SUPPLEMENTARY SEARCH FOR A BACHELOR'S DEGREE IN SCENIC EDUCATION

Antimicrobial Activity of Carum carvi against stander micro organism

Prepaid By:
Hadeel Mohammed El-mahi

Supervision:
Dr. Somia Basher

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Dedication

To my parent
To my friends
To my Brothers
To my colleges
To all whom put the education in front of their eyes
Acknowledgement

Thanks, gratitude and praise to the almighty Allah who gives me health and patience's to complete this work.
First of all, thanks and praise always will be to Allah and great thanks to my supervisor Dr. Somia Basher.
I also wish to extend my thanks to all members of the Center Research for their help during the laboratory work. Special thanks and appreciations to Dr. Muddathir Siddig & Mohamed Omer for their continuous help and support.
Thanks are also extended to my friends. Special words of thanks are due to my family for their help and encouragement.
Finally, and in order not to forget any one who had helped me, thanks for all of them.
Abstract

This study is to investigate Antibacterial Activity of carum carvi extract in different solvent (oil – methanol – petroleum ether – water) Against some pathogen such as the results indicate that carum carvi oil have Antimicrobial activity Against s.aureus only.

The pathogen inculcate that oil carum carvi extract all substance that have Antimicrobial Activity when companed with other.
الخلاصة:

تناولت هذه الدراسة اختبار النشاط الميكروبي لمستخلص نبات الكرآيا في مذيبات مختلفة (الزيت – الميثانول – البنزوليم إيثيل – الماء) وآثار التنازج إلى فعالية الزيت الطيار في استخلاص المواد التي لها نشاط ميكروبي ضد البكتريا العنقودية الذهبية فقط.
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Chapter one

Introduction & Literature review

1: 1: Introduction:

1: 1: 1: *Carum carvi*

Caraway known as meridian fennel, or Persian cumin, is a biennial plant in the family Apiaceae, native to western Asia, Europe and Northern Africa. The plant is similar in appearance to other members of the carrot family, with finely divided, feathery leaves with thread-like divisions, growing on 20–30 cm stems. The main flower stem is 40–60 cm tall, with small white or pink flowers in umbels. Caraway fruits (erroneously called seeds) are crescent-shaped achenes, around 2 mm long, with five pale ridges.

1: 1: 2: **Scientific classification:**

<table>
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<tbody>
<tr>
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<tr>
<td>(unranked):</td>
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<tr>
<td>Order:</td>
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<tr>
<td>Family:</td>
</tr>
<tr>
<td>Genus:</td>
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*Carum* carvi

L.
1:1:3: Names and history:

The etymology of caraway is complex and poorly understood. Caraway has been called by many names in different regions, with names deriving from the Latin *cuminum* (cumin), the Greek *karon* (again, cumin), which was adapted into Latin as *carum* (now meaning caraway), and the Sanskrit *karavi*, sometimes translated as "caraway" but other times understood to mean "fennel". The Italian *finochchio meridionale* (meridian fennel) suggests these shared roots, while *cumino tedesco* (German cumin) again points towards cumin—though caraway also has its own name in Italian, *caro*. Other languages share similar peculiarities, with the Norwegian name *karve*, Yiddish borrowing the German *Kümmel* (caraway) as *kimmel* to mean caraway, yet using the Semitic term *kamoone* for cumin, which is *Kreuzkümmel* in German. In Iran it is known as *zīr* or *zīre siyāh* ("black cumin"), and is cultivated mostly in Kerman province in the southeast of the country. In India it is commonly called *vilāyaṭīrā* ("foreign cumin").

English usage of the term caraway dates back to at least 1440 and is considered by Skeat to be of Arabic origin, though Katzer believes the Arabic *al-karawya* (cf. Spanish *alcaravea*) to be derived from the Latin *carum*.
1:1:4: Origin of plant:
Spreads plant in areas of the Mediterranean basin and the central and northern Europe and Asia original home to him and spread grown in many parts of the world, and we can say that the Netherlands is the most important country producing caraway are grown primarily for the extraction of essential oil of improved breeds with high proportions of oils.

Caraway plant grass around me height of up to one meter and his severe Altfsas papers, inflorescence tent and white fruit and smooth curved structure with a length of 4-7 mm.

Caraway and enjoys cultivating the soil nutrient-rich loam and organic material and succeed grown in saline soils and wetlands to be good drainage.

1:1:5: Agriculture:-

Deadline Agriculture and quantity of seed:
Caraway grown in the loop during the winter months of October and early November, and the delay in agriculture leads to the production of plants a few weak growth and the necessary crop per acre seed 4-6 kg of high-dynamic where multiply caraway seed and seed should be used from good sources and Related Content High volatile oil and is common to be sustainable agriculture in the land directly but it can also be a method of transplanting in agriculture where mobility seedlings after about
45 days from planting nursery when it reaches the length of the seedling to about 10-12 cm.

- **Processing of land for agriculture and farming method:**

  Preparing the ground for planting Bhrthha twice Mtaamdtan with the addition of organic fertilizer and Old Jb. rate of 15-20 m³ per acre with 200 kg super phosphate, calcium and so in the old lands to be increasing this quantity in the new lands for up to 20-25 m³ organic fertilizer Old Kthll + 300 kg Super calcium phosphate, seeds are planted in Gore on a single feather (badminton Eastern or tribal) in the middle of the line where placed in each Jourct 4-5 seeds then covered with a thin layer of injustice silt and Jura away from the other a distance of 25 cm and then irrigated land immediately after agriculture Ri protector and so the Nha water reaches the top of the line.

**1:1:6: Plant Description:**

The caraway plant is a biennial herb usually with a fleshy root and slender, branched stems. It has feather-like leaves divided into very narrow segments and small white flowers. The fruit, when ripe, splits into narrow, elongated carpels, which are curved, pointed at the ends and have four longitudinal ridges on the surface. The dried fruits or seeds, brown in color, are hard and sharp to touch. They have a pleasant odor, aromatic flavor, somewhat sharp taste and leave a somewhat warm feeling in the mouth.
**1:1:7: Active ingredient**

Caraway fruits contain volatile oil (3-7%) and the most important compounds is the carvone and 60-40 Limonene material and are considered of digestive substances. And also fruits contain materials Flavonyh and protein materials and many sugary and greasy oil a fixed rate of up to 20%

**1:1:8: Medical uses and benefits:**
1) Has an aromatic smell active the digestion and Tkra of the burp, and removes indigestion
2) strengthens the body with the opening of a nice appetite, and nausea and dizziness addresses
3) mixed with other medications to remove the bad effects of them or to add flavor, and an example used caraway with laxatives
4) Removes flatus and colic in adults and children
5) Ground (powder) caraway seed puton bruises, Wesera cured
6) Thoracic and addresses and cough, cold and flu, expectorant of
7) Diuretic also benefit for hiccups, palpitations
8) Diuretic breast milk when nursing mother
9) Helps to relax the muscles of the stomach and intestines and is successfully used in the treatment of flatulence
10) Used as an adjunct in the treatment of gastric ulcer and duodenal and treat infections of the colon.
Caraway oil has a significant influence in bronchitis also has a strong influence in relieving asthma and lowers thyroid
Frequent use of boiled caraway prevents transformation of normal cells into cancerous cells
This disease has a close relationship with heart disease

1:1:9: Other uses:

Caraway is widely used for flavoring breads, biscuits, cakes and cheese. It is also used as an ingredient in sausages and as a seasoning and pickling spices. Caraway oil is useful chiefly for flavoring purposes.
The roots may be cooked as a root vegetable like parsnips or carrots. Caraway fruit oil is also used as a fragrance component in soaps, lotions, and perfumes.
1:2: Tested organism:

1:2:1: Staphylococcus aureus

1:2:1:1: Scientific classification:

*Staphylococcus aureus*

![Scanning electron micrograph of *S. aureus*; false color added.](image)

Domain: Bacteria
Kingdom: Eubacteria
Phylum: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Staphylococcaceae
Genus: *Staphylococcus*
Species: *S. aureus*

1:2:1:2: Definition:

The main species of medical importance is *Staphylococcus aureus*. Several other species may also cause disease including *Staphylococcus*...
epidermidis and staphylococcus saprophyticus( formerly micrococcus subgroup).

Staphylococci are widely distributed in the environment. They form part of the normal microbial flora of the skin, upper respiratory tract, and intestinal tract.

S. aureus is carried in nose of 40% or more of healthy people.

1:2:1:3: Pathogenicity:

S. aureus

a) Abscesses, boils, styes, and impetigo. It may also cause secondary infections of insect bites, ulcers, burns, wounds, and skin disorders.

b) Conjunctivitis, especially of the newborn.

c) Cross-infections in hospitals.

d) Septicaemia, endocarditis, and osteomyelitis.

e) Pneumonia and empyema.

f) Mastitis (inflammation of the breast).

g) Antibiotic-associated enteritis.

H) Food-poisoning from enterotoxin B produced by S. aureus in foods such as cooked meats and milk products (e.g ice-cream).

The food is often contaminated by a carrier of S. aureus.

i) Scalded skin syndrome in young children due to the toxin exfoliatn.

j) Toxic shock syndrome due to a colonization of S. aureus especially in the vagina.
**1:2:1:4: Diagnosis:**

Specimens: pus and sputum for microscopy and culture. Blood for culture. Faeces, vomit, and the remains of food if food-poisoning is suspected. An anterior nasal swab is required to detect S. aureus carriers. For the collection of specimens see chapter 38.

**Microscopy:**

Staphylococcus species are non-motile, non-capsulate, gram positive cocci of uniform size (about 1 μm in diameter), that occur characteristically in groups but also singly and in pairs.

**Culture:**

Staphylococci grow well aerobically and in a carbon dioxide enriched atmosphere. Most strains also grow anaerobically, but less well. Temperature range for growth is 10-42 °C, with an optimum of 35-37 °C. S. aureus. On blood agar and chocolate (heated blood) agar, S. aureus produces yellow to cream or occasionally with 1-2 mm in diameter colonies. Some strains are beta-haemolytic when grown aerobically. Colonies are slightly raised and easily emulsified on a slide.

On MacConkey agar, smaller (0.1-0.5 mm) colonies are produced after overnight incubation at 35-37 °C. Most strains are non-lactose fermenting.
Mannitol salt agar is a useful differential and selective medium for recovering *S. aureus* from faecal specimens when investigating staphylococcal food-poisoning.

**1:2:1:5: Treatment:**

Treatment for *Staphylococcus aureus* depends upon the seriousness of the infection. As there is no vaccine for *Staphylococcus aureus* doctors recommend antibiotics to cure it.

**Examples of antibiotic:**

**Penicillin:**

- Penicillin is the classic antibiotic your doctor may prescribe for treating bacterial infections, although only about 10 percent of staph infections can be cured with it.

Because there is a group of *Staphylococcus aureus* bacteria which have become resistant. This group is known as *Methicillin*-resistant *Staphylococcus aureus* or MRSA.

**Vancomycin**

- Vancomycin is a more powerful antibiotic than penicillin that your doctor may prescribe for staph infections, although it may cause more severe side effects including diarrhea and thrush.

Flucloxacillin – *methicillin* – and the cephalosporins can often be used to treat penicillin resistant strains of *S. aureus*
Ciprofloxacin:

- Ciprofloxacin is a type of antibiotic usually reserved as a drug of last resort for treating infections, including those caused by staph bacteria, due to its serious side effects.

It is very important for a doctor to know about the patient's medical background, as in some cases, if the infection is deep and antibiotic is not able to cure it, doctors go for surgeries. This is done to make the wound clean and a proper drainage of the pus from the wound. After that, the doctor cleans the wound properly. This process is painful but in some cases it becomes necessary. After the surgery also the patient has to take care of the wound properly. Surgery cannot be done on the patients who have chronic disorders like.
1:2:2: Pseudomonas aeruginosa

1:2:2:1: Scientific classification:

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gamma Proteobacteria
Order: Pseudomonadales
Family: Pseudomonadaceae
Genus: Pseudomonas
Species: P. aeruginosa

1:2:2:2: Definition:

The main species of medical importance are: Pseudomonas aeruginosa (formerly Pseudomonas pyocyanea). Pseudomonas pseudomallei (Whitmore's bacillus).
Occasionally pseudomonas maltophilia and other pseudomonas species may also cause disease. Pseudomonas species can be found in water, soil, sewage, and vegetation. They can also be found in the intestinal tract. P. aeruginosa is frequently present in hospital environments, especially in moist places such as sink, bowls, drains, cleaning buckets, and humidifiers. It can also be found growing in eye drops, ointments, and weak antiseptic solution.

P. pseudomallei is naturally found in rice paddy fields, the mud of river banks, and surface stagnant water. The organism can infect cattle, pigs, and other animals.

1:2:2:3: Pathogenicity:

P. aeruginosa

a) Skin infections especially at burn sites, wounds, pressure roses, and ulcers, often as a secondary invader.
b) Urinary infection, usually following catheterization or associated with chronic urinary infections.
c) Respiratory infection especially in patients with cystic fibrosis or conditions that cause immunosuppression.
d) External ear infections (otitis externa).
e) Eye infections (usually hospital-acquired).
f) Septicaemia especially in persons already in poor health.
1:2:2:4: Diagnosis:

Specimens: Depending on the site of infection, specimens include pus, urine, sputum, and effusion for microscopy and culture, and occasionally blood for culture. Specimens that may contain P. pseudomallei or P. mallei must be marked HIGH RISK and handled with great care. Both these species are highly infectious.

Microscopy:
Pseudomonas species are gram negative motile rods. P. pseudomallei shows bipolar staining, that can be seen in a garm stained preparation or more clearly in a giemsa stained smear. P. mallei occurs as a rod or coccobacillus.

Culture:
P. aeruginosa is an obligatory aerobe and is usually recognized by the yellow-green pyocyanin pigment it produces. About 4% of strains, however, do not produce pyocyanin culture have a distinctive smell due to 2-aminoacetophenon production.

P. aeruginosausually produce large, flat, haemolytic colonies on blood agar. Small colonies are formed by some strains (all strains are strongly oxidase positive). If the culture is left at room temperature, the yellow-green of the pyocyanin is intensified. P. aeruginosa also grows well on nutrient agar, MacConkey agar and other media containing bile salts and also
on cetrimide agar. When grown on Kliger iron agar, it produces a characteristic pink-red slope and butt.

1:2:2:5: Treatment:

*P. aeruginosa* is frequently isolated from nonsterile sites (mouth swabs, sputum, etc.), and, under these circumstances, it often represents colonization and not infection. The isolation of *P. aeruginosa* from nonsterile specimens should, therefore, be interpreted cautiously, and the advice of a microbiologist or infectious diseases physician/pharmacist should be sought prior to starting treatment. Often no treatment is needed.

When *P. aeruginosa* is isolated from a sterile site (blood, bone, deep collections), it should be taken seriously, and almost always requires treatment.

*P. aeruginosa* is naturally resistant to a large range of antibiotics and may demonstrate additional resistance after unsuccessful treatment, in particular, through modification of a porin. It should usually be possible to guide treatment according to laboratory sensitivities, rather than choosing an antibiotic empirically. If antibiotics are started empirically, then every effort should be made to obtain cultures, and the choice of antibiotic used should be reviewed when the culture results are available.
Phage therapy against *P. aeruginosa* remains one of the most effective treatments, which can be combined with antibiotics, has no contraindications and minimal adverse effects. Phages are produced as sterile liquid, suitable for intake, applications etc Phage therapy against ear infections caused by *P. aeruginosawas* reported in the journal *Clinical Otolaryngology* in August 2009

Antibiotics that have activity against *P. aeruginosa* may include:

- aminoglycosides (gentamicin, amikacin, tobramycin, but *not* kanamycin)
- quinolones (ciprofloxacin, levofloxacin, but *not* moxifloxacin)
- cephalosporins (ceftazidime, cefepime, cefoperazone, cefpirome, cefbibrople, but *not* cefuroxime, ceftriaxone, cefotaxime)
- antipseudomonalpenicillins: carboxypenicillins (carbenicillin and ticarcillin), and ureidopenicillins (mezlocillin, azlocillin, and piperacillin). *P. aeruginosa* is intrinsically resistant to all other penicillins.
- carbapenems (meropenem, imipenem, doripenem, but *not*ertapenem)
- polymyxins (polymyxin B and colistin)
- monobactams (aztreonam)
These antibiotics must all be given by injection, with the exceptions of fluoroquinolones, aerosolized tobramycin and aerosolized aztreonam. For this reason, in some hospitals, fluoroquinolone use is severely restricted to avoid the development of resistant strains of \textit{P. aeruginosa}. In the rare occasions where infection is superficial and limited (for example, ear infections or nail infections), topical gentamicin or colistin may be used.
1:2:3: Escherichia coli

1:2:3:1: Scientific classification:

Domain: Bacteria
Kingdom: Eubacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: Escherichia
Species: E. coli

1:2:3:2: Definition:

The main species of medical importance is: Escherichia coli The species E. coli also includes the organisms that were formerly known as the Alkalescens –dispargroup (send of submit42:6)
E. coli organisms form part of the normal microbial flora of the intestinal tract of humans and animals. They can also be found in water, soil, and vegetation.

**1:2:3:3: Pathogenicity:**

E. coli causes:

a) Urinary infections including cystitis, pyelitis, and pyelonephritis. E. coli is the commonest pathogen isolated from patients with cystitis. Recurrent infections are common in women.

b) Wound infections, appendicitis, peritonitis, and infection of the gall bladder.

c) Bacteraemia, and meningitis especially of the newborn.

d) Diarrhoeal disease especially in infants but also in adults.

E. coli is the name of a type of bacteria that lives in your intestines. Most types of E. coli are harmless. However, some types can make you sick and cause diarrhea. One type causes travelers' diarrhea. The worst type of E. coli causes bloody diarrhea, and can sometimes cause kidney failure and even death. These problems are most likely to occur in children and in adults with weak immune systems.

You can get E. coli infections by eating foods containing the bacteria. Symptoms of infection include
- Nausea or vomiting
- Severe abdominal cramps
- Watery or very bloody diarrhea
- Fatigue
- Fever

To help avoid food poisoning and prevent infection, handle food safely. Cook meat well, wash fruits and vegetables before eating or cooking them, and avoid unpasteurized milk and juices. You can also get the infection by swallowing water in a swimming pool contaminated with human waste. Most cases of E. coli infection get better without treatment in 5 to 10 days.

1:2:3:4: Diagnosis:
Specimens: Depending on the site of infection specimens include urine, pus, and blood. If ETEC, infection is suspected, and facilities are available, feces should be sent for toxin detection.

Microscopy:
E. coli is a Gram negative usually motile rod. Organisms formerly referred to as the \textit{ALkalescens dispar} group are non-motile. Some E. coli strains are capsulated.

Culture:
E. coli is an aerobe and facultative anaerobe. It produces 1-4 mm in diameter colonies on blood agar after overnight incubation at 35-37 C. The colonies may appear mucoid and some strains are haemolytic.
On MacConkey agar, most E.coli strains produce lactose fermenting colonies. Some EPEC strains are late or non-lactose fermenting. Isolates formerly of the Alkalescens-dispar group are non-lactose fermenting. Most strains of E. coli do not grow, or are markedly inhibited, on XLD agar, DCA, SS agar, or other selective media used to isolate shigllae salmonellae.

The culture of E.coli in MacConkey membrane broth is described under, the microbiological analysis of water supplies on p. 219.

1:2:3:5: Treatment:

Antimicrobials that are used to treat E.coli urinary and other infections include those with activity against Gram negative organisms such as sulphonamides, trimethoprim cotrimoxazole, nalidixic acid, nitrofurantoin, tetracycline, ampicillin, amoxycillin, cephalosporin's, and aminoglycosides. In the treatment of E. coli diarrhoea, however, the use of antibiotics is in general only of minor importance. Rehydration of the patient is always the most important measure taken.
Bacillus subtilis

Scientific classification:

<table>
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<th>Bacteria</th>
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<tr>
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<td>Bacilli</td>
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<tr>
<td>Order</td>
<td>Bacillales</td>
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<tr>
<td>Family</td>
<td>Bacillaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Bacillus</td>
</tr>
<tr>
<td>Species</td>
<td>B. subtilis</td>
</tr>
</tbody>
</table>

Definition:

Bacillus species are widely distributed in nature. Most live as saprophytes in the soil, dust, water, and on vegetation. They are able to form resistant spores. Various species occur as common contaminants of laboratory cultures.
1:2:4:3: Pathogenicity:

*Bacillus subtilis* bacteria are non-pathogenic. They can contaminate food, however, they seldom result in food poisoning. They are used on plants as a fungicide. They are also used on agricultural seeds, such as vegetable and soybean seeds, as a fungicide. The bacteria, colonized on root systems, compete with disease causing fungal organisms. *Bacillus subtilis* use as a fungicide fortunately does not affect humans (EMBL EBI). Some strains of *Bacillus subtilis* cause rots in potatoes. It grows in food that is non-acidic, and can cause ropiness in bread that is spoiled (Todar). Some strains related to *Bacillus subtilis* are capable of producing toxins for insects. Those strains can also be used for protecting crops as well. *Bacillus thuringiensis*, for example, is another bacterium in the same genus that is used for insect control.
Chapter two

Materials and Methods

2:1: Plant materials:
Plant sample was brought from Omdurman market and identified by the taxonomist of medicinal and aromatic plants research institute.

2: 2: Chemicals:
Petroleum ether  S D Fine  India.
Methanol  S D Fine  India.

2: 3: Apparatus:
Soxhelt  Duran  England.
Rotary evaporator  Buchi  Switzerland.
Clevenger  Duran  England.
Balance  A and B  England

2.1.3 Culture media:-
Blood agar base  Oxoid limited, England
Cetrimide agar  Oxoid limited, England
Eosin methylene blue agar  Oxoid limited, England
Koser citrate agar  Oxoid limited, England
Lactose  Oxoid limited, England
MacConkey’s agar  Oxoid limited, England
Mannitol salt  Oxoid limited, England
agar  Oxoid limited, England
Nutrient agar  Oxoid limited, England
Nutrient broth  Oxoid limited, England
Nutrient gelatin  Oxoid limited, England
Peptone  Oxoid limited, England
Starch agar  Oxoid limited, England
Urea agar  Oxoid limited, England
2:1:4: Equipment and Instruments:

- Autoclave
- Balance type H 6T
- Colony counters
- Glass ware
- Harvard organ bath
- Hot air oven
- Incubators
- Microscope
- Water bath

Griffin and George Ltd, England
E. Mettler, England
Gallenkamp, England
Griffin And George Ltd.
Harvard apparatus Ltd., U.S.A
Gallenkamp, England
Baird and Tatlock Ltd, England
Will Wetzlar, Germany
Grant Instruments Ltd.

2:1:5: Test Bacterial organisms:

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

National Collection of Type Culture (NCTC), Colindale, England.
American Type Culture Collection (ATCC) Rockville, Maryland, USA.
2:1:6: Preparation of the extracts:

Extraction was carried out according to method described by (Sukhdevet . al 2008):
50 g of each part of *Carum carvi* was successively extracted with petroleum ether and methanol using soxhelt extractor apparatus. Extraction carried out for about four hours for petroleum ether and eight hours for methanol. Solvents were evaporated under reduced pressure using rotary evaporator apparatus. Finally extracts allowed to air in Petri dishes till complete dryness and the yield percentages were calculated as followed:

Weight of extract obtained / weight of plant sample * 100

2:1:7: Preparation of the aqueous extract:

The marc obtained from the above extraction was sacked in 100 ml hot distilled water, and left till cooled down with continuous stirring at room temperature. Extract was then filtered through cotton and stored in a refrigerator till used.

2:1:8: Volatile oil Distillation:

Distillation of volatile oils was carried out using the method described by (Sukhdevet . al 2008):
250 g of each sample was placed in 2000 ml rounded bottom capacity flask. 1000 ml of distilled water was added and the Clevenger receiver (lighter than water) (Duran west Germany) and condenser attached to the top of the flask. System was heated at 100 C for about four hours till the volume of oil above
water layer at the receiver constant. Oil was pipetted, dried over sodium sulphate anhydrous and stored in a dark container in a refrigerator till used. Yield percentages were calculated as followed:

\[
\text{Volume of oil} / \text{weight of plant sample} \times 100
\]

**2:1:9: Preparation of bacterial suspensions:**

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed on to nutrient agar slopes and incubated, at a 37°C for 24 hours. The bacterial growth was harvested and washed and washed off with 100 ml sterile normal saline, to produce a 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 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.

Each time a fresh stock suspension was prepared all the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

2:1:10: In vitro testing of extracts for antimicrobial activity:—

The cup-plate agar diffusion method (Cavanaugh, 1972) was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts.

One ml of the standardized bacterial stock suspension $10^8$- $10^9$ C.F.U/ml were thoroughly mixed with 100ml of molten sterile nutrient agar which was maintained at 45°C. 20ml aliquots of 1h inoculated nutrient agar were distributed into sterile Petri-dishes.

The agar was left to set and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed.

Alternate cups were filled with 0.1 ml sample of each extracts using automatic microlitre pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 18 hours.

Two replicates were carried out for each extract against each of the test organisms. After incubation the diameters of the
resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

2:1:11: Determination of minimum inhibitory concentration (MIC) by agar plate dilution method:

The principle of the agar plate dilution is the inhibitory of growth on the surface of the agar by the plant extracts incorporated into the medium.

Plates were prepared in the series of increasing concentrations of the plant extract. The bottom of each plate was marked off into 6 segments. The organisms tested were grown in broth over night to contain $10^8$ C.F.U/ml.

Loop-full of diluted culture is spotted with a standard loop that delivers 0.001 ml on the surface of segment.

The end point (MIC) is the least concentration antimicrobial that inhibits the growth. Results are reported as the MIC in mg/ml.
Chapter Three
Chapter three

Results and discussion

3: 1: yield percentages of solvent extraction:

Yield percent of extracts:

<table>
<thead>
<tr>
<th>Weight of sample</th>
<th>Petroleum ether</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight g</td>
<td>Yield %</td>
</tr>
<tr>
<td>50 g</td>
<td>3.914 g</td>
<td>7.828 %</td>
</tr>
</tbody>
</table>

3: 2: yield percentage of oil distillation:

<table>
<thead>
<tr>
<th>Weight of sample</th>
<th>Volume of oil</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>190 g</td>
<td>2.1 ml</td>
<td>1.105 %</td>
</tr>
</tbody>
</table>
3:3: Antimicrobial Activity of *carum carvi* Against some bacterial pathogen:

<table>
<thead>
<tr>
<th>Extract</th>
<th>E.coli</th>
<th>P.a</th>
<th>B.s</th>
<th>S.a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- oil (100)</td>
<td>14–15</td>
<td>14–14</td>
<td>14–14</td>
<td>17–18</td>
</tr>
<tr>
<td>2- methanol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3- P.Ether</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4- water</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

3:4: Antimicrobial Activity of *carum carvi* Against some bacterial pathogen of MIC:

<table>
<thead>
<tr>
<th>Extract</th>
<th>E.coli</th>
<th>P.a</th>
<th>B.s</th>
<th>S.a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>15</td>
<td>13</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>25</td>
<td>16</td>
<td>13</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>12.5</td>
<td>17</td>
<td>14</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>6.25</td>
<td>15</td>
<td>14</td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td>Methanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>11</td>
<td>12</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>25</td>
<td>12</td>
<td>13</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>12.5</td>
<td>12</td>
<td>13</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>6.25</td>
<td>12</td>
<td>13</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>PEther</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>50</td>
<td>-</td>
<td>-</td>
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<td>25</td>
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<td>12.5</td>
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<tr>
<td>6.25</td>
<td>-</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
3:5:1: Antimicrobial Activity of *carum carvi* oil extraction
Against some bacterial pathogen in different concentration

**Table (1)**

<table>
<thead>
<tr>
<th>concentration</th>
<th>100%</th>
<th>50%</th>
<th>25%</th>
<th>12.5%</th>
<th>6.25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>pathogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.coli</td>
<td>15</td>
<td>I</td>
<td>15</td>
<td>I</td>
<td>16</td>
</tr>
<tr>
<td>p.s</td>
<td>14</td>
<td>I</td>
<td>13</td>
<td>R</td>
<td>13</td>
</tr>
<tr>
<td>s.a</td>
<td>18</td>
<td>S</td>
<td>18</td>
<td>S</td>
<td>20</td>
</tr>
<tr>
<td>B.s</td>
<td>14</td>
<td>I</td>
<td>15</td>
<td>I</td>
<td>16</td>
</tr>
</tbody>
</table>

E.coli \( \left( \frac{5}{5} \times 100 \right) = 100\% \ I \\
p.s \quad \left( \frac{2}{5} \times 100 \right) \left( \frac{2}{5} \times 100 \right) \left( \frac{1}{5} \times 100 \right) = 40\% \ I \text{ and } 40\% \ R, \ 20\% \ S \\
S.a \quad \left( \frac{4}{5} \times 100 \right) \left( \frac{1}{5} \times 100 \right) = 80\% \ S, \ 20\% \ I \\
B.s \quad \left( \frac{4}{5} \times 100 \right) \left( \frac{1}{5} \times 100 \right) = 80\% \ I, \ 20\% \ S \\

2:5:2: Antimicrobiciel Activity of *carum carvi* Methanol extraction Against some bacterial pathogen in different concentration

**Table (2)**

<table>
<thead>
<tr>
<th>concentration</th>
<th>100%</th>
<th>50%</th>
<th>25%</th>
<th>12.5%</th>
<th>6.25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>pathogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.coli</td>
<td>—</td>
<td></td>
<td>11</td>
<td>R</td>
<td>12</td>
</tr>
<tr>
<td>p.s</td>
<td>—</td>
<td></td>
<td>12</td>
<td>R</td>
<td>13</td>
</tr>
<tr>
<td>s.a</td>
<td>—</td>
<td></td>
<td>15</td>
<td>I</td>
<td>14</td>
</tr>
<tr>
<td>B.s</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>12</td>
</tr>
</tbody>
</table>
2:5:3: Antimicrobial Activity of *carum carvi* P.ether extraction
Against some bacterial pathogrin in different concentration

**Table (3)**

<table>
<thead>
<tr>
<th>concentration</th>
<th>100%</th>
<th>50%</th>
<th>25%</th>
<th>12.5</th>
<th>6.25</th>
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</thead>
<tbody>
<tr>
<td><strong>pathogen</strong></td>
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<td></td>
</tr>
<tr>
<td>E.coli</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>PS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>S.a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>B.s</td>
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</table>

3:5:4: Antimicrobial Activity of *carum carvi* water extraction
Against some bacterial pathogrin in different concentration

**Table (4)**

<table>
<thead>
<tr>
<th>concentration</th>
<th>100%</th>
<th>50%</th>
<th>25%</th>
<th>12.5</th>
<th>6.25</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pathogen</strong></td>
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</tr>
<tr>
<td>E.coli</td>
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<td>-</td>
</tr>
<tr>
<td>PS</td>
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<td>-</td>
</tr>
<tr>
<td>S.a</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B.s</td>
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</tbody>
</table>
Chapter (Three)

Results and discussion

The Result obtained refer to the tables (1-4) as following :-

Table (1)

*Carum carvi* oil extraction in different concentration suggested that most of the pathogen used are resistant or no response where as *s-aureus* is sensitive to *carum carvi* oil extraction in different concentration ($\frac{4}{5} \times 100 = 80\%$)

Table (2)

All pathogen give no response *carum carvi* methanol extraction except *S-aureus* which gave Intermediate response (oil - methanol - petroleum ether - water )

Table (3 and 4)

With reference to the table (3-4) no response determined in all concentration used.

The above results suggest that not all strain used in this study are senctive to *carum carvi* extraction in all solvents . but *carum carvi* oil extraction is successfully inhibit the growth of *s-aureus* by(80%) which suggest that oil can be used to extract *carum carvi* subsastances. That have Antimicrobial activity aganist some pathogen.

On the other hand *carum carvi* a has not Antimicrobial activity Aagainst most strains used. plates ( 1- 7 )
This study suggested further investigation must be done on *carum carvi* antimicrobial activity agents other pathogens using others solvents.

The extract of oil *carum carvi* showed activity against tested organism. This result found to be online with:

(Hacri Rohani A (Ph.D.) et.al, 2010).
(Rahimian, et.al, 2012)
(ALICE GRIGORE, et.al, 2011)
References


2. "Global Crops Database: Meridian Fennel"

3. Benefits of Caraway Seeds: Meridian Fennel, a biennial herb with a fleshy root ...

4. "USDA Plants Classification Report: Apiaceae"

5. "Katzer's Spice Pages: Caraway Caraway (Carumcarvi L.)"


8. Words of Arabic Origin


21. Internet Wikipedia.
Appendices
Seeds Carum Carvi

Carum Carvi
Geographical location for the herb Carum Carvi