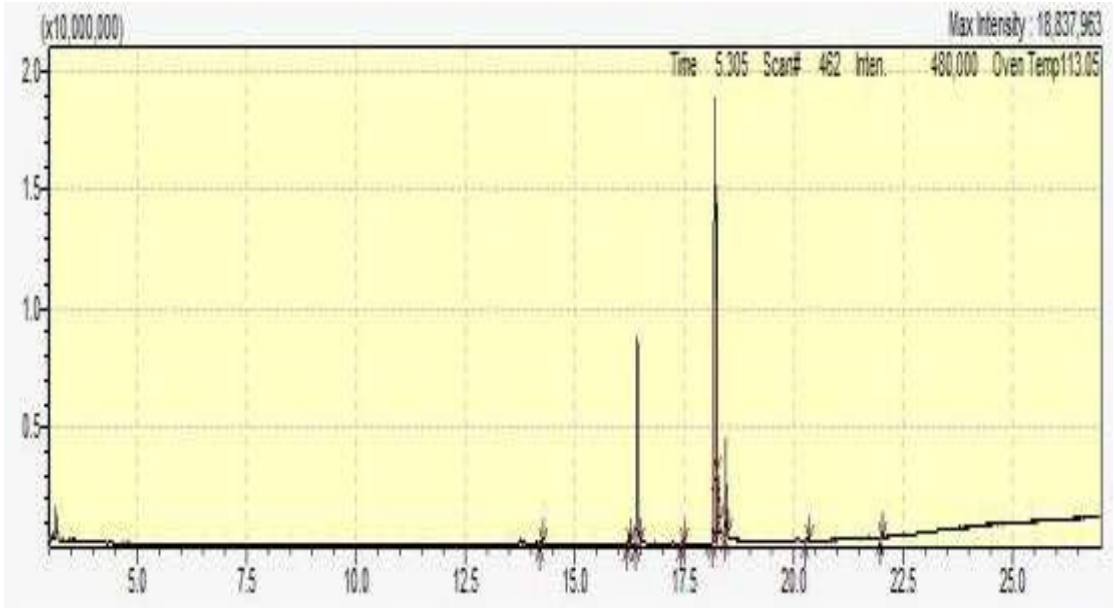
Fig(3.3): Activity of Candida albicans

.**Fig(3.4):**Activity of Staphylococcus aureus.

Fig(3.5): Activity of Bacillus subtilis.

Antimicrobial activity of the one species of fungal microorganism (candida albicans) was measured that is found response. The microbial activity toward the oil is found identical with the national committee for clinical laboratory standards guidelines (Nccls, 1999).

3-5 Results of GC-MS:



Fig(3.6) :GC-MS peak of Gubbain seeds oil.

Table (3.4) GC-MS profile fatty acid composition

ID#	Name	Ret.Time	Area	Area%
1	Methyl tetradecanoate	14.214	243059	0.25
2	9-Hexadecenoic acid, methyl ester, (Z)-	16.226	675323	0.70
3	Hexadecanoic acid, methyl ester	16.426	17279059	17.83
4	Heptadecanoic acid, methyl ester	17.458	181773	0.19
5	9,12-Octadecadienoic acid (Z,Z)-,	18.198	52503122	54.17
6	9-Octadecenoic acid (Z)-, methyl ester	18.234	15332357	15.82
7	9-Octadecenoic acid, methyl ester, (E)-	18.267	2918246	3.01
8	Methyl stearate	18.443	7261230	7.49
9	Heneicosanoic acid, methyl ester	20.299	346256	0.36
10	Triacontanoic acid, methyl ester	22.007	177782	0.18

Figure (3.6) and Table (3.4) show the fatty acid composition of S. dubun oil. Results indicates that 9-12 Octadecadienoic acid (54.17%), Hexadecanoic acid (17.83%), 9-Odecenoic acid (15.82%), Methylstearate (7.49%), 9-Octadecenoic acid (3.01%) as the major fatty acids (98%).

According to the GC-MS results *solanum dubium* oil is rich by variety of unsaturated oilic acid , the results showed present of variety oilic acid by long carbon chain (high molecular weight), such (9-hexadecenoic acid) and (hexadecanoic acid) and (heptadecanoic acid) and (9-12-Octadecadienoic acid) and (9-Octadecenoic acid (z) methyl ester) and (9-Octadecenoic acid (E) methyl ester) and (heneicosanoic acid) and (triacontanoic acid).

3-6 Conclusions:

- *S.dubium* fast growing plant which is affected by drought so can be easily grown in a wide range in Sudan.
- Characterization of oil from *S.dubium* seeds oil showed that oil could be utilize as successfully as source of the Antibiotic.
- The GC-MS showed that the *S.dubium* oil it contains high saturated and unsaturated fatty acid ratio.
- The qualitative and quantitative determination of major and minor constituents of seeds oil is done by GC-MS which are the technique widely applied for analysis of oils and fats.
- The detailed scientific knowledge regarding the physio-chemical properties of the seeds oil considerable importance for the pharmaceutical development.

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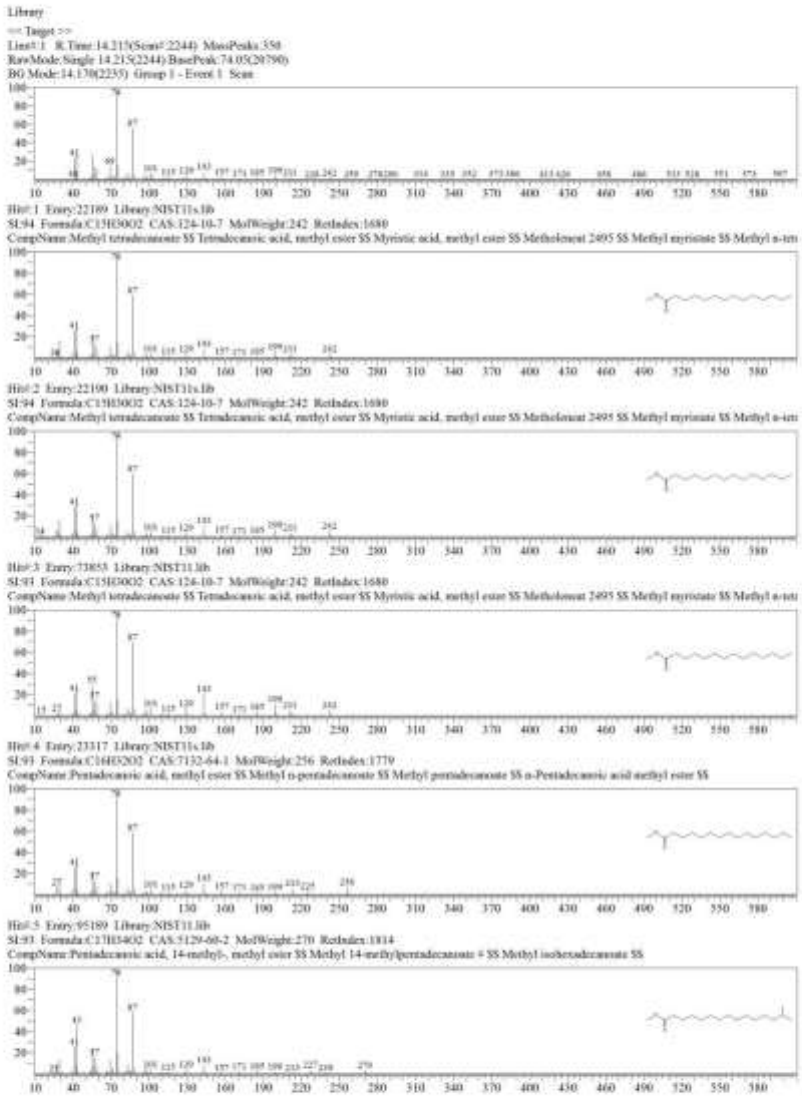
National Collection of Type Culture (NCTC), Colindale, England.

American Type Culture Collection (ATCC) Rockville, Maryland, USA.

Appendix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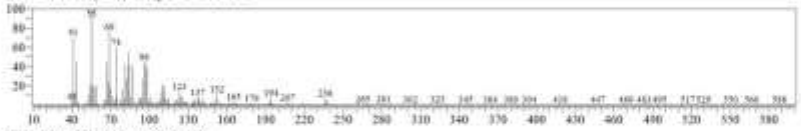

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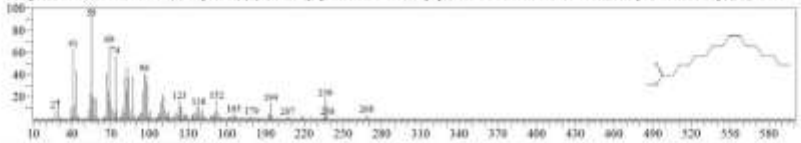
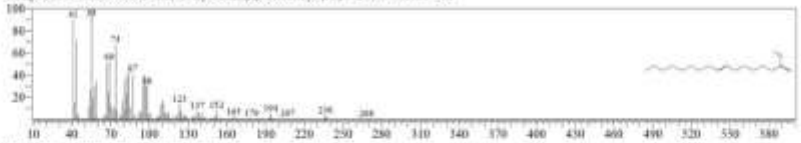
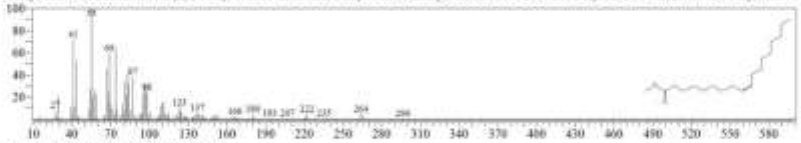
SI:95 Formula:C17H32O2 CAS:1120-25-8 MolWeight:268 RetIndex:1886
CompName:9-Hexadecenoic acid, methyl ester, (Z)- $$ Methyl palmitoleate $$ Methyl palmitoleinate $$ Palmitoleic acid, methyl ester $$ Methyl (9Z)-9-hexad



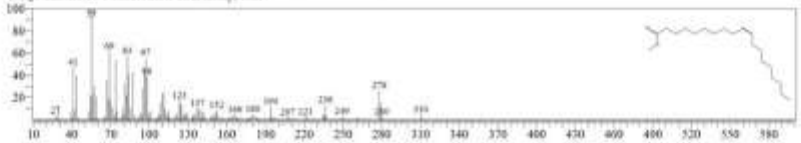
Hit#:2 Entry:93476 Library:NIST11.lib
SI:94 Formula:C17H32O2 CAS:56875-67-3 MolWeight:268 RetIndex:1886
CompName:7-Hexadecenoic acid, methyl ester, (Z)- $$ Methyl (7E)-7-hexadecenoate = $$



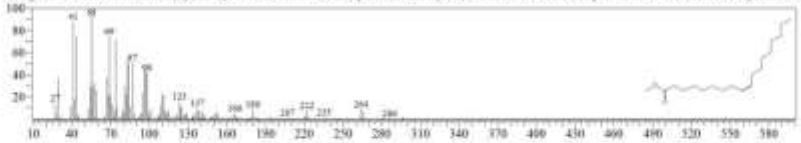
Hit#:3 Entry:25928 Library:NIST11s.lib
SI:93 Formula:C19H36O2 CAS:112-62-9 MolWeight:296 RetIndex:2085
CompName:9-Octadecenoic acid (Z)-, methyl ester $$ Oleic acid, methyl ester $$ Emery oleic acid ester 2301 $$ Methyl cis-9-octadecenoate $$ Methyl oleate $



Hit#:4 Entry:126499 Library:NIST11.lib
SI:92 Formula:C20H38O2 CAS:0-00-0 MolWeight:310 RetIndex:2183
CompName:cis-10-Nonadecenoic acid, methyl ester

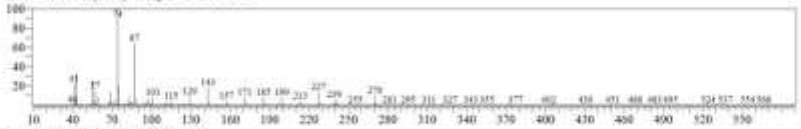


Hit#:5 Entry:25927 Library:NIST11s.lib
SI:92 Formula:C19H36O2 CAS:112-62-9 MolWeight:296 RetIndex:2085
CompName:9-Octadecenoic acid (Z)-, methyl ester $$ Oleic acid, methyl ester $$ Emery oleic acid ester 2301 $$ Methyl cis-9-octadecenoate $$ Methyl oleate $

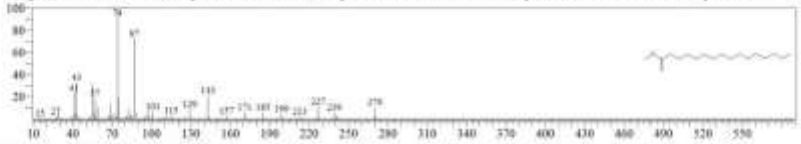


.

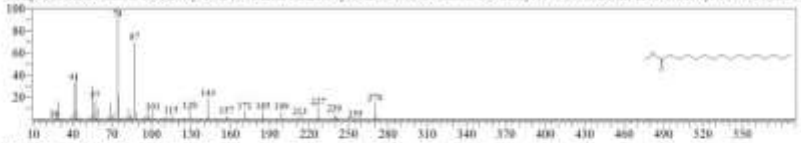
<< Target >>
Line#:3 R.Time:16.425(Scan#:2686) MassPeaks:430
RawMode:Single 16.425(2686) BasePeak:74.05(1673907)
BG Mode:16.380(2677) Group 1 - Event 1 Scan



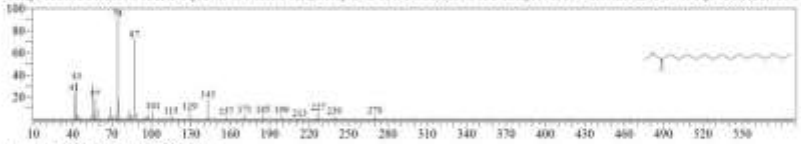
Hit#:1 Entry:95188 Library:NIST11.lib
SI:97 Formula:C17H34O2 CAS:112-39-0 MolWeight:270 RetIndex:1878
CompName:Hexadecanoic acid, methyl ester $$ Palmitic acid, methyl ester $$ n-Hexadecanoic acid methyl ester $$ Metholene 2216 $$ Methyl hexadecanoate $



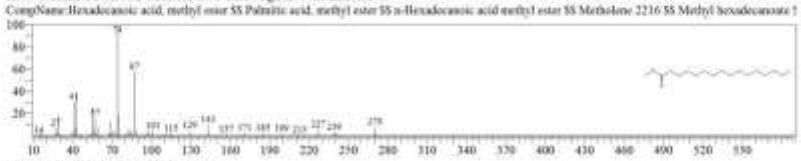
Hit#:2 Entry:24298 Library:NIST11s.lib
SI:95 Formula:C17H34O2 CAS:112-39-0 MolWeight:270 RetIndex:1878
CompName:Hexadecanoic acid, methyl ester $$ Palmitic acid, methyl ester $$ n-Hexadecanoic acid methyl ester $$ Metholene 2216 $$ Methyl hexadecanoate $



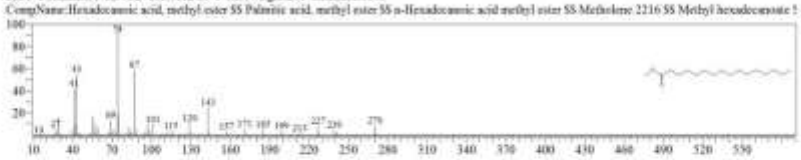
Hit#:3 Entry:24299 Library:NIST11s.lib
SI:94 Formula:C17H34O2 CAS:112-39-0 MolWeight:270 RetIndex:1878
CompName:Hexadecanoic acid, methyl ester $$ Palmitic acid, methyl ester $$ n-Hexadecanoic acid methyl ester $$ Metholene 2216 $$ Methyl hexadecanoate $



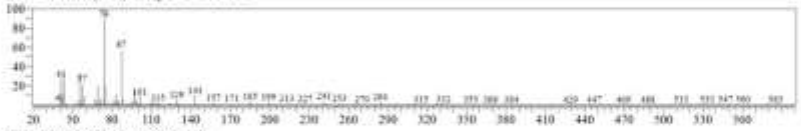
Hit#:4 Entry:24296 Library:NIST11s.lib
SI:93 Formula:C17H34O2 CAS:112-39-0 MolWeight:270 RetIndex:1878
CompName:Hexadecanoic acid, methyl ester $$ Palmitic acid, methyl ester $$ n-Hexadecanoic acid methyl ester $$ Metholene 2216 $$ Methyl hexadecanoate $



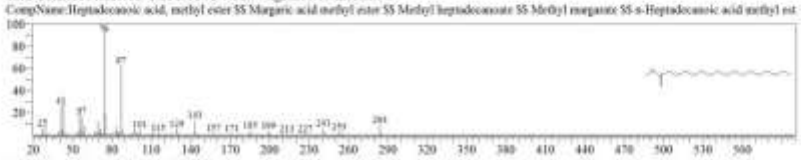
Hit#:5 Entry:24297 Library:NIST11s.lib
SI:93 Formula:C17H34O2 CAS:112-39-0 MolWeight:270 RetIndex:1878
CompName:Hexadecanoic acid, methyl ester $$ Palmitic acid, methyl ester $$ n-Hexadecanoic acid methyl ester $$ Metholene 2216 $$ Methyl hexadecanoate $



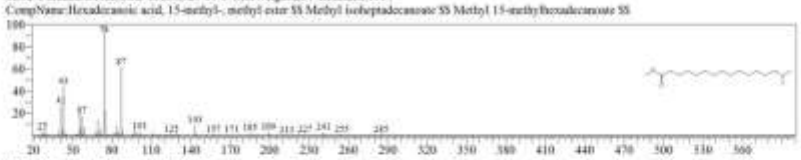
<< Target >>
Line#:4 R.Time:17.455(Scan#:2892) MassPeaks:389
RawMode:Single 17.455(2892) BasePeak:74.0(17809)
BG Mode:17.415(2884) Group 1 - Event 1 Scan



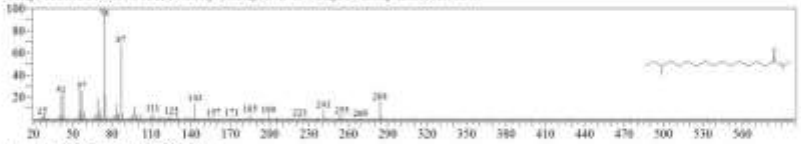
Hit#:1 Entry:25172 Library:NIST11s.lib
SI:92 Formula:C18H36O2 CAS:1731-92-6 MolWeight:284 RetIndex:1978
CompName:Heptadecanoic acid, methyl ester $$ Margaric acid methyl ester $$ Methyl heptadecanoate $$ Methyl margarate $$ n-Heptadecanoic acid methyl est



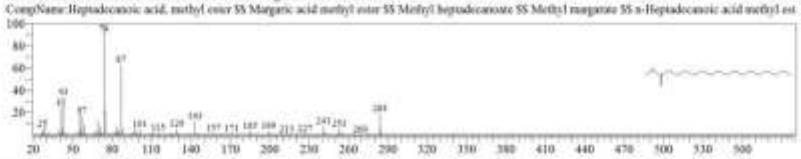
Hit#:2 Entry:106180 Library:NIST11.lib
SI:92 Formula:C18H36O2 CAS:6929-04-0 MolWeight:284 RetIndex:1914
CompName:Hexadecanoic acid, 15-methyl-, methyl ester $$ Methyl isoheptadecanoate $$ Methyl 15-methylhexadecanoate $$



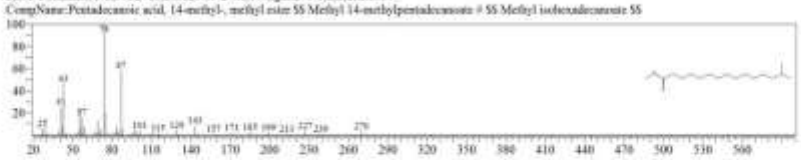
Hit#:3 Entry:106179 Library:NIST11.lib
SI:92 Formula:C18H36O2 CAS:2490-49-5 MolWeight:284 RetIndex:1914
CompName:Hexadecanoic acid, 14-methyl-, methyl ester $$ Methyl 14-methylhexadecanoate $$



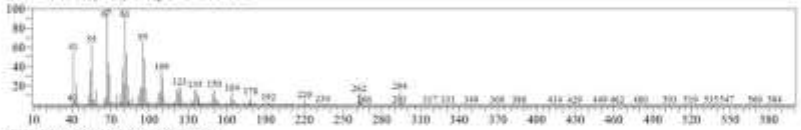
Hit#:4 Entry:25171 Library:NIST11s.lib
SI:91 Formula:C18H36O2 CAS:1731-92-6 MolWeight:284 RetIndex:1978
CompName:Heptadecanoic acid, methyl ester $$ Margaric acid methyl ester $$ Methyl heptadecanoate $$ Methyl margarate $$ n-Heptadecanoic acid methyl est



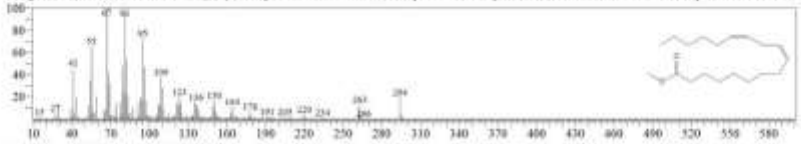
Hit#:5 Entry:95189 Library:NIST11.lib
SI:91 Formula:C17H34O2 CAS:5129-60-2 MolWeight:270 RetIndex:1814
CompName:Pentadecanoic acid, 14-methyl-, methyl ester $$ Methyl 14-methylpentadecanoate # $$ Methyl isohexadecanoate $$



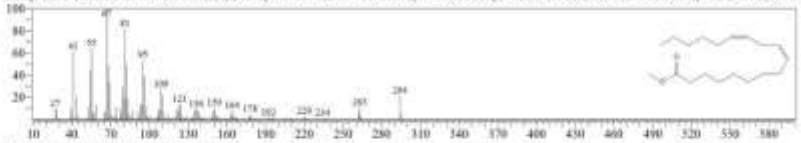
<< Target >>
Line#:3 R.Time:18.200(Scan#:3041) MassPeaks:423
RawMode:Single 18.200(3041) BasePeak:67.05(1375440)
BG Mode:18.335(3028) Group 1 - Event 1 Scan



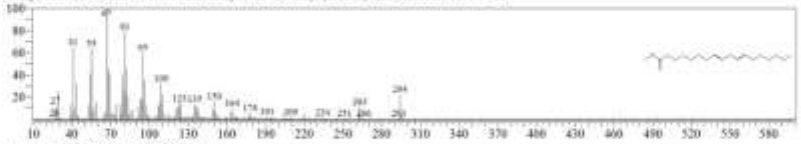
Hit#:1 Entry:113931 Library:NIST11.lib
SI:97 Formula:C19H34O2 CAS:112-63-0 MolWeight:294 RetIndex:2093
CompName:9,12-Octadecadienoic acid (Z,Z)-, methyl ester $$ Linoleic acid, methyl ester $$ Methyl cis,cis-9,12-octadecadienoate $$ Methyl linoleate $$ Meth



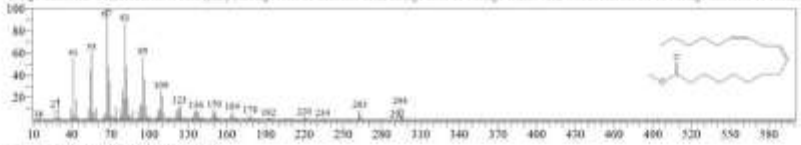
Hit#:2 Entry:25809 Library:NIST11s.lib
SI:95 Formula:C19H34O2 CAS:112-63-0 MolWeight:294 RetIndex:2093
CompName:9,12-Octadecadienoic acid (Z,Z)-, methyl ester $$ Linoleic acid, methyl ester $$ Methyl cis,cis-9,12-octadecadienoate $$ Methyl linoleate $$ Meth



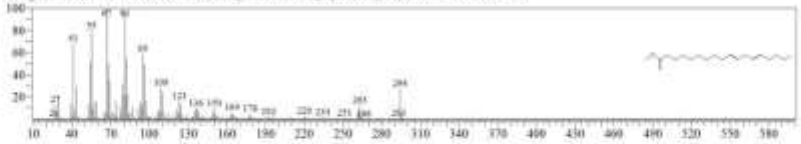
Hit#:3 Entry:113940 Library:NIST11.lib
SI:95 Formula:C19H34O2 CAS:56599-58-7 MolWeight:294 RetIndex:2093
CompName:8,11-Octadecadienoic acid, methyl ester $$ Methyl (8E,11E)-8,11-octadecadienoate # $$



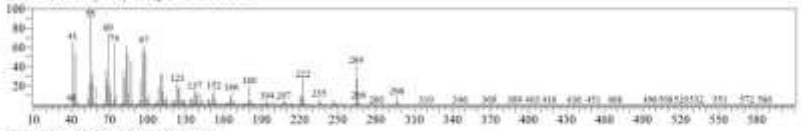
Hit#:4 Entry:25810 Library:NIST11s.lib
SI:94 Formula:C19H34O2 CAS:112-63-0 MolWeight:294 RetIndex:2093
CompName:9,12-Octadecadienoic acid (Z,Z)-, methyl ester $$ Linoleic acid, methyl ester $$ Methyl cis,cis-9,12-octadecadienoate $$ Methyl linoleate $$ Meth



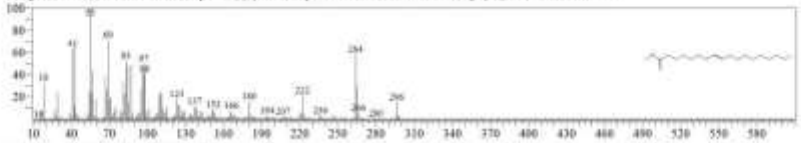
Hit#:5 Entry:113946 Library:NIST11.lib
SI:94 Formula:C19H34O2 CAS:56554-62-2 MolWeight:294 RetIndex:2093
CompName:10,13-Octadecadienoic acid, methyl ester $$ Methyl (10E,13E)-10,13-octadecadienoate # $$



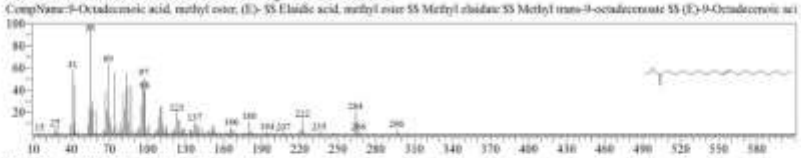
<< Target >>
Line#:6 R.Time:18.255(Scan#:3048) MassPeaks:389
RawMode:Single 18.255(3048) BasePeak:55.05(309582)
BG Mode:18.255(3052) Group 1 - Event 1 Scan



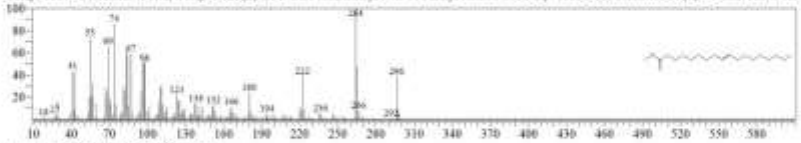
Hit#:1 Entry:115416 Library:NIST11.lib
SI:93 Formula:C19H36O2 CAS:20528-50-7 MolWeight:296 RetIndex:2085
CompName:9-Octadecenoic acid, methyl ester, (E)- $$ Methyl trans-8-octadecenoate $$ Methyl (8E)-8-octadecenoate # $$



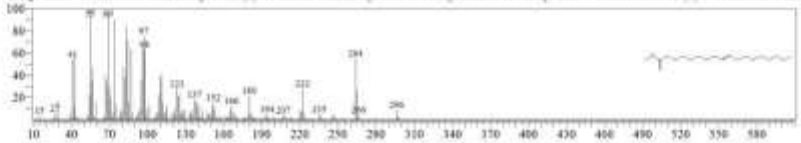
Hit#:2 Entry:25930 Library:NIST11s.lib
SI:93 Formula:C19H36O2 CAS:1937-62-8 MolWeight:296 RetIndex:2085
CompName:9-Octadecenoic acid, methyl ester, (E)- $$ Elaidic acid, methyl ester $$ Methyl elaidate $$ Methyl trans-9-octadecenoate $$ (E)-9-Octadecenoic ac



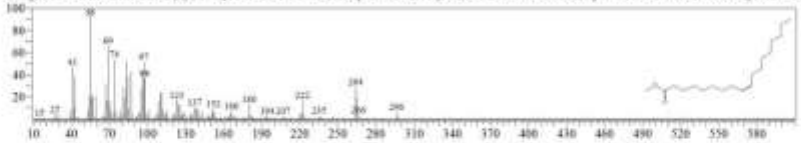
Hit#:3 Entry:25935 Library:NIST11s.lib
SI:93 Formula:C19H36O2 CAS:1937-62-8 MolWeight:296 RetIndex:2085
CompName:9-Octadecenoic acid, methyl ester, (E)- $$ Elaidic acid, methyl ester $$ Methyl elaidate $$ Methyl trans-9-octadecenoate $$ (E)-9-Octadecenoic ac

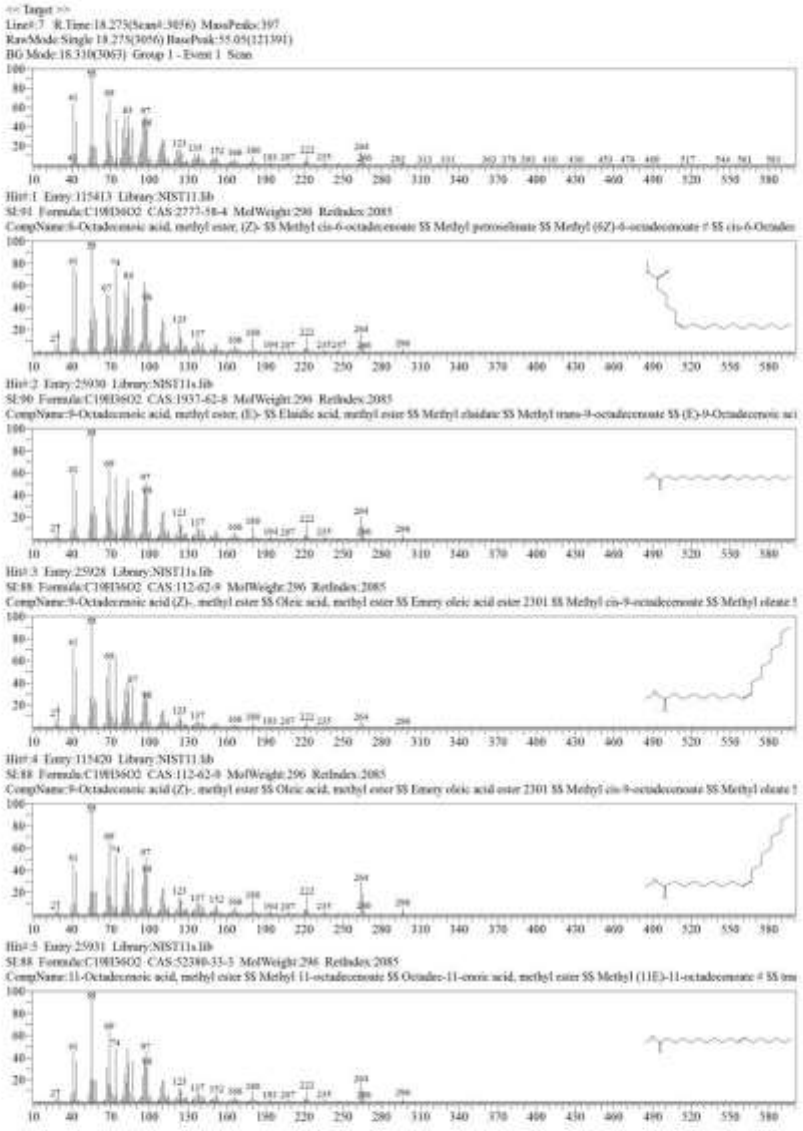


Hit#:4 Entry:115419 Library:NIST11.lib
SI:92 Formula:C19H36O2 CAS:1937-62-8 MolWeight:296 RetIndex:2085
CompName:9-Octadecenoic acid, methyl ester, (E)- $$ Elaidic acid, methyl ester $$ Methyl elaidate $$ Methyl trans-9-octadecenoate $$ (E)-9-Octadecenoic ac

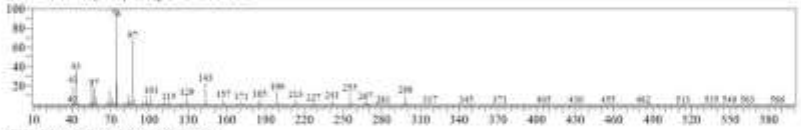


Hit#:5 Entry:115420 Library:NIST11.lib
SI:92 Formula:C19H36O2 CAS:112-62-9 MolWeight:296 RetIndex:2085
CompName:9-Octadecenoic acid (Z)-, methyl ester $$ Oleic acid, methyl ester $$ Emery oleic acid ester 2301 $$ Methyl cis-9-octadecenoate $$ Methyl oleate $

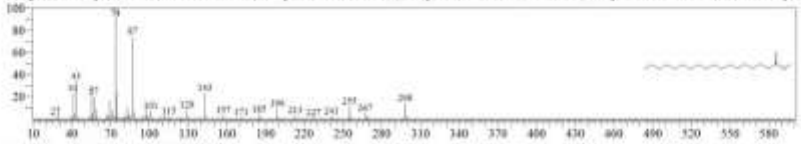



>> Target >>
Line#:7 R.Time:18.275(Scan#:3056) MassPeaks:397
RawMode:Single 18.275(3056) BasePeak:55.05(121391)
BG Mode:18.310(3063) Group 1 - Event 1 Scan
Hit#:1 Entry:115813 Library:NIST11.lib
SI:91 Formula:C19H36O2 CAS:2777-58-4 MolWeight:296 RetIndex:2085
CompName:9-Octadecenoic acid, methyl ester, (Z)- $$ Methyl cis-6-octadecenoate $$ Methyl petroselinate $$ Methyl (6Z)-6-octadecenoate # $$ cis-6-Octadec
Hit#:2 Entry:25930 Library:NIST11s.lib
SI:90 Formula:C19H36O2 CAS:1937-62-8 MolWeight:296 RetIndex:2085
CompName:9-Octadecenoic acid, methyl ester, (E)- $$ Elaidic acid, methyl ester $$ Methyl elaidate $$ Methyl trans-9-octadecenoate $$ (E)-9-Octadecenoic ac
Hit#:3 Entry:25928 Library:NIST11s.lib
SI:88 Formula:C19H36O2 CAS:112-62-9 MolWeight:296 RetIndex:2085
CompName:9-Octadecenoic acid (Z)-, methyl ester $$ Oleic acid, methyl ester $$ Emery oleic acid ester 2301 $$ Methyl cis-9-octadecenoate $$ Methyl oleate $
Hit#:4 Entry:115420 Library:NIST11.lib
SI:88 Formula:C19H36O2 CAS:112-62-9 MolWeight:296 RetIndex:2085
CompName:9-Octadecenoic acid (Z)-, methyl ester $$ Oleic acid, methyl ester $$ Emery oleic acid ester 2301 $$ Methyl cis-9-octadecenoate $$ Methyl oleate $
Hit#:5 Entry:25931 Library:NIST11s.lib
SI:88 Formula:C19H36O2 CAS:52380-33-3 MolWeight:296 RetIndex:2085
CompName:11-Octadecenoic acid, methyl ester $$ Methyl 11-octadecenoate $$ Octadec-11-enoic acid, methyl ester $$ Methyl (11E)-11-octadecenoate # $$ tra

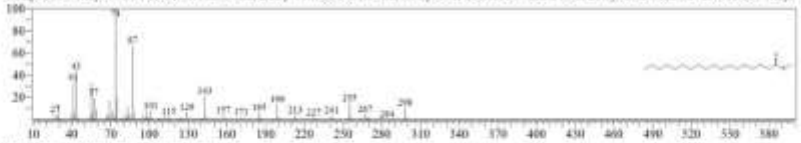
<< Target >>
Line#:8 R.Time:18.445(Scan#:3090) MassPeaks:371
RawMode:Single 18.445(3090) BasePeak:74.05(718629)
BG Mode:18.400(3081) Group 1 - Event 1 Scan



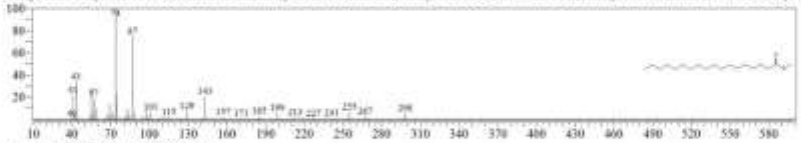
Hit#:1 Entry:117152 Library:NIST11.lib
SI:96 Formula:C19H38O2 CAS:112-61-8 MolWeight:298 RetIndex:2077
CompName:Methyl stearate $$ Octadecanoic acid, methyl ester $$ Stearic acid, methyl ester $$ n-Octadecanoic acid, methyl ester $$ Kemester 9718 $$ Methyl



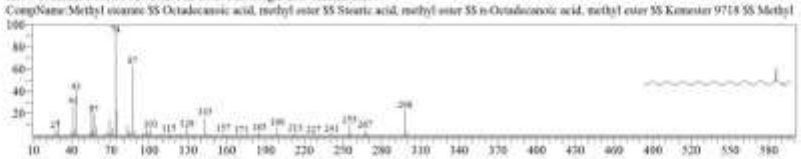
Hit#:2 Entry:26031 Library:NIST11s.lib
SI:96 Formula:C19H38O2 CAS:112-61-8 MolWeight:298 RetIndex:2077
CompName:Methyl stearate $$ Octadecanoic acid, methyl ester $$ Stearic acid, methyl ester $$ n-Octadecanoic acid, methyl ester $$ Kemester 9718 $$ Methyl



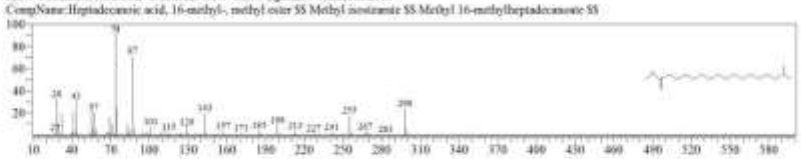
Hit#:3 Entry:26033 Library:NIST11s.lib
SI:95 Formula:C19H38O2 CAS:112-61-8 MolWeight:298 RetIndex:2077
CompName:Methyl stearate $$ Octadecanoic acid, methyl ester $$ Stearic acid, methyl ester $$ n-Octadecanoic acid, methyl ester $$ Kemester 9718 $$ Methyl

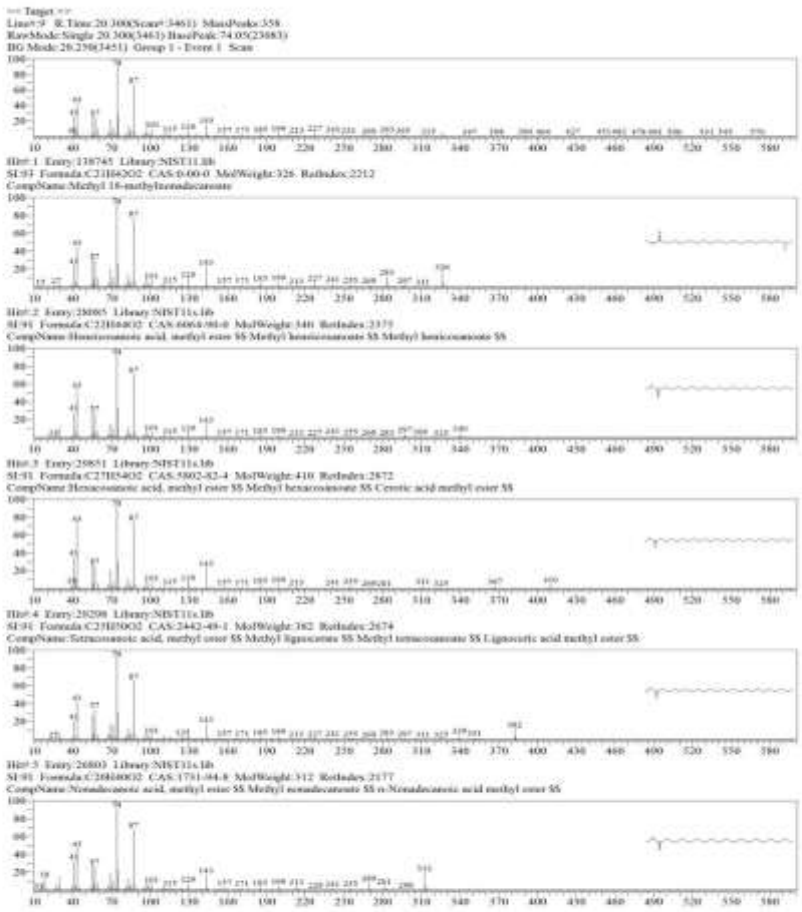


Hit#:4 Entry:26032 Library:NIST11s.lib
SI:94 Formula:C19H38O2 CAS:112-61-8 MolWeight:298 RetIndex:2077
CompName:Methyl stearate $$ Octadecanoic acid, methyl ester $$ Stearic acid, methyl ester $$ n-Octadecanoic acid, methyl ester $$ Kemester 9718 $$ Methyl



Hit#:5 Entry:26029 Library:NIST11s.lib
SI:94 Formula:C19H38O2 CAS:5129-61-3 MolWeight:298 RetIndex:2013
CompName:Heptadecanoic acid, 16-methyl-, methyl ester $$ Methyl isostearate $$ Methyl 16-methylheptadecanoate $$

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Line#:9 R.Time:20.300(Scan#:34611) MassPeaks:358
RawMode:Single 20.300(34611) BasePeak:74.05(230883)
BG Mode:20.250(34551) Group 1 - Event 1 Scan
Hit#:1 Entry:138745 Library:NIST11.lib
SI:93 Formula:C21H42O2 CAS:0-00-0 MolWeight:326 RetIndex:2212
CompName:Methyl 18-methyleicosanoate
Hit#:2 Entry:28065 Library:NIST11s.lib
SI:91 Formula:C22H44O2 CAS:6064-90-0 MolWeight:340 RetIndex:2375
CompName:Henicosanoic acid, methyl ester $$ Methyl henicosanoate $$ Methyl henicosanoate $$
Hit#:3 Entry:29851 Library:NIST11s.lib
SI:91 Formula:C27H54O2 CAS:5802-82-4 MolWeight:410 RetIndex:2872
CompName:Hexacosanoic acid, methyl ester $$ Methyl hexacosanoate $$ Cerotic acid methyl ester $$
Hit#:4 Entry:28298 Library:NIST11s.lib
SI:91 Formula:C25H50O2 CAS:2442-49-1 MolWeight:382 RetIndex:2674
CompName:Tetracosanoic acid, methyl ester $$ Methyl lignocerate $$ Methyl tetracosanoate $$ Lignoceric acid methyl ester $$
Hit#:5 Entry:26803 Library:NIST11s.lib
SI:91 Formula:C20H40O2 CAS:1731-94-8 MolWeight:312 RetIndex:2177
CompName:Nonadecanoic acid, methyl ester $$ Methyl nonadecanoate $$ n-Nonadecanoic acid methyl ester $$