Comparative Investigation of the Antimicrobial Properties of the Root, Leaf, and Seed Oils of Carrot Plant Growing in Sierra Leone

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Research Article

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Abstract

*Daucus carota* L. (Carrot) is a biennial herbaceous plant which has various nutritional, cosmetic, and medicinal benefits. This study determined the antimicrobial activity of the oil extract from the leaves, roots, and seeds of carrot plants against four selected bacterial isolates.

The leaves, roots, and seeds of the carrot plant were weighed and the oils were extracted by hydrodistillation using the Clevenger apparatus. The antimicrobial susceptibility testing was performed by disc and well diffusion methods against *Streptococcus pneumoniae, Staphylococcus aureus, Salmonella typhi*, and *Pseudomonas aeruginosa*. Nigella sativa oil was used as the positive control.

The extraction process of essential oils from the part of the carrot plants yielded varying quantities of oil, spanning from the roots (0.3%), the leaves (0.4%) and the seeds (0.8%). The results of the study showed that all the extracted essential oils had some activity against the selected bacterial isolates, with varying degrees of inhibition. The root oil showed the highest antimicrobial activity (14mm) against *Streptococcus Pneumoniae*, matching the control oil using the well diffusion method and a higher activity (17mm) than the control when the disc diffusion method was used. The seed oil had the highest activity (15mm) against *Pseudomonas aeruginosa*, which is slightly less than the control oil using the disc diffusion method. The zones of inhibition were generally higher by the disc diffusion method than the well diffusion method for most of the isolates.

The essential oils extracted from *Daucus carota* L. oils had antimicrobial activity against the four bacterial isolates used, with higher activity against gram-positive bacteria than gram-negative. The antimicrobial activity varied according to the extracted plant parts, the bacterial isolate and the susceptibility test methods used.

Introduction

In the realm of modern medicine, the development of antimicrobial resistance (AMR) has become a major threat to global health, as it increases the burden of diseases and mortality rate (Jasovský et al., 2016). The threats posed by AMR have urged more researchers to explore natural sources for potential antimicrobial agents. Among the numerous plants investigated for their therapeutic potential, the *Daucus carota* L. (Carrots plants) stand out as an intriguing candidate owing to their widespread cultivation and medicinal uses across diverse cultures.

Carrot is known for its medicinal properties, which are attributed to various bioactive compounds present in different parts of the plant. Some of these compounds include phenolic acids, flavonoids, terpenoids, coumarins, alkaloids, and polyacetylenes (Kataria, Kaur Chahal, et al., 2016). However, these bioactive compounds vary in composition according to the part of the plant used for extraction.

The benefits of carrot fruit are quite understood by different people and its consumption for medicinal reasons is a common practice. Frequent consumption of this fruit vegetable is associated with longevity.
and a strong immune system (Conner et al., 2013). Carrot plants have been reported to have antimicrobial, antioxidant, anti-diabetic, anti-inflammatory, hepatoprotective, wound healing, and anticancer activities (Kataria, Chahal, et al., 2016; Maxia et al., 2009; Sharma et al., 2012).

Among the different parts of the carrot plant, the root is the most commonly consumed and studied for its nutritional and health benefits (Conner et al., 2013). However, the leaves and seeds of carrots are also important sources of bioactive compounds that may have significant antimicrobial potential. For instance, the essential oils extracted from carrot leaves and seeds have been shown to exhibit antibacterial and antifungal activities against various microorganisms (Soković et al., 2009). According to the study done by Soković et al., essential oil extracts from various parts of *Daucus carota* L. were more effective than the commercial drug streptomycin, against *Pseudomonas aeruginosa* and *Escherichia coli* (Soković et al., 2009).

Moreover, the essential oils from carrot leaves and seeds have some varying chemical compositions than the root oil, which may result in different modes of action and spectra of activity (Alves-Silva et al., 2016; Kataria, Chahal, et al., 2016). This study therefore aimed to determine the antimicrobial properties of the oil extracts of the roots, leaves, and seeds of carrot plants grown in Sierra Leone.

**Methods**

**Study Setting, Design, and Period**

An experimental study design was conducted between April and September 2022. This study was conducted at the Faculty of Pharmaceutical Sciences Laboratory, College of Medicine and Allied Health Sciences (COMAHS) and the Microbiology Laboratory of the Pharmacy Board of Sierra Leone (PBSL).

**Plant materials**

Samples of *Daucus carota* L. [carrot seeds, leaves, and root] were obtained from Sokobalia village, Koinadugu district, Northern Province of Sierra Leone between May and June, and were authenticated at the botany laboratory at Fourah Bay College (FBC), University of Sierra Leone (USL).

**Extraction of the essential oils from the Daucus carota L.**

The leaves, roots, and seeds were individually weighed and placed in separate round bottom flasks. These flasks were connected to a heating mantle, along with the Clevenger apparatus and condenser. Hydro-distillation was conducted for 4 hours for each plant part.

The oil extract was collected when distinct water and oil layers were formed in the apparatus. The oil extract from each plant part was stored in sealed containers, enclosed in black polythene bags, and kept in a dark cupboard at room temperature for subsequent analysis. The yielded oil quantities are presented in the results section.

**Preparation of Culture Medium**
Mueller-Hinton agar served as the designated medium in this study. The preparation adhered strictly to the manufacturer’s guidelines. Electronic beam balance was used to weigh the required quantity (9.5g), then poured into distilled water and boiled on a hot plate for proper dissolution of particles.

Sterilization was done with the aid of a sterilized pot at 121°C for 15 minutes. The prepared medium was cooled in a water bath before pouring onto Petri dishes for solidification. Quality control measures were executed to affirm sterility. Two medium-containing plates were randomly chosen. One plate remained at room temperature, while the other underwent 24-hour incubation at 22°C. Following this period, a comprehensive growth assessment was conducted on both plates.

**Sourcing of isolates**

Isolates such as Staphylococcus, Salmonella, Streptococcus, and Pseudomonas banked at the microbiology unit of the Pharmacy Board of Sierra Leone were used for the study.

**Identification of bacteria using gram staining**

An inoculating loop was used to transfer the bacteria from the culture to a slide in which gram staining was done using crystal violet, iodine, alcohol for decolourization, and safranin, and four different slides were used numbered 1–4 for ease of identification.

Bacteria were collected and distributed evenly on slides with distilled water. After adherence, crystal violet (0.5%) was applied, followed by a 20-second incubation. Subsequent steps involved washing with distilled water, application of Gram’s iodine for one minute, rinsing, and decolourization with 95% ethanol. Safranin drops were then introduced, followed by gentle washing, and air drying, and later examination was done under a microscope (oil immersion, plan C 100x) for morphological evaluation. This procedure was reiterated thrice.

Slide observations indicated *Streptococcus spp.* through mauve rod-like structures on slide two, and mauve cocci on slide four, signifying *Pseudomonas spp.* *Staphylococcus spp.* and *Salmonella spp.* were identified on slides three and one, respectively. Further investigation involved sub-culturing the stained bacteria.

**Preparation of Sub-Cultures Using Streaking Method**

Four different liquid media were prepared using Mueller-Hinton agar as a growth medium.

A 9.5g of Mueller-Hinton agar was weighed and placed in a measuring cylinder, and 250 ml of distilled water was added and stirred gently with a string rod. The measuring cylinder containing the mixture was then placed on a heating mantle to boil and dissolved completely. The dissolved mixture was then transferred into a bottle, and placed in a sterile pot for autoclaving (used in place of an autoclaving machine which was unavailable), then a sterile tape was placed on the cover of the bottle to help detect the completion of the autoclaving process. The bottle with the dissolved mixture was taken to a water
bath at 45°C for removal of bubbles before being poured into four different Petri dishes that were labelled *Staphylococci, Streptococci, Salmonella, and Pseudomonas* spp.

The differently labelled Petri dishes were then placed into an aseptic cabinet, for the inoculation of the bacteria and then streaked with a sterile bacteriological loop which was then left for 15mins; the sub-culture was later transferred to an incubator at 37°C for 24 hours. After 24 hours, the growth of the organisms was seen.

**Determination of antimicrobial properties *Daucus carota L.* oil extracts**

**Well diffusion method**

Different sub-cultures were prepared using the same method illustrated above, except, instead of streaking the inoculum was carpeted using the lawn culture method. The inoculums obtained from the streaking method were dissolved into labelled (*Staphylococci, Streptococci, Salmonella, and Pseudomonas* spp) four test tubes each containing sodium chloride solution to maintain the stability of the organism, which was then poured into different petri dishes; and then carpeted and was left to solidify. A pipette tip was used to create a well of 8mm in all of the new sub-cultures. Two drops of the oil samples were then placed on each formed well using a 2ml dropper, which was left for 15 minutes in the cabinet and later transferred to the incubator for 24 hours at 37°C.

**Disc Method**

The procedure outlined above was replicated with a modification in the technique. Instead of well formation, a disc with a circumference of 6mm was created using filter paper. The oil extracts of the leaves, seed, and root were dropped in the disc using a 2ml dropper, the disc was then placed in the different Petri dishes containing the inoculum, which was left for 15mins in the cabinet and later transferred to the incubator for 24 hours at 37°C.

**Results and discussion**

**Quantification of the oil extracts from the different parts of *Daucus carota L.* samples**

The oil extract yield of the seeds, leaves, and roots of *Daucus carota L.* was calculated based on the weight of the respective samples after extraction by steam distillation. The yields of essential oil obtained from the three parts of *Daucus carota L.* vary, ranging from 0.3–0.8%. The results are presented in Table 1.
Table 1
Oil yield from the Seed, Root, and Leaves of *Daucus carota L.*

<table>
<thead>
<tr>
<th>Plant</th>
<th>Weight Used (grams)</th>
<th>Oil Yield (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed</td>
<td>107.42</td>
<td>0.8</td>
</tr>
<tr>
<td>Root</td>
<td>100</td>
<td>0.4</td>
</tr>
<tr>
<td>Leaves</td>
<td>90.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

The results show that the seeds had the highest essential oil yield (0.8%), followed by the leaves (0.4%) and the roots (0.3%). Comparatively, similar research studies have also demonstrated varying oil yields from plant sources. For instance, a study conducted in Vienna reported essential oil yields from *Daucus carota* ssp. as 0.2, 0.1–0.3 and 0.8–1.6% (v/w) for roots, leaves, and fruits, respectively (Chizzola, 2013).

The variation in essential oil yield may be attributed to several factors, such as genetic diversity, environmental conditions, harvesting time, extraction method, and sample preparation (Chizzola, 2013; Kataria, Kaur Chahal, et al., 2016). It is important to optimize these factors to obtain the maximum essential oil yield from *Daucus carota L.*

### Antimicrobial Activity of Carrot Oils

The antimicrobial activity of the root, leaf, and seed oils of carrot plants was determined by two methods: well diffusion and disc diffusion. All the isolates showed sensitivity to the three oils, with varying degrees of inhibition.

#### Zones of inhibition produced by Well diffusion method

The root, leaf, and seed oils of the carrot plant were tested for their antimicrobial activity against four bacterial isolates (*Streptococcus pneumoniae*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa*) by well diffusion method. Nigella sativa oil was used as a positive control. The results are shown in Table 2.

Table 2
Zone of inhibition (mm) produced by the oil extracts using the well diffusion method

<table>
<thead>
<tr>
<th>Names of microbial isolates</th>
<th>Zones of inhibition produced by oil extracts (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf extract</td>
</tr>
<tr>
<td><em>Strep. Pneumoniae</em></td>
<td>12</td>
</tr>
<tr>
<td><em>Staph. Aureus</em></td>
<td>12</td>
</tr>
<tr>
<td><em>S. Typhi</em></td>
<td>11</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>8</td>
</tr>
</tbody>
</table>
The root oil showed the highest activity against *Strep. Pneumoniae*, with a zone of inhibition of 14 mm, equal to that of the control oil. The root oil showed slightly higher activity than the leaf and seed oils against *P. aeruginosa*. However, the result is almost half of that of the control.

Both the leaf and root oils showed similar and higher activity than the seed oil against *Staph. Aureus*, but lower activity than the seed oil against *S. typhi*. In both cases, the control oil had higher activity than all the other oils against *Staph. Aureus* and *S. typhi*, with a zone of inhibition of 19 mm and 25 mm respectively.

The results indicate that the root oil had a more consistent and higher antimicrobial activity when determined by the Well diffusion method among the three oil extracts, especially against *Strep. Pneumoniae* and *P. aeruginosa*. The seed oil had moderately low activity against all the isolates except against *S. typhi*. The control oil had superior activity than all the other oil extracts. These results are consistent with previous studies conducted in Serbia, which reported the antimicrobial activity of essential oils of carrot fruits and roots to be higher than the other investigated essential oils from different parts of the Carrot plant (Soković et al., 2009).

The variation in antimicrobial activity among the different parts of the carrot plant may be attributed to their different chemical compositions and concentrations of bioactive compounds. For instance, carrot root oil is rich in carotenoids and tocopherols, which may have antioxidant and antibacterial properties (El et al., 2014; Stanojević et al., 2023). Carrot seed oil is rich in sesquiterpenes and esters, which may also have antioxidant and antibacterial properties (Kataria, Kaur Chahal, et al., 2016).

### Zones of inhibition produced by disc diffusion method

The *Daucus carota* *L.* root, leaf, and seed oils were also tested for their antimicrobial activity against the four bacterial isolates using the disc diffusion method. Nigella sativa oil was also used as a positive control. The results are shown in Table 3.

<table>
<thead>
<tr>
<th>Names of Microbial isolates</th>
<th>Zones of inhibition produced by oil extracts (mm)</th>
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</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>11</td>
</tr>
</tbody>
</table>

These results are similar to those obtained by the Well diffusion method, except for some differences in the zones of inhibition. The zones of inhibition were generally higher by the disc diffusion method than...
by the well diffusion method for most of the isolates and oils. This may be due to the different diffusion rates and concentrations of the oils in the two methods.

The root oil exhibited the highest activity against *Strep. Pneumoniae*, surpassing the control oil. The leaf oil had slightly higher activity than the seed and root oils against *S. aureus* but less than the control.

Remarkably, Pseudomonas's susceptibility to all three oils increased significantly with the disc diffusion method as compared to the well diffusion method. The seed oil showcased the most extensive zone of inhibition (15mm), nearly doubling the well method's effect. The leaf and root oils also exhibited larger zones of inhibition, measuring 11mm and 14mm, respectively. These results are consistent with previous studies conducted in northern Tunisia, which reported the antimicrobial activity of carrot oils by disc diffusion method against different bacteria showed more activity against gram-positive than gram-negative (Rokbeni et al., 2013).

The results obtained from both the Well and disc diffusion methods indicate that gram-positive bacteria (Streptococcus and Staphylococcus species) were more sensitive to carrot oil extract especially the roots as compared to the tested gram-negative bacteria (Salmonella and Pseudomonas species). The decreased susceptibility of gram-negative bacteria to the oil extract could be a result of its structural modification, which has an additional outer layer (lipopolysaccharide) that prevents the penetration of the oils to their cell walls and makes them less susceptible to the oil extracts (Breijyeh et al., 2020; Ewansiha JU et al., 2020). This result is similar to a study conducted at the Federal University of Technology, Nigeria which revealed that gram-positive bacteria are more susceptible to carrot oils than gram-negative bacteria (Ewansiha JU et al., 2020). However, this is contrary to a study at the University of Abou Bekr Belkaid, Algeria, where the aqueous extract of carrot oils does not show any effect against the tested gram-positive bacteria (Bacillus cereus, Staphylococcus aureus) up to the value of 3000µg/mL (Dib et al., 2010). Staphylococcus aureus used in our study also showed a minimum zone of inhibition to most of the oil extract as compared to that of *Streptococcus* spp.

**CONCLUSION**

This study reveals that the oil extracts used from the different parts of the *Daucus carota* L. oils have antimicrobial activity against the four bacterial isolates, with higher activity against gram-positive bacteria than gram-negative bacteria. Among the different oil extracts, the root oil showed the most consistent and potent activity against most of the isolates, while the leaf oil showed the least activity. The disc diffusion method revealed higher activity of the oils than the well diffusion method, especially against Pseudomonas. The results suggest that the antimicrobial activity of *Daucus carota* L. oils could depend on the plant part, the bacterial isolate, and the test method used.

**Abbreviations**

AMR - antimicrobial resistance
P. aeruginosa - Pseudomonas aeruginosa

Strep. – Streptococcus

Mm – millimetre

N. sativa - Nigella sativa

Spp - Species

Declarations

Author contributions

AV, MK, and SCM were responsible for the conceptual and design of the study, and laboratory work. They also played a crucial role in drafting the manuscript and providing approval for the final version to be published. SCM, MK, TB, FM, MM, ML and EC contributed to data analysis and interpretation and also participated in the drafting of the manuscript. KMS, FSK, HS, ZG, and MSS were involved in recording data, ensuring the accuracy and completeness of the dataset. AV, JO, RC and DEL III reviewed the final version to be published. All authors have reviewed the manuscript, contributed their expertise, and endorsed its content before publication.

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Conflict of interest statement

The author declares that they have no competing interests.

Ethics statement

Permission to conduct the study was obtained from the Research and Innovation Committee of the Faculty of Pharmaceutical Sciences, College of Medicine, and the Allied Health Sciences University of Sierra Leone,

Availability of data and materials

Most of the data is included in the manuscript. Additional can be found from the corresponding author based on reasonable request.

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