بسم الله الرحمن الرحيم

قال تعالى :

اقْرَأْ بِاسْمِ رَبِّكَ الّذِي خَلَقَ (1)خَلَقَ الإنْسَانَ مِنْ عَلَقٍ (2) اقْرَأْ وَرَبُّكَ الأكْرَمُ (3)الّذِي عَلّمَ بِالْقَلَمِ (4) عَلّمَ الإنْسَانَ مَا لَمْ يَعْلَمْ (5)

صدق الله العظيم

سورة العلق الآيات من (1-5)

Dedication

To

Father and Mother
My Husband
Brother and Sisters

Acknowledgment

First of all my sincere thanks to Almighty**Allah**For helping me to complete this work.

It is a pleasure to record my deep appreciation, and thanks to prof. Mohammed abdelkarim Mohammed for his wise guidance, which helped me to present this project in this shape. I am very grateful to the staff of the department of chemistry. Sudan University of science and technology for all facilities,Thanks to my family for their continual support.

Abstract

The fixed oil of Rumexacetosa was extracted by maceration. GC-MS analysis was conducted. Major constituents are :
9-octadecanoic acid methyl Ester (38.41%) , Ethyl oleate (21.16%) , Hexadecanoic 1,2,3-propane-triyl ester (6.41%) , 9,12-octadecanoic acid methyl ester (4.28%).
The oil was assessed for antimicrobial potency via cup plate agar diffusion bioassay against a panel of bacterial isolates. The oil showed significant activity against *Pseudomonas aeruginosa*and good activity against *Escherichia coli.* However it was partially active against *staphylococcusaureus* and fungus *candida albicans.*

المستخلص

استخلص الزيت الثابت لبذور الحميض بواسطه تقنية الكروموتوغرافية الغازية ـ طيف الكتله حيث اتضح أن الزيت يحتوي علي 14مكونا والمكونات الرئيسيه هي :

9-octadecanoic acid methyl Ester (38.41%) , Ethyl oleate (21.16%) , Hexadecanoic 1,2,3-propane-triyl ester (6.41%) , 9,12-octadecanoic acid methyl ester (4.28%).

ثماخضع الزيت لاختبارات بيولوجية كمضاد للميكروبات حيث استخدمت خمسه انواع من البكتريا القياسيه وقد ابدى الزيت فعاليه عاليه ضد الفطر

*Pseudomonas aeruginosa*and*Escherichia coli.*

وفعاليه ضعيفه ضد

staphylococcusaureus and fungus *candida albicans.*

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1-Introduction

1.1-Essential oil (Eos)

An essential oil is a concentrated hydrophobic liquid containing volatile aromatic compound from plants. Essential oils are generally extracted by distillation. Steam distillation is often used . Other processes include expression or solvent extraction. They are used in perfumes, cosmetics, soaps and other products, for flavoring food and drink, and for adding scent to incense and household cleaning products. [1 -2-3]

1.1.1-Histortry of essential oils

Throughout history, the essential oils of plants were used in many cultures for their medicinal and therapeutic benefits. 4-5Medical applications proposed by those who sell medicinal oils range from skin treatments to remedies for cancer and often are based solely on historical accounts of use of essential oils for these purposes. Claims for the efficacy of medical treatments, and treatment of cancers in particular, are now subject to regulation in most cou1ntries. As the use of essential oils has declined in evidence-based medicine, one must consult older textbooks for much information on their use. Modern works are less inclined to generalize, rather than refer to “essential oils” as a class at all, they prefer to prefer to discuss specific compounds, such as methyl salicylate , rather than “oil of wintergreen”. Interest in essential oils has

revived in recent decades with the popularity of aromatherapy, a branch of alternative medicine that claims that essential oil and other aromatic compounds have curative effects. Oils are volatilized or diluted in carrier oil and used in massage, diffused in the air by nebulizer, heated over a candle flame, or burned as incense. The earliest recorded mention of the techniques and methods used to produce essential oils is believed to be that of Ibn al-Baitar(1188-1248), an Andalusian physician, pharmacist and chemist [6].

1.1.2-Chemicals constituents of essential oils

In general, essential oils can be subdivided into two distinct groups of chemical constituents:

i) Volatile fraction

Hydrocarbons and oxygenated derivatives may constitute up to 90-95% of essential oil weight. The hydrocarbons are the molecules constituted of H and C atom arranged in chains. These hydrocarbons may be acyclic, alicylic (monocyclic,bicyclic, or tricyclic), or aromatic. Basic hydrocarbon found in plants are 90% terpenes made from isoprene unites (several 5 carbon basic unites, C5). A combination of 2 isoprene unites is called a “terpene unit”. Essential oils consist of mainly (mono-terpenes (have a structure of 10 carbon atoms and at least one double bond. The 10 carbon atoms are derived from two isoprene units.) and sesquiterpene (consisting of 15 carbon atoms) the di terpenes (C20),

triterpenes (C30), and tetraterpenes (C40) exist in essential oils at low concentrations. 5Terpenoids (terpenes containing oxygen) is also found in essential oils . 5% of oxygenated compound are the combination of C, H, and O, and there are variety of compounds found in essential oils. Oxygenated derivatives are: aliphatic aldehydes, alcohols, ketones, phenols and esters.

ii) Non volatile residue or fixed oils

Non volatile comprises 1-10% of the oil, containing hydrocarbons, fatty acid, sterols, carotenoids, waxes, and flavonoids. [7]

31.1.3-Properties of essential oils

In plants the amount of essential oils is different and this determines the price of essential oil. Apart from aromatic compounds, indigenous pigments contribute to varying color of essential oil 8. This can affect the applications as the ingredient in some particular foods.

Essential oils are good source of several bio active compounds which possess antioxidant and antimicrobial activities, thereby serving as natural additives in food and food product. It can be used as active compounds in packaging materials, in which the properties of those materials, particularly water vapor barrier property associated with hydrophobicity in nature of essential oils can be improved [9]

1.1.4-Pharmacological properties of essential oils

Essential oils have antiseptic properties and are active against a wide range of bacteria. Moreover, they are also known to be active against fungi and yeast (Candida). The most common sources of essential oils used as antiseptics are: cinnamon, thymol; clover; eucalyptus; citral, geraniol; linalool. However, thymol and linalool are much more potent than phenol [10] .

When used externally, essential oils like (L essence de terebenthine) increase microcirculation and provide a slight local anesthetic action. Till now, essential oils are used in a number of ointments, cream and gels, whereby they are known to be very effective in relieving sprains and other articular pains. Oral administration of essential oils like eucalyptus or pin oils, stimulate ciliated epithelial cells to secrete mucus. On the renal system, these are known to increase vasodilation and in consequence bring about a diuretic effect.

Essential oils from the Umbellifereae family and especially Mentha species and Verbena are reputed to decrease or eliminate gastrointestinal spasms. These essential oils increase secretion of gastric juice. In other cases, they are known to be effective against insomnia.

1.1.5- Method of extracting essential oils

The way in which oils are extracted from **4**|P a g e plants is important because some processes use solvents that can destroy the therapeutic

properties.

The value of the newer processing methods depends greatly on the experience of the distiller, as well as the intended application of the final product. Some of the extraction methods are given below:

1.1.5.1-By distillation

Most oils are distilled in a single process. One exception is Ylang-Ylang (Canangodorata), which takes 22 hours to complete through a fractional distillation.

The re condensed water is referred to as a hydrosol, hydrolat, herbal distillate or plant water essence, which may be sold as another fragrant product.

Popular hydrosols include rose water, lavender water, lemon balm, clary sage and orange blossom water. The use of herbal distillates in cosmetics is increasing. Some plant hydrosols have unpleasant smells and are therefore not sold.

1.1.5.2- By pressing

Most citrus peel oils are expressed mechanically or cold-pressed (similar to olive oil extraction). Due to the relatively large quantities of oil in citrus peel and low cost to grow and harvest the raw materials, citrus-fruit oils are cheaper than most other essential oils. Lemon or sweet orange oils that are obtained as byproducts of the citrus industry are even cheaper. Before the discovery of distillation, all essential oils were

extracted by pressing.

1.1.5.3-By solvent extraction

Most flowers contain to be extracted by pressing their chemical components are too delicate and easily denatured by the high heat used in steam distillation. Instead, a solvent such as hexane or supercritical carbon dioxide is used to extract the oils. Extracts from hexane and other hydrophobic solvent are called concretes, which are a mixture of essential oil, waxes, resins, and other lipophilic (oil soluble) plant material “Although highly fragment, concretes contain large quantities for non fragrant waxes and resins. Often, another solvent, such as ethyl alcohol, which is more polar in nature, is used to extract the fragrant oil from the concrete. The alcohol is removed by evaporation, leaving behind the absolute [11].

1.1.5.4-By Maceration

In this process, the whole or coarsely powderd plant material is placed in a stopper container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the mare (the damp solid material) is pressed, and the combined liquid are clarified by filteration or decantation after standing [12].

1.1.6- Uses of essential oils

Essential oils have been used for thousands of years in various cultures

for medicinal and health purpose. Essential oil uses range from aromatherapy, household cleaning product personal beauty care and nature medicine treatment. Essential oil benefits come from their antioxidant, antimicrobial and anti-inflammatory properties.

The amount of essential oil from different plants and this determines the price of essential oil. Apart from aromatic compound, indigenous pigment contribute to varying colors of essential oil. This can affect the applications as the ingredient in some particular foods.

The essential oils a good source of several bioactive compounds which possess antioxidant and antimicrobial activities, there by serving as natural additives in foods and food product. It can be used as active compounds in packing materials, in which the properties of those materials, particularly water vapor barrier property associated with hydrophobicity in nature of essential oils, can be improved [13].

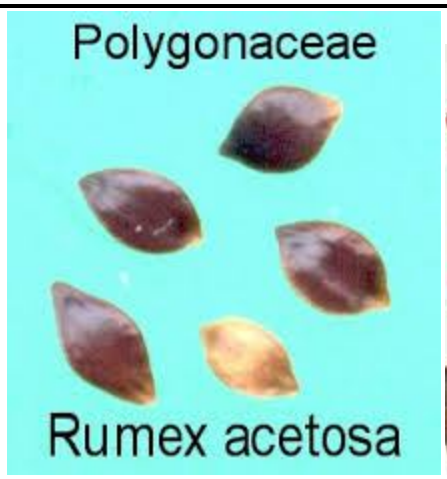
Essential oils are used in perfumes, cosmetics, soap and other product, for flavoring food and drink, and for adding scents to incense and household cleaning product and have been used medicinally in history. Medical application proposed by those who sell medicinal oils range from skin treatment to remedies for cancer and often are based solely on historical accounts of use of essential oils for these purpose. Claims for the efficacy of medical treatment, and treatment of cancers in particular, are now subject to regulation in most countries [14] .

1.2-Rumex oil

1.2.1-Scientific classification of Rumex

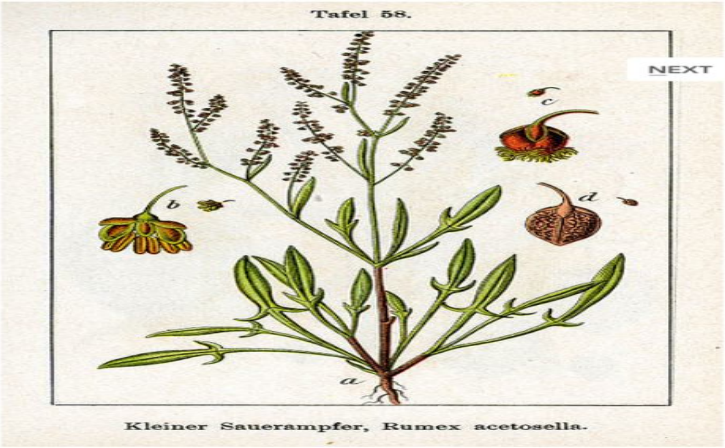
Kingdom:	Plantae
(Unranked):	Angiosperms
(Unranked):	Eudicots
(Unranked):	Core eudicots
Order:	Caryophyllales
Family;	Polygonaceae
Genus:	Rumex
Species:	R. acetosa

Binominal name
Rumexacetosa



Common sorrel or garden sorrel (Rumexacetosa), often simply called Sorrel, is a Perennial herb in the family Polygonaceae. It is a common plant in grassland habitats and cultivated as a garden herb or leaf vegetable (pot herb).

It is a common plant in grassland habitats and cultivated as a garden herb or leaf vegetable (pot herb) [15].



Sorrel is a slender herbaceous perennial plant about 60 centimeters (24 in) high, with roots that run deep into the ground, as well as juicy stems and edible, arrow-shaped (sagittate) leaves. The leaves, when consumed raw, have a sour taste. The lower leaves are 7 to 15 centimeters (2.8 to 5.9 in) in length with long petioles and membranous ocrea formed of fused, sheathing stipules. The upper ones are sessile, and frequently become crimson. It has whorled spikes of reddish-green flower, which bloom in early summer, becoming purplish. The species is dioeciously, with stamens and pistils on different plants [16].

1.2.3-Distribution

Rumexacetosa occurs in grassland habitats throughout Europe from the northern Mediterranean coast to the north of Scandinavia and in part of Central Asia. It occurs as an introduced species in part of north America.

1.2.4-Uses

Common sorrel has been cultivated for centuries. The leaves may be pureed in soup and sauces or added to salads; they have a flavor that is similar to kiwifruit or sour wild strawberries. The plants sharp taste is due to oxalic acid, which is mildly toxic [17].

1.2.5-Subspecies

Several subspecies have been named. Not all are cultivated:

.Rumexacetosassp.acetosa

.Rumexacetosa ssp. ambiguous

.Rumexacetosa ssp. arifolius

.Rumexacetosa ssp. hibernicus

.Rumexacetosa ssp. hirtulus

.Rumexacetosa ssp. vineal

Rumexacetosella is a species of sorrel bearing the common names

sheep's sorrel , red sorrel , sour weed , and field sorrel . The plant and its subspecies are common perennial weeds . It has green arrowhead-shaped leaves and red-tinted deeply ridged stems, and it sprouts from an aggressive rhizome . The flowers emerge from a tall, upright stem. Female flowers are maroon in color [18]

A common, invasive perennial weed, to most, with a spreading rootstock; arrowhead-shaped leaves; small reddish flowers throughout summer, produces lots of seed; native to Europe and Asia, common in North America.

The leaves can be used as thickeners in soups etc, they can also be dried for later use.

Sheep's sorrel is a detoxifying herb, the fresh juice of the leaves having a pronounced diuretic effect. Like other members of the genus, it is mildly laxative and holds out potential as a long term treatment for chronic disease, in particular that of the gastro-intestinal tract [19].

The plant is also part of a North American formula called essiac which is a popular treatment for cancer. Its effectiveness has never been reliably proven or disproven since controlled studies have not been carried out. The other herbs included in the formula are Arctiumlappa, Ulmusrubra and Rheum palmatum.

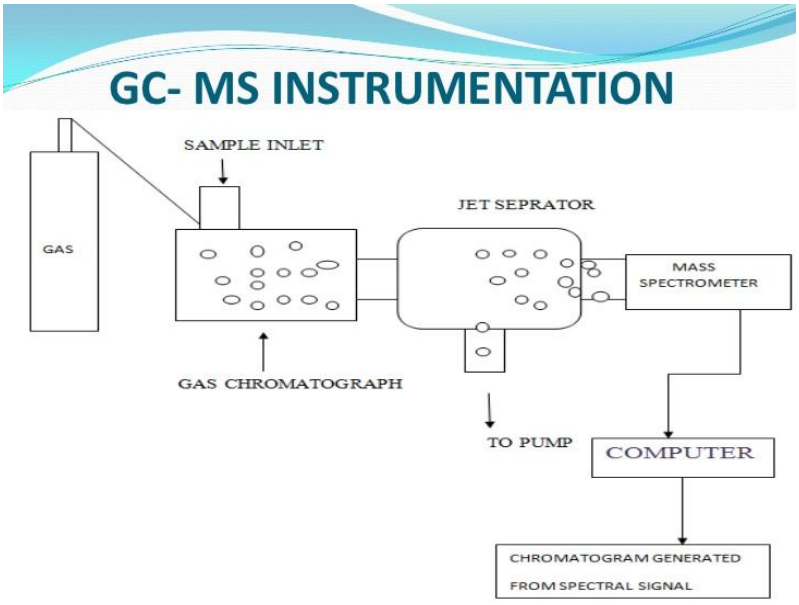
Take a large amount of Sorrel and saute in some butter till wilted and falling apart. Add enough chicken stock or water to cover the Sorrel Bring to a boil and let simmer a minute or two When cool add to a

blender a puree till smooth Add salt, pepper and heavy cream to taste Enjoy this soup hot or cold [20]

1.3-Gas chromatography mass spectrophotometer (GC-MS)

GC-MS is an analytical method that combines the features of gas-liquid and chromatography and mass spectrometry to identify different substances with in a test sample. Application of GC-MS includes drug detection fire investigation and identification of unknown samples [21]. GC-MS include drug detection fire investigation environmental analysis explosives investigation and identification of unknown samples-GC can also be used in air port security to detect substances in luggage on human being additionally it can identify trace elements in materials that were previously through to have disintegrated beyond identification GC-MS has been widely heralded as a gold a standard forensic substance identification because it is used to perform a specific test. A specific test positively identifies that actual presence of a particular substance in a given sample a non specific test merely indicates that a substance falls into a category of substance. Although a non specific test could statistically suggest the identification of the substances this could lead. To false positive identification [22]. The gas chromatograph utilizes capillary column which depend on the columns dimensions (length diameter film thickness) as well as the phase properties (e: g 5%phenyl poly siloxane) the difference in the chemical properties between

different molecules in a mixture and their relative affinity for the stationary phase [23].



1.3.1-Advantages of GC-MS

GC-MS has become a highly recommended tool for monitoring and tracking organic pollutants in the environment. The cost of GC/MS

equipment has decrease whereas the reliability has markedly increased. The determination of chloro-phenols in water and soils, polycyclic aromatic hydrocarbons (PAH), unleaded gasoline, dioxins, dibenzofurans, organo-chlorine pesticides, herbicides, phenols, halogenated pesticides, sulphur in air is very convenient to be screened by this technique. It can be used to screen the degradation products of lignin in bio-mass research, pesticides in spinach. Analysis of decacyclene, ovalene and even C60 degradation analysis of carbamazepine and its metabolites in treated sewage water and steroid can be done without derivatization[24, 25].

Food and beverages have several aromatic compounds existing naturally in native state or formed while processing. GC-MS is also used to detect and measure contaminant, spoilage and adulteration of food, oil, butter, ghee that could be harmful and should be controlled and checked as regulated by governmental agencies. It is used in the analysis of piperine, spearmint oil, essential oil, fragrance reference standards, perfumes, chiral compounds in essential oils, fragrance, menthol, allergens, olive oil, lemon oil, peppermint oil, straw berry syrup, butter triglycerides residual pesticides in food and wine [26, 27].

In criminal cases GC-MS can analyze the particles from suspect to correlate his involvement in case. The analysis of fire debris using GC-MS can be established by American Society for Testing Materials (ASTM) standard for fire debris analysis. It is the key tool used in sport

anti-doping laboratories to test athlete's urine samples for prohibited performance enhancing drugs like anabolic steroids. It is also commonly used in forensic toxicology to find poisons, steroids in biological specimens of suspects or victims [28, 29].

In biology, GC-MS is exclusively used in bio-analysis of blood, urine for the presence of barbiturates, narcotics, alcohol, and residual solvent, drug like anesthetics, anticonvulsant, antihistamine, anti-epileptic drug, sedative hypnotics, narcotics, and food items. This technique could be used for detecting adulterations, fatty acid profiling in microbes, presence of free steroids, blood pollutants, metabolites in serum, organo-chlorinated pesticides in river water, soft drinks by head space, pesticides in sunflower oil etc[30].

GC-MS is also involved in security affairs. Explosive detection systems have become a part of all international airports. GC-MS is an essential part of chemical analysis unit. For enhancing capability in homeland security and public health preparedness, traditional GC-MS units with the transmission quadrupole mass spectrometer [31]

Several GC-MS have left earth for the astro- chemical studies. Two GC-MS instruments were taken to Mars planet by the Viking program. Scientist analyzed the atmosphere of Venus with GC-MS. The Huygens probe of the cassini-Huygens mission landed one GC-MS on Saturn”s largest moon, Titan.

The materials in the comet 67lChuryumov-Gerasimenko were analyzed

by the Rosetta mission with a chiral GC-MS in 2014.

Significantly enhanced molecular ions, major isomer and structurally significant mass spectral peaks, extended range of low volatility hydrocarbons that are amenable for analysis and unique isotope ratio information make GC-MS valuable for organic geochemical applications [32, 33]

1.3.2-Applications of GC-MS

GC-MS is widely used in pharmaceutical industries for analytical research and development, quality control, quality assurance, production, pilot plant departments for active pharmaceutical ingredients (API), bulk drug and formulations. It is used for process and method development, identification of impurities in API. It is an integral part of research associated with medicinal chemistry (synthesis and characterization of compounds), pharmacognosy, pharmaceutical process control, pharmaceutical biotechnology etc[34]

GC-MS provides a rare opportunity to perform the analysis of new compound for characterization and identification of synthesized or derivatized compound [35].

GC-MS is widely used in pure and applied sciences like chemistry, polymers, nanotechnology and biotechnology etc. It yields useful information that can be used in research publication.

GC-MS is used for the analysis of aromatic solvent, sulphur, impurities in polypropylene, sulphur in menthane, natural gases, 1,3 butadiene,

ethylene, gas oil, unleaded gasoline, polyethene, diesel oil, unleaded gasoline, polyethylene, diesel, modified biomass, grafted polymers etc[36] GC-MS has triggered a new arena of research and taken to new heights of impactful presentation and characterization of compound by its wide range of applications.

1.4-Antimicrobial

An antimicrobial is an agent that kills or microorganisms inhibit their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria and antifungal are used against fungi. They can also be classified according to their function. Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are called biostatic. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis.

The main classes of antimicrobial agents are disinfectants ("nonselective antimicrobials" such as bleach), which kill a wide range of microbes on non-living surfaces to prevent the spread of illness, antiseptics (which are applied to living tissue and help reduce infection during surgery), and antibiotics (which destroy microorganisms within the body). The term "antibiotic" originally described only those formulations derived

from living organisms but is now also applied to synthetic antimicrobials, such as the sulphonamides, or fluoroquinolones. The term also used to be restricted to antibacterial (and is often used as a synonym for them by medical professionals and in medical literature), but its context has broadened to include all antimicrobials. Antibacterial agents can be further subdivided into bactericidal agents, who kill bacteria, and bacteriostatic agents, which slow down or stall bacterial growth [37].

Use of substances with antimicrobial properties is known to have been common practice for at least 2000 years. Ancient Egyptians and ancient Greeks used specific molds and plant extracts to treat infection [38].More recently, microbiologists such as Louis Pasteur and Jules Francois Joubert observed antagonism between some bacteria and discussed the merits of controlling these interactions in medicine. [39]In 1928, Alexander Fleming became the first to discover a natural antimicrobial fungus known as *Penicilliumrubens*. The substance extracted from the fungus he named penicillin and in 1942 it was successfully used to treat a *Streptococcus* infection. [40] Penicillin also proved successful in the treatment of many other infectious diseases such as gonorrhea, strep throat and pneumonia, which were potentially fatal to patients until then.

Many antimicrobial agents exist, for use against a wide range of infectious diseases.

Aim of this study

This study was designed to:

-Extraction of fixed oil from seeds of the medicinally important Rumexacetosa.

-Analysis of extracted oil by GC-MS

-Screening the oil for anti microbial.

3-Results and Discussion

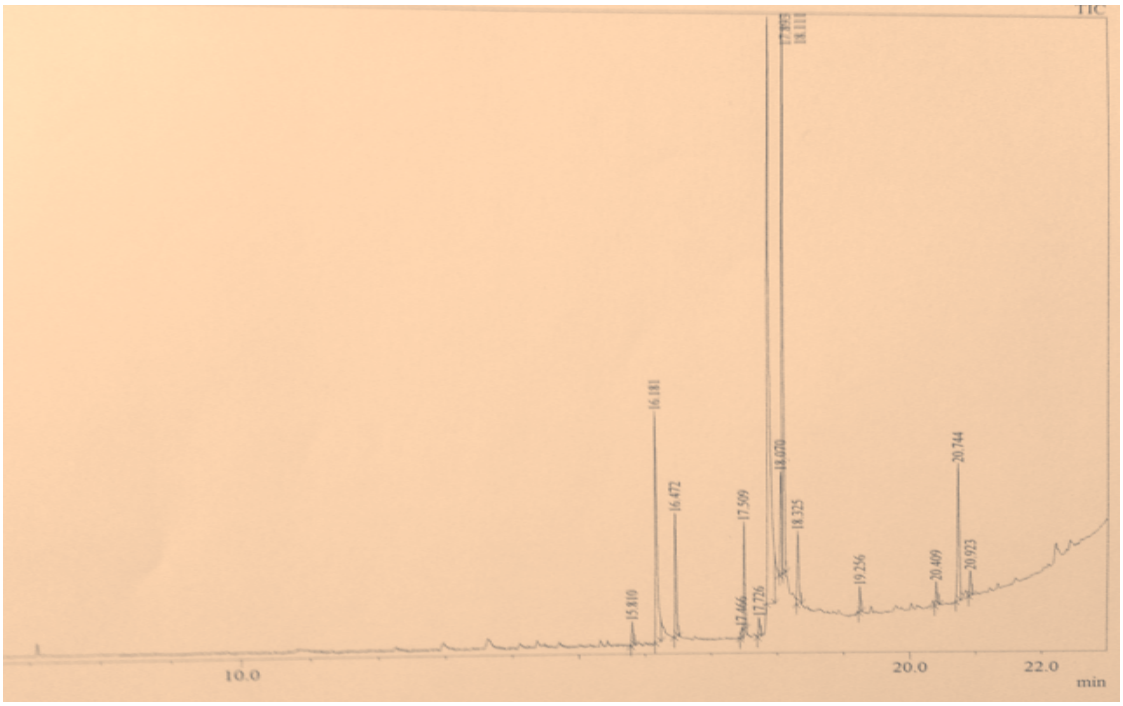
3.1-GC-MS analysis

Rumexacetosa oil was extracted by maceration from fruits and analyzed by GC-MS where 14 components were detected in total ions chromatogram(Fig.1).

Different constituents of the oil were quantified and identified by retention time and their characteristic fragmentation pattern. A tabulation of these components is given in Table (1).

Table 1:Constiruents of *Rumexacetosa* oil

R.Time	Area	Area%	Name
15.810	198353	0.98	Hexadecanoic acid, methyl ester
16.181	2448358	12.11	n-Hexadecanoic acid
16.472	1034482	5.11	Hexadecanoic acid, ethyl ester
17.466	98972	0.49	Methyl 9-cis,11-trans-octadecadienoate
17.509	794019	3.93	9-Octadecenoic acid (Z)-, methyl ester
17.726	171686	0.85	Methyl stearate
17.893	7769370	38.41	9-Octadecenoic acid, (E)-
18.070	866586	4.28	9,12-Octadecadienoic acid (Z,Z)-
18.111	4280204	21.16	Ethyl Oleate
18.325	642632	3.18	Octadecanoic acid, 17-methyl-, methyl est
19.256	198950	0.98	Heptadecanoic acid, heptadecyl ester
20.409	195894	0.97	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl
20.744	1297150	6.41	9-Octadecenoic acid, 1,2,3-propanetriyl es
20.923	228406	1.13	Tristearin
	20225062	100.00	



Total ions chromatogram Fig.1:

Major constituents of the oil are discussed below:

i) 9-Octadecanoic acid methyl ester(38.41%)

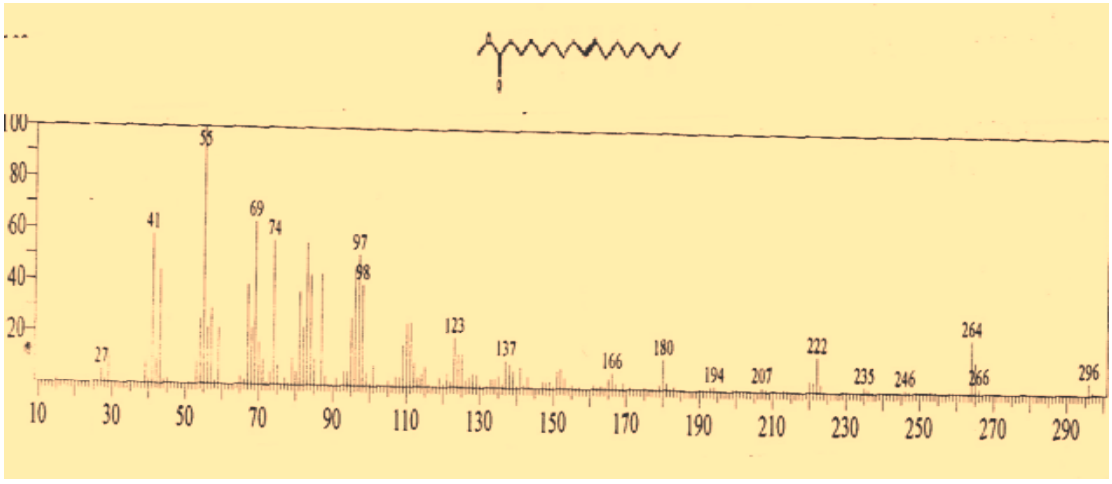


Fig.3.16: Mass spectrum of 9-octadecanoic acid methyl ester

The EI mass spectrum of 9-octadecanoic acid methyl ester is shown in Fig. 3.16.The peak at m/z 296 , which appeared at R.T.17.893 in total

ion chromatogram, corresponds M^+ $[C_{19}H_{36}O_2]^+$ .The peaks at m/z 254 correspond to loss of a ,methoxyl .

ii) Ethyl oleate (21.16%)

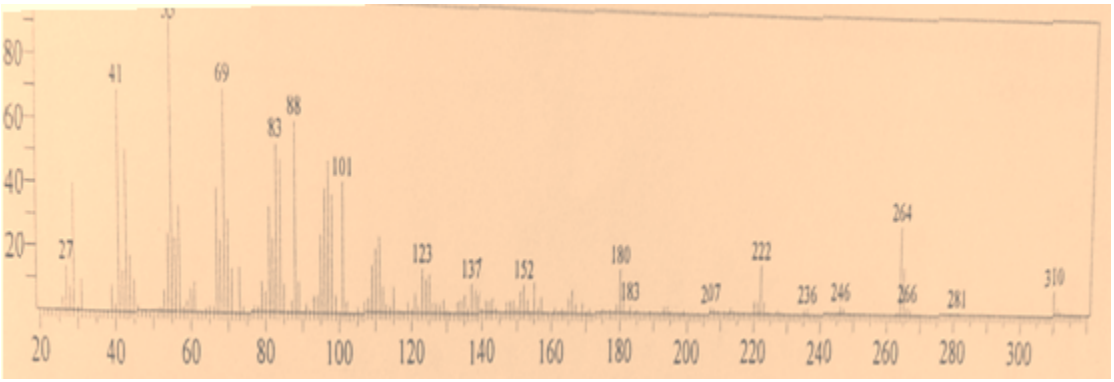


Fig. : Mass spectrum of ethyl oleate

The molecular ion , $M^+ (C_{20} H_{38} O_2)^+$, for ethyl oleate appeared at m/z310(RT,18.111).The signal at m/z281 is due to loss of a methoxyl function.

Hexadecanoic acid methyl ester(12.11%)

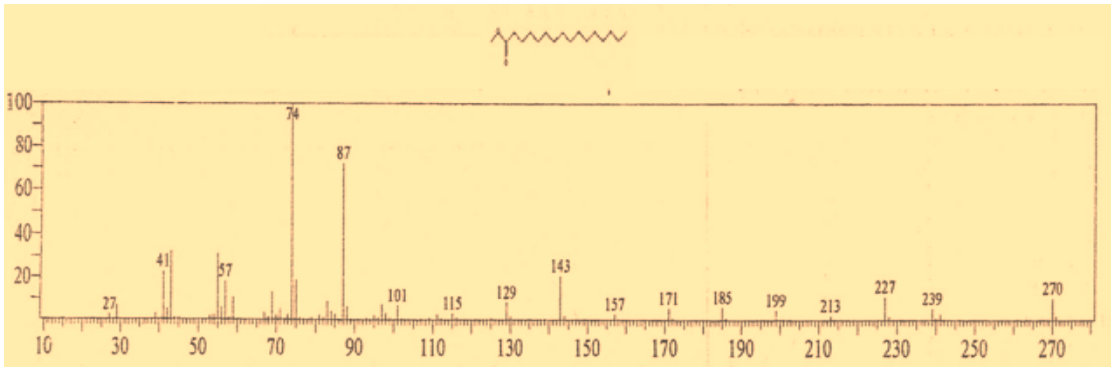
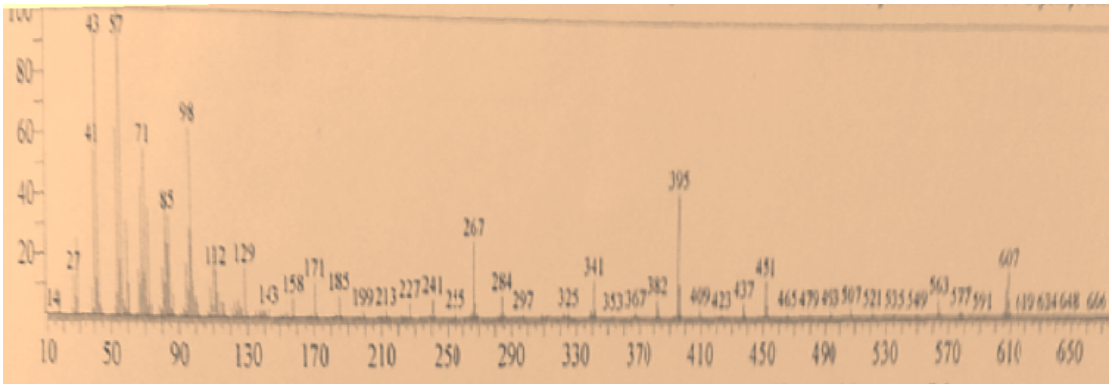


Fig. 3.5: Mass spectrum of hexadecanoic methyl ester

The EI mass spectrum of hexadecanoic acid methyl ester is shown in Fig. 3.5.The peak at m/z 270, which appeared at R.T. 16.181 in total ion chromatogram, corresponds to $M^+[C_{17}H_{34}O_2]^+$ .The peak at m/z239 corresponds to loss of a methoxyl function.

Octadecanoic 1,2,3-propane-triyl ester(6.41%)



Themass spectrum of octadecanoic 1,2,3-propane-triyl ester is displayed in Fig. . The peak at m/z890 corresponds $M^{+}(C_{57}H_{110}O_6)$.

9,12-Octadecanoic acid methyl ester(4.28%)

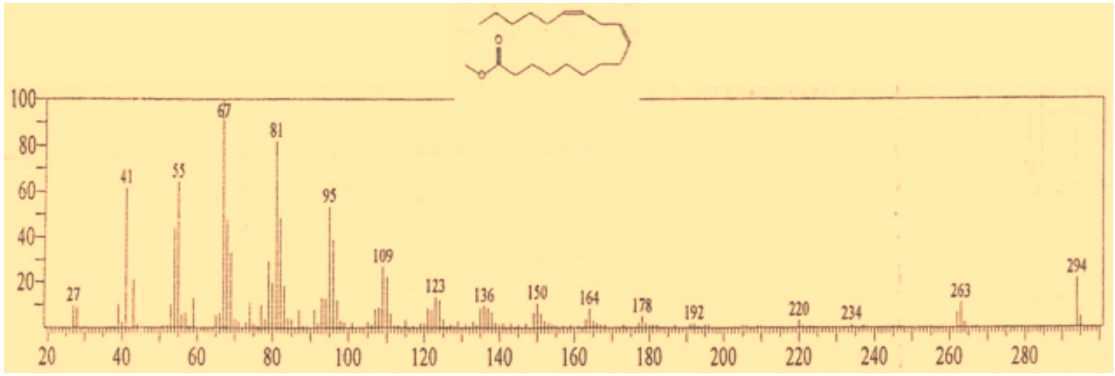


Fig. 3.10: Mass spectrum of9,12-octadecanoic acid methyl ester

The EI mass spectrum of 9,12-octadecanoic acid methyl ester is shown in Fig. 3.10.The peak at m/z 294, which appeared at R.T. 18.070 in total ion chromatogram, corresponds to $M^{+}[C_{19}H_{34}O_2]^{+}$ .The peak at m/z263 corresponds to loss of a methoxyl function.

3. – Antimicrobial suceptability

The oil was screened for antimicrobial activity against standard organisms. The average of the diameters of the growth inhibition zones are shown in Table (2) .The results were interpreted in terms of the

commonly used terms (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active).Tables (3) and (4) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 2 : Antibacterial activity of *Rumexacetosa*oil :M.D.I.Z (mm)

Drug	Conc.(mg/ml)	Ec	Ps	Sa	Bs	Ca
Rumexacetosa	100	15	20	14	9	13

Table 3 : Antibacterial activity of standard chemotherapeutic agents :M.D.I.Z (mm)

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 4 : Antifungal activity of standard chemotherapeutic agents against standard fungi

Drug	Conc. mg/ml	An.	Ca.
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

- Sa.*: Staphylococcus aureus*
- Ec.*: Escherichia coli*
- Pa.: *Pseudomonas aeruginosa*
- An.: *Aspergillusniger*

- Ca.: *Candida albicans*
- *Bs.: Bacillus subtilis*
- M.D.I.Z: Mean diameter or growth inhibition zone (mm). Average or two replicates, inhibition zone >=15: sensitive, <15: resistant.

The oil showed excellent activity against *Pseudomonas aeruginosa*and good activity against *Escherichia coli*,but it was partially active against *Staphylococcus aureus* and the fungus *Candida albicans.*

Reference

Reference

1. "essential oil". Oxford English Dictionary (online, American English ed.). Retrieved 2014-07-21.

2. Reeds, P. J. *The Journal of nutrition*.**130 (7)**: 1835S (2000).

3. Houtsma,E. J. Brill's Brill. pp. 1011–.ISBN **978-90-04-09790-2**(1993).

4. Gilman, A. G.; Rall, T. W.; Nies, Alan S.; Taylor, Palmer, eds. Goodman & Gilman's .New York: Pergamon. ISBN **0-08-040296-8** (1990).

5. Klaassen, Casarett and Doull'sToxicology . New York: McGraw-Hill. ISBN **0071052399** (1991).

6. Ryman, Century Publishing CO. Ltd. pp. Chapter 3. ISBN **9780852072158**(1984).

7. Forster, Cambridge University Press(2007).

8. Refrigerant 1234YF's Potential Impact in Automotive Applications.

9. "ISO TC 54 Business Plan – Essential oils" (PDF). Retrieved 2006-09-14.

10. "Carvacrol data sheet from Sigma-Aldrich".

11. Soares, I.H.; Loreto, É.S.; Rossato, L.; Mario, D.N.; Venturini, T.P.; Baldissera, F.; Santurio, J.M.; Alves, S.H. *Journal de MycologieMédicale / Journal of Medical Mycology.* **25 (3)**: 213–7. PMID 26281965 (2015).

12. Mandras, Narcisa; Nostro, Antonia; Roana, Janira; Scalas, Daniela; Banche, Giuliana; Ghisetti, Valeria; Del Re, Simonetta; Fucale, Giacomo; Cuffini, Anna Maria; Tullio, **16(1)**: 330 PMID 27576581 (2016).

13. Warnke, Patrick H.; Becker, Stephan T.; Podschun, Rainer; Sivananthan, Sureshan; Springer, Ingo N.; Russo, Paul A.J.; Wiltfang, Joerg; Fickenscher, Helmut; Sherry, Eugene, *Journal of Cranio-Maxillofacial Surgery*. **37(7)**: 392–7. PMID 19473851(2009).

14. Norman, Actions and Uses of Drugs. A.A. Balkema (1963).

15. The Plant List: *A Working List of All Plant Species*, retrieved 10 May 2016

16. Blamey, M.; Fitter, R.; Fitter, London: A & C Black. p. 64.ISBN **978-1408179505** (2003).

17. [a b c]Stace, *New Flora of the British Isles* . *Cambridge*, U.K.: Cambridge University Press. p. 446.ISBN **9780521707725** (2010).

18. International Organization for Plant Information (IOPI). Retrieved 2008-07-01.

19. Germplasm Resources Information Network , Beltsville, Maryland.

Retrieved 2008-07-02 .

20. "Rumex". Index NominumGenericorum.International Association for Plant Taxonomy.2006-02-20.

21. O. David Sparkman; Zelda Penton; Fulton G. Kitson . ISBN **978-0-08-092015-3**(17 May 2011).

22. Gohlke, Analytical Chemistry. ISSN **0003-2700** (1959).

23. Gohlke, Roland S.; McLafferty, Fred W. *Journal of the American Society for Mass Spectrometry.* ISSN **1044-0305**(1993).

24. Hites, Ronald A. **88 (14)**: 6955–6961. ISSN 0003-2700 (2016).

25. [a b c] Brock, David C.**29 (1)**.(2014).

26. Webb-Halpern, Chemical Heritage Magazine. **26(2)**: 31.(2008)

27. Thermo Instrument Systems Inc. pp. 513–514. Retrieved 23 January 2015.

28. JeromeJeyakumar, *International Journal ofPharmaceutical Science Invention*: 37–41. Retrieved 23 January 2015.

29. "Optimizing the Analysis of Volatile Organic Compounds – Technical Guide" RestekCorporation, Lit. Cat. 59887A

30. Wang, T.; Lenahan, **32 (1)**: 429–438. ISSN 0007-4861(1984).

31. McLafferty, F. W.; Hertel, R. H.; Villwock, **9 (7)**: 690–702 (1974).

32. Stein, SE; J Am Soc Mass Spectrom. **5 (9):** 859–866. PMID 24222034 (1994).

33. [a b] Amirav A, Gordin A, Poliak M, Mass Spectrom. **43 (2)** PMID 18225851.

34. Alon, T.; Amirav, Rapid Communications in Mass Spectrometry. **20 (17)**:PMID 16897787 (2006).
35. Tsivou, M.; Kioukia-Fougia, N.; Lyris, E.; Aggelis, Y.; Fragkaki, A.; Kiousi, X.; Simitsek, P.; Dimopoulou, H.; Leontiou, I. -P.; Stamou, M.; Spyridaki, M. -H.; Georgakopoulos, 555: 1–13. (2006).
36. Smith, P. A.; Lepage, C. J.; Lukacs, M.; Martin, N.; Shufutinsky, *International Journal of Mass Spectrometry.* **295(3)**: 113–118. (2010)
37. Antimicrobial".Merriam-Webster Online Dictionary.Retrieved 2009-05-02.
38. "Antimicrobial Porous Media | Microbicidal Technology | Porex Barrier Technology". Retrieved 2017-02-16.
39. M. Wainwright ."Moulds in ancient and more recent medicine".Mycologist.**3 (1)**: 21–23 (1989).
40. A. Smith-Palmer; J. Stewart; L.Letters in Applied Microbiology.**26(2):** PMID 9569693 (1998).