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RESEARCH

Antioxidant and Antibacterial Activities of Crude Extracts of *Carissa spinarum* L. Leave

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Abstract

This study's goal was to assess the antioxidant potential of *Carissa spinarum* leaf extracts in methanol and ethyl acetate. Petroleum ether, chloroform, acetone, ethyl acetate, and methanol were among the solvents used to extract the *Carissa spinarum* leaf powder. Biochemical tests, specifically diphenylpicrylhydrazyl (DPPH) and hydrogen peroxide radical scavenging activity, were used to evaluate the extracts' antioxidant capacity. The findings showed that the majority of extracts had antibacterial activity and all extracts demonstrated antioxidant activity. Peroxide values ranged from 19.8 meq/kg (90% methanol) to 379 meq/kg (defatted with petroleum ether) at 70 °C, and from 3.1 meq/kg (90% methanol) to 119 meq/kg (defatted with petroleum ether) at room temperature. Ferric reducing antioxidant power was found to range between 336.58 ± 0.052 mgAAE/100 g extract (90% methanol) and 172.94 ± 0.032 mgAAE/100 g (petroleum ether). With a mean zone of inhibition ranging from 0 to 26 mm, crude extracts of *Carissa spinarum* leaves demonstrated varying degrees of antibacterial activity against a few common and drug-resistant microbial infections. The crude extract of *Carissa spinarum* leaves was found to have the strongest bactericidal activities on specific bacterial populations in 90% methanol and pure methanol solvent extracts, while having a weak antibacterial effect on *S. aureus*, *E. coli*, *S. aurens*, and *K. pneumoniae* in both petroleum ether and chloroform extracts.

Keywords: *Carissa spinarum*, free radicals, antioxidant activity, peroxide value, antimicrobial activity.

1. Introduction

Electrons typically form pairs in the structure of atoms and molecules, each of which moves inside a specific area of space known as an atomic or molecular orbital. Each pair of electrons has a spin quantum number of +1/2 for one and -1/2 for the other. Any species with one or more unpaired electrons that is capable of independent existence—thus the word "free"—is considered a free radical (Zeppilli and Orian, 2025). A significant amount of reactivity to the free radical is often provided by this unpaired electron or electrons (Ragi et al., 2025). Reactive oxygen species (ROS) include oxygen-centered radicals like superoxide anion (O_2•-), hydroxyl (HO•), alkoxyl (RO•), and peroxyl (ROO•) radicals; reactive nitrogen species (RNS) include agents like peroxynitrite anion (ONOO-) and nitric oxide (NO•) radical, among others; and non-free radical species (oxygen-centered non radical derivatives) include hydrogen peroxide (H_2O_2), singlet oxygen $1O_2$ (O-O:), nitric oxide (NO), and hypochlorous acid (HClO)(Ozcan and Ogun, 2015). Free radicals have a well-established dual function in our bodies as both harmful and helpful organisms. Free radicals are necessary for normal physiological processes at low to moderate quantities, but their overproduction is detrimental and leads to oxidative stress. Numerous disorders can result from oxidative stress's destruction to cell components, including lipids, proteins, RNA, and DNA (Tumilaar et al., 2024). A class of substances known as antioxidants can stop the harmful

effects of free radicals (Alkadi, 2020). As the name suggests, "antioxidants" are substances that have the ability to inhibit or stop other molecules from oxidizing. The protective mechanism of antioxidant compounds is based on neutralizing these reactive species by pairing the highly energetic reaction odd electron that causes the oxidation process, thereby preventing or delaying damage to cells and tissues (Flora, 2009).

Many natural medical plants and herbs have been studied as naturally occurring antioxidants, and the results have indicated that the raw extracts or isolated pure components from these plants worked better as antioxidants in vitro than well-known synthetic ones like vitamin E or BHT (Xu et al., 2017). Antioxidant qualities are found in substances including flavonoids, anthocyanins, tannins, dietary glutathioline, vitamins, and endogenous metabolites(Asif, 2015). By squelching singlet and triplet oxygen, breaking down hydrogen peroxide, and inhibiting enzymes, they can disrupt the generation of free radicals and render them inactive(Sundaram Sanjay and Shukla, 2021).

Additionally, plant phyto-constituents possess antibacterial qualities. The most significant naturally occurring antibacterial substances that come from plants are helpful as a backup plan to manage infection and illness. In vitro, compounds with antibacterial properties have been identified, including flavonoids, alkaloids, terpenoids, and tannins (Gurib-Fakim, 2006).

The World Health Organization (WHO) reports that over 80% of people in underdeveloped nations rely primarily on traditional medicines, the majority of which are derived from plants, for basic healthcare, with the remaining 20% also depending on plant-based goods (Dutta et al., 2012). The genus Otostegia is well-known for its therapeutic qualities among plants that have been utilized as traditional remedies. Otostegia species are obviously medicinally significant and have long been utilized for their antibacterial, antihyperglycemic, ophthalmia, mosquito-repelling, and antioxidant properties to prevent a variety of illnesses and conditions. Additionally, its chemical components have demonstrated sedative, anxiolytic, antidepressant, antiulcer, and antispasmodic properties (Abate and Yayinie, 2018). The plant *Carissa spinarum* is widely recognized in Ethiopia as "Tinjute" in the local Amharic language. It is prized for its odor, strong therapeutic benefits, and insecticidal qualities (Uddin and Rauf, 2012).

Numerous research indicate that Agam, or *Carissa spinarum*, may be utilized as a natural remedy or as a health-promoting substance for a range of conditions. Due to its insecticidal qualities, the plant is frequently used as a fumigant in homes and containers (Ayalew Tiruneh et al., 2022). The roots are used to cure lung conditions and are well known for their ability to repel insects, especially mosquitoes in the early evening, which are known to carry infections. Nevertheless, the plant leaf extract's antibacterial and antioxidant properties remained unaltered (Singh et al., 2012). Several studies revealed that *Carissa spinarum* extracts included a variety of phyochemicals, including flavonoids and tannin (Kemal et al., 2020). The many phyochemicals found in plants react differently to different solvents, which affects how the antioxidant and antibacterial properties of the plant leaf extract vary (Mehmood et al., 2022). Otostegia species have long been utilized in traditional medicine to treat a wide range of illnesses throughout Ethiopia (Karunamoorthi, 2014). Despite being an old plant, no documented records have demonstrated the antioxidant properties of *Carissa spinarum* leaf extract in various solvents. The number of bacterial species that are resistant to several drugs and antibiotics has increased recently. The use of commercial antimicrobial medications, which are frequently used to treat infectious disorders, has led to the development of human harmful microbes (Chinemerem Nwobodo et al., 2022). Scientists were compelled by this circumstance to look for novel antibacterial compounds from a variety of sources, including medicinal plants. Thus, the purpose of this work was to test the antibacterial properties of plant leaf extracts against gram positive and gram-negative bacterial species as well as to examine the antioxidant activity of *Carissa spinarum* leaf extracts and their impact on various solvents. Researchers will be able to utilize the current work as a hint to synthesize a medicine that will address the issue of drug-resistant bacterial species. Even though the plant is a well-known medicinal plant in Ethiopia, people cannot grow it on well-prepared ground because of a lack of education, awareness, and understanding about it. This study offers a possibility to increase people's awareness about growing the plant on small or large farms and using its leaves for their antibacterial and antioxidant properties.

2. Materials and Methods

2.1. Plant Material

Sus. Sci. Lett. (2025), 1(1): 55-63
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The *Carissa spinarum* leaves utilized in this study were collected from the area surrounding Debre Tabor town following the plant's September–October flowering season. After the *Carissa spinarum* leaves separated from the parent plant, they were cleaned with tape water to get rid of waste materials and dust. For nine days, the leaves were left in an open, shaded area at room temperature (23°C) to dry with fresh air away from heat and sunlight. The *Carissa spinarum* leaves were air-dried, sliced into small pieces, and ground into a consistent powder using a coffee grinder. They were then packaged in plastic bags and kept in the refrigerator until they were needed. Gram positive and gram negative bacteria, including *Shigella boydii*, *Escherichia coli*, *Staphylococcus aureus*, and *S. pneumonie*, were acquired from Bahir Dar and will be used to assess the antibacterial activity of *Carissa spinarum* leaf extracts.

2.2 Extraction of Plant Material

Four distinct solvents were used to extract the *Carissa spinarum* powdered leaves: petroleum ether, pure methanol, chloroform, and 90% methanol. For every extraction procedure, 30 grams of dry powder (ground material) leaves were left at room temperature with 300 milliliters of the solvents added to *Spinarum Carissa*. Following 48 hours of shaking the solutions with an electronic shaker, the extracts were filtered through Whatmann filter paper. Dried magnesium sulfate ($MgSO_4$) was then added to the extracts to eliminate any remaining water. A rotary evaporation process with reduced pressure and a maximum temperature of 35°C was used to concentrate the extracts. The yields were computed and stored in airtight bottles or in a refrigerator at 5°C. OI1, OI2, OI3, and OF4 are the names of the crude extracts that were produced using various solvents. For *Carissa spinarum* leaf extracts, these solvents were 90% methanol, 100% methanol, chloroform, and petroleum ether, respectively.

2.3 Defatting procedure

Petroleum ether and chloroform solvent were used in Soxhlet extraction procedures to defatten the dried powdered *Carissa spinarum* leaves. Two distinct thimble chambers were filled with thirty grams of the ground materials, which were then put inside the Soxhlet extractor. A 50 mL round-bottomed flask was filled with around 300 mL of petroleum ether and chloroform, each separately. In each flask, three boiling chips (sands) were added. On top of the flask, the soxhlet extractors were connected. The water flow was activated by connecting the condensers to the top of the Soxhlet extractor. For four to five hours, the equipment was kept above 65 degrees Celsius on the lampmaster. At last, the filtrate and residue were separated. To fully evaporate the solvents, the defatted components were placed in clean, dry paper at room temperature (23 °C) for 12 hours. 30 grams of defatted *Carissa spinarum* leaves were extracted using 90% methanol using the same protocol as non-defatted extraction techniques. For defatted samples made with petroleum and chloroform solvents, respectively, the samples were liable as ODPE and ODCF.

2.4 Procedure to measure the antioxidant activities

2.4.1 Reducing power assay

With few modifications, the Oyaizu method was used to determine the produced extracts' reduction power (Abate et al., 2017). For every solvent (90% methanol, pure methanol, chloroform, and petroleum ether), extracts of *Carissa spinarum* leaves at varying concentrations (12%, 24%, 36%, 48%, and (v/v)) were made. 2.5 ml of extract was extracted from each sample and combined with 2.5 ml of 1% potassium ferricyanide solution and 2.5 ml of 200 mM sodium phosphate buffer (PH = 6.6). For 20 minutes, the combinations were incubated at 50 degrees Celsius in a water bath. After adding 2.5 ml of a 10% w/v trichloroacetic acid (TCA) solution, the mixture was centrifuged for 10 minutes at 3000 rpm. Absorbance was measured at 700 nm after the top layer (2.5 ml) was combined with 2.5 ml of distilled water and 0.5 ml of a ferric chloride solution (0.1% w/v).

2.4.2. Peroxide value determination

Based on the procedures outlined below, the peroxide value is the number that represents the amount of peroxide present in 1000 g of the material in milliequivalents of active oxygen. Because Niger seed oil is a valuable edible oil source in Ethiopia and includes linolic acid, a crucial component for antioxidant studies, it was used to determine the peroxide value (McCord, 2000). Niger seed oil and OI1, Niger seed oil and OI2, Niger seed oil and OI3, Niger seed oil and OI4, Niger seed oil and OPE, Niger seed oil and OCF, and Niger seed oil and AA are in fourteen distinct samples. Seven samples were kept at room temperature, while the remaining seven were kept at 70 degrees Celsius. Five grams were extracted from each sample and put into twenty-one separate 250 ml conical flasks. Each sample received 30

Sus. Sci. Lett. (2025), 1(1): 55-63
https://doi.org/10.59983/ssl2025xxxx

milliliters of a glacial acetic acid and chloroform (3:2) combination. In addition to adding 0.5 ml of saturated potassium iodide solution to each flask that was kept at room temperature (22 °C), 75 °C, and 100 °C, the mixtures were shaken to dissolve them and then shook for one minute. After adding 30 milliliters of water, a 0.01N sodium thiosulfate solution was used for titration. To signal the conclusion of the titration, 5 ml of starch solutions were added to each sample once the yellow tint had vanished. With constant shaking, the titrant was gradually added till the blue hue was released. The same conditions were used for a blank determination. The levels of peroxide were computed.

2.5 Antibacterial Activity Determination procedures

2.5.1 Disk diffusion methods

The antibacterial activity of six samples of *Carissa spinarum* leaf extracts (OI1, OI2, OI3, and OF4), ODPE, and ODCF, was assessed using the agar well diffusion test on Muller agar (MHA) medium. To create a solid plate, the MHA was melted, cooled, and then transferred into sterilized Petri dishes. The agar plate surface was then streaked with standardized inoculums (0.5 McFarland). A

sterile cork borer (6 mm diameter) was used to create wells in the seeded agar plates. Carefully, 100 µl of the test chemical or crude extract was poured into each well. In tandem with other control antibiotics, this was carried out three times. At 37°C, the extracts were left to diffuse for roughly two hours. Following an overnight incubation period, the plates were examined for the zone of inhibition, and measurements were made of the inhibition zone's diameter against *Shigella boydii*, *Escherichia coli*, *Staphylococcus aureus*, and *S. pneumoniae*.

Statistical methods

Values represent the mean of three repetitions ± standard deviation (SD), and all measurements were performed in triplicate (n=3). Analysis of variance (ANVOA) was performed on the results. Origin 8 software was used to display the graphs and the correlation coefficient (R^2). The least significant difference test was used to calculate the mean difference, and a significance level of $P < 0.05$ was considered to be significant.

3. Results and Discussion

Phytochemical Screening tests

The phytochemical analyses of different solvent extract of the *Carissa spinarum* leaves were showed in table 1.

Table 1. Results of the screening test extracted by various solvents

S.No.	Solvents used for extraction and test results				
	Phytochemicals	**Pet. ether**	**Chloroform**	**Pure CH_3OH**	**90% CH_3OH**
1	Phenol	-	-	++	+++
2	Glycoside	+++	++	-	-
3	Saponin	-	-	-	-
4	Terpenoid	+++	++	-	-
5	Alkaloid	+++	+	-	-
6	Steroid	++	-	-	-

Antioxidant potential determination of extracts of *Carissa spinarum* leaves. Antioxidant activity by ferric reducing power, FRAP

To calculate the antioxidant activity of *Carissa spinarum* leaf extracts in terms of ascorbic acid equivalent, a calibration curve was created. The calibration curve was plotted as uv-response (absorbance) verses concentration of ascorbic acid (0.1, 0.3, 0.5, 0.7) mg/ml and the value of the absorbance obtained corresponding to concentration are given in Table 2 with linear regression coefficient (R^2) 0.99437.

Table 2: Absorbance of AA at various concentrations at 700 nm

Concentration (mg/ml)	Absorbance
0.09	0.1525 ± 0.001^a
0.29	0.287 ± 0.011^b
0.48	0.474 ± 0.023^c
0.69	0.619 ± 0.012^d

Values are mean ±SD of triplicate analysis. Different superscript letters within columns showed significant

Using the potassium ferricyanide reduction method, the reducing power of several solvent extracts of *Carissa spinarum* leaves—which could be a major indicator of the

Sus. Sci. Lett. (2025), 1(1): 55-63
https://doi.org/10.59983/ssl2025xxxx

antioxidant activity—was assessed. Absorbance per particular amount of *Carissa spinarum* leaf extracts (12%, 24%, 36%, and 48%) (v/v) was used to express the reducing power. Every extract showed some lowering power, according to the graph and data analysis. As concentration rises, so does the decreasing power. Color intensity is a qualitative way to convey it.

The 90% methanolic extract showed the highest reductive capacity among the various extracts, followed by pure methanol. The order of the reduction power of the various extracts was OI1 > OI2 > ODPE > ODCF > OI3 > OI4. This suggests that polar molecules, which are better extracted using highly polar solvents, are more responsible for antioxidant actions. This conclusion is supported by the data that Bushra Sultana and colleagues provided. The antioxidant activity of several medicinal plant extracts' leaves, barks, and fruits has been shown to have a lower value in pure methanol extract and a higher value in aqueous 80% methanol extracts (Birben et al., 2012). The strength of solvent polarity is to blame for this. The plant extracts' increased absorbance suggests that the tested sample's reducing power and reductive capacity have increased. The reduction power of the *Carissa spinarum* leaf extracts serve as a key indicator of its antioxidant potential action.

The FRAP method was used to assess the antioxidant activity of extracts from defatted and non-defatted *Carissa spinarum* leaves. Plant leave extracts which is defatted with chloroform had higher absorbance than defatted with petroleum ether at particular concentration. The absorbance rises with increasing concentration in every sample. This demonstrates how concentration and defatting have a significant impact on the antioxidant properties of *Carissa spinarum* leaf extracts.

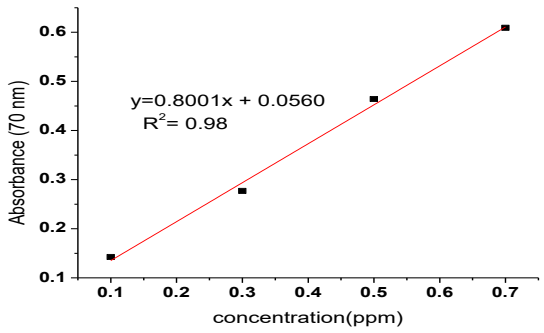

Figure 1: Calibration curve that shows the absorbance of ascorbic acid at different concentration at 700 nm.

Figure 2: The absorbance of different solvent leave extracts of O. integrifolia at 700 nm.

In comparison to a 90% methanolic extract of the plant's leaves without defatting, the reducing power of the *Carissa spinarum* leaf extract made with 90% methanol after the powder was defatted using petroleum ether and chloroform was examined. The outcome demonstrated that *Carissa spinarum* leaf extracts, both defatted and non-defatted, have the ability to lower ions. The sample's capacity to convert Fe^{3+} to Fe^{2+} was demonstrated by the increase in absorbance that accompanied concentration. The results also demonstrated that *Carissa spinarum* did not exhibit greater ferric ion reduction activity in any of the defatted leaf extracts compared to petroleum ether and chloroform defatted extracts. The higher heat used to defatten the plant's powdered leaves may have forced some antioxidant compounds into the sample and mixed them with solvent or inactive bioactive compounds, which could explain why petroleum ether and chloroform defatted extracts have a lower reduction capacity than the majority of non-defatted extracts.

Both the defatted and non-defatted extracts of *Carissa spinarum* leaves had reduction powers that were measured in milligrams of ascorbic acid equivalent per 1000 grams of day weight of ascorbic acid equivalent capacity (mgAAE/1000 g).

Table 3: Ferric reducing antioxidant power (FRAP) of the *Carissa spinarum* leaves extracts with different solvent (mgAAE/1000 g ext.).

FRAP	Samples					
	OI_1	OI_2	OI_3	OI_4	ODCF	ODPE
mgAAE/1000g ext.	330.0 ± 0.039	261.8±0.048	36.69 ±0.02	23.39 ±0.01	62.4 ±0.05	109.4 ±0.04

The ascorbic acid equivalent capacity (AAE) was used to express the reduction power of various solvent extracts of *Carissa spinarum* leaves. OI1 had the highest score (330 ± 0.04), while OI4 had the lowest (23.35 ± 0.01). These demonstrated that 90% aqueous methanol extracts had the highest reducing power (highest antioxidant capabilities), while PE extracts had the lowest reducing

Sus. Sci. Lett. (2025), 1(1): 55-63
https://doi.org/10.59983/ssl2025xxxx

power. This reduces the capacity to change Fe3+ into Fe2+.

Determination of peroxide value

Using the parameters peroxide value and value, the oxidation stability of sun flower oil was measured at three different temperatures (23, 70, and 100 degrees Celsius).

Figure 3 displays the sunflower oil's peroxide levels at 22 °C with and without antioxidant. The peroxide value (POV) increased for the last two weak treatments that included extracts from *Carissa spinarum* leaves, as the figure and table shown. Oils with OI1, OI2, and ODCF had lower peroxide values than the ascorbic acid value across all treatments, while oils with OI3, OI4, ODCF, and ODPE showed greater peroxide values than the ascorbic acid (AA) POV throughout all treatments. The peroxide value of oil, both with and without antioxidants, increases with the number of storage days. This indicates that the oxidation of sunflower oil resulted in an increase in the primary product (peroxide) and secondary products (aldehyde and ketone) (Alisi et al., 2018). This suggests that at normal temperature, OI1 and OI2 significantly limit the oxidation of sunflower oil, which is then followed by AA. OI1 and OI2 exhibited the highest antioxidant activity, respectively, based on this data. At normal temperature (22°C), 90% methanol extracts and pure methanol extracts exhibited higher antioxidant values than the well-known synthetic antioxidant ascorbic acid (AA).

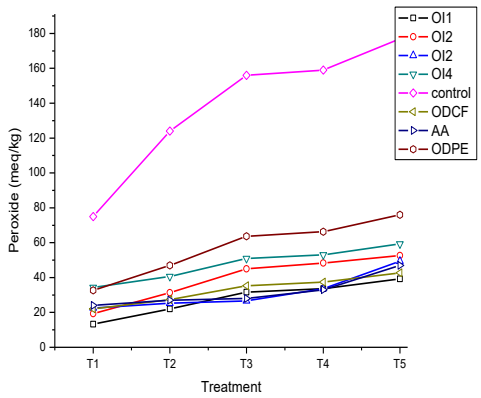

Figure 3: Change in peroxide value (meq/kg) during storage at 22oc with different treatments.

In practically all treatments, the POV of sun flower oil containing OI1, OI2, ODPE, and AA did not significantly differ during storage at 70°C; however, the oil including OI1 had the lowest POV value. After the fourth treatment, the POV of oil containing OI3, OI4, ODCF, and oil without extracts did not significantly differ from one another. The highest value was observed with samples that were not extracted, followed by OI4 in all treatments. The POV of the sun flower oil was 0.2 meq/kg when it was first acquired, but it rose to 247.8 meq/kg in the fifth treatment at 70°C and 370 at 100°C. Overall, this shift in point of view revealed a discernible oxidation of sunflower oil. The graphs and data showed that the control's point of view (POV) was higher than that of all other treatments throughout all storage days and temperature ranges.

Sunflower oil with all extracts had a greater POV at 100°C than it did at 70°C and 22°C, indicating that more iodine was librated at higher temperatures. The amount of sodium thiosulfate ($Na_2S_4O_6$) solution is also large, which results in a higher peroxide value and changes the color of the purple iodine to colorless. However, as the temperature rises and the extract's antioxidant activation decreases, the value difference between samples gets smaller. Both the control and OI1 POVs were nearly identical, and they required nearly the same quantity of sodium thiosulfate (($Na_2S_4O_6$) to react with I2 Scheme 1.

According to the aforementioned observation, sunflower oil's oxidation decreased at room temperature. As temperature and storage days rise, more titrant (S2O32-) is required to alter the sample's color. This is because peroxide and iodine ions in aqueous medium reacted to form an increasing amount of I2. The amount of PV increases (decreases the antioxidant property) when iodine synthesis is high because it requires a high amount of S2O32- to fully react with it.

Peroxide + KI + H+ I_2 ⟶

I_2 (purple) + $2Na_2S_2O_3$ $Na_2S_4O_6$ + 2NaI (colorless)

Scheme 1: The reaction of peroxide with iodine ion in aqueous media.

Sus. Sci. Lett. (2025), 1(1): 55-63
https://doi.org/10.59983/ssl2025xxxx

According to the aforementioned observation, sunflower oil's oxidation decreased at room temperature. As temperature and storage days rise, more titrant ($S_2O_3$2-) is required to alter the sample's color. This is because peroxide and iodine ions in aqueous medium reacted to form an increasing amount of I_2. The amount of PV increases (decreases the antioxidant property) when iodine synthesis is high because it requires a high amount of $S_2O_3$2- to fully react with it.

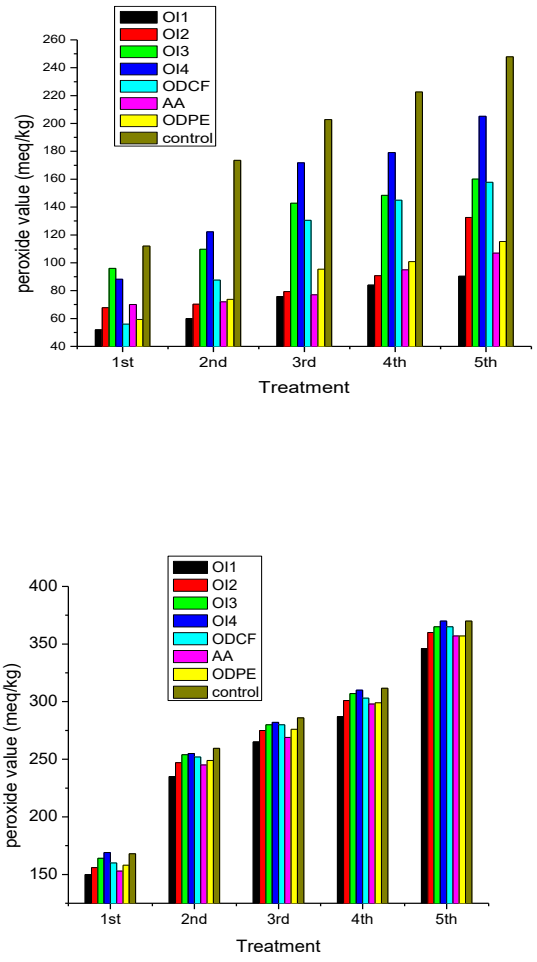

Figure 4: Change in peroxide value (meq/Kg) during storage at different temperature range 70oc (above) and 100oc (below)

Determination of antimicrobial activity of *Carissa spinarum* leave extracts

Agar well diffusion method

Gram positive and gram negative bacterial species were significantly inhibited by several leaf extracts from *Carissa spinarum*. The inhibitory zones against each examined microorganism (gram positive and gram negative) were produced by each solvent extract of *Carissa spinarum* leaves and a control.

A good inhibitory zone against each bacterium species (*K. pneumonia*, *E. coli*, *S. pneumonia*, and *S. aurens*) was demonstrated by several solvent extracts of Carissa spinarum leaves, including 90% methanol, pure methanol, petroleum ether, and chloroform. The inhibitory zone of *Carissa spinarum* leaf extracts in 90% methanol, pure methanol, chloroform, and petroleum ether was greater than that of penicillin (8 ± 0.00) against K. pneumonia, E. coli, *S. pneumonia*, and *S. aurens*. With the exception of S. *aurens* species, none of the extracts showed an inhibitory zone against *K. pneumonia, E. coli*, or *S. pneumonia*.

In comparison to other more polar solvents like pure methanol and chloroform, the inhibition zone evaluated with 90% methanol extracts was larger. Aqueous methanolic leaf extracts of *Carissa spinarum* have superior antibacterial activity compared to other solvents. It suggests that either the bioactive component is more miscible in aqueous methanol than the other two polar solvent extracts of *Carissa spinarum* leaves, or the microorganisms may be comparatively resistant to the plant molecule that is extracted by $HCCl_3$ and CH_3OH. The non-polar petroleum ether leaf extracts of *Carissa spinarum* have the lowest inhibition zone against K. pneumonia and the highest against S. aurens. This suggests a strong antibacterial response from non-polar solvent extracts against S. aurens.

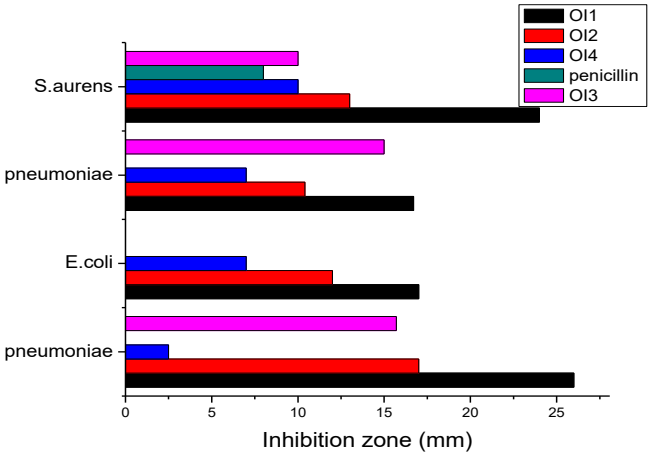

Figure 5: Comparison of inhibition zone among crude extracts of leaves of Carissa spinarum using different solvent.

The study's findings demonstrated that, in the agar well diffusion assay, every isolated bacterium was vulnerable to every solvent extract of *Carissa spinarum* leaves. Extracts from *Carissa spinarum* leaves will therefore offer efficient means of managing microbial illness. Compared to the control, all of the solvents utilized to extract *Carissa spinarum* leaves exhibited potent antibacterial properties. Therefore, the fact that plants have antibacterial substances that can potentially suppress the development of tested pathogens before commercial antibiotics are employed in clinical settings is encouraging. It also provides a clue for the synthesis of antibacterial medications that are more effective than commercial antibiotics.

4. Conclusion

The antioxidant activity of several extracts of *Carissa spinarum* leaves, both defatted and non-defatted, was assessed in this work. The study's findings unequivocally showed that all examined extracts, both defatted and non-defatted, exhibited antioxidant activity when tested using the peroxide technique and reduced antioxidant capacity. 90% methanol, the extraction solvent, demonstrated the highest reducing capacity and the lowest peroxide value in all antioxidant activity measurements. When compared to the corresponding defatted leaf extracts of *Carissa spinarum*, which were obtained using 90% methanol and pure methanol, none of the defatted *Carissa spinarum* leaf extracts exhibited the highest

antioxidant properties; however, none of the defatted extracts displayed lower antioxidant activities than the defatted ones, which were obtained using petroleum solvent and chloroform. 90% methanol, the extraction solvent, demonstrated the highest reducing capacity and the lowest peroxide value in all antioxidant activity measurements. When compared to the corresponding defatted leaf extracts of *Carissa spinarum*, which were obtained using 90% methanol and pure methanol, none of the defatted *Carissa spinarum* leaf extracts exhibited the highest antioxidant properties; however, none of the defatted extracts displayed lower antioxidant activities than the defatted ones, which were obtained using petroleum solvent and chloroform.

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References

Abate, L., Abebe, A., Mekonnen, A. (2017) Studies on antioxidant and antibacterial activities of crude extracts of Plantago lanceolata leaves. Chem. Int 3:277-287.

Abate, L., Yayinie, M. (2018) Effect of solvent on antioxidant activity of crude extracts of Otostegia integrifolia leave. Chem Int 4:183-188.

Alisi, C., Asiwe, E., Ene, C., Alisi P. (2018) Antioxidant and free radical scavenging properties of aqueous extract of Psidium guajava leaf. FUTO J Ser 4:222-234.

Alkadi, H. (2020) A review on free radicals and antioxidants. Infectious disorders-drug targets (Formerly current drug targets-infectious disorders) 20:16-26.

Asif, M. (2015) Chemistry and antioxidant activity of plants containing some phenolic compounds. Chemistry international 1:35-52.

Ayalew Tiruneh T., Ayalew Tiruneh G., Chekol Abebe E., Mengie Ayele T. (2022) Phytochemical Investigation and determination of antibacterial activity of Solvent leave extracts of *Carissa Spinarum*. Infection and Drug Resistance:807-819.

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S., Kalayci O. (2012) Oxidative stress and antioxidant defense. World allergy organization journal 5:9-19.

Chinemerem, Nwobodo, D., Ugwu, M.C., Oliseloke Anie, C., Al-Ouqaili, M.T., Chinedu Ikem, J., Victor Chigozie, U., Saki M. (2022) Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. Journal of clinical laboratory analysis 36:e24655.

Dutta, A.K., Gope, P.S., Makhnoon, S., Rahman, S., Siddiquee, M.A., Kabir, Y. (2012) Effect of solvent extraction on phenolic content, antioxidant and α-amylase inhibition activities of *Swertia chirata*. Int J Drug Dev Res, 4:317-325.

Flora, S.J. (2009) Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. Oxidative medicine and cellular longevity, 2:191-206.

Gurib-Fakim, A. (2006) Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular aspects of Medicine, 27:1-93.

Karunamoorthi, K. (2014) Tinjute [Labiatae;(Otostegia integrifolia)]: A versatile Ethiopian ethnomedicinal plant-a systematic review of the scientific evidences. CellMed, 4:8.1-8.6.

Kemal, J., Alemu, S., Tsegaye, B., Tamerat, N. (2020) Study on ruminant tick infestation, phytochemical analysis and in vitro acaricidal effect of Calpurnia aurea and Otostegia integrifolia extracts on Amblyomma variegatum. Ethiopian Veterinary Journal 24.

McCord, J.M. (2000) The evolution of free radicals and oxidative stress. The American journal of medicine 108:652-659.

Mehmood, A., Javid, S., Khan, M.F., Ahmad, K.S., Mustafa, A. (2022) In vitro total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems. BMC chemistry, 16:64.

Ozcan, A., Ogun, M. (2015) Biochemistry of reactive oxygen and nitrogen species. Basic principles and clinical significance of oxidative stress, 3:37-58.

Ragi, C., Sumayya, P., Muraleedharan, K. (2025) Thermodynamics of free radical scavenging activity of deprotonated hibiscetin towards reactive oxygen and nitrogen species. Chemical Physics:112768.

Singh, B., Singh, P.R., Mohanty, M.K. (2012) Toxicity of a plant based mosquito repellent/killer. interdisciplinary toxicology, 5:184.

Sundaram Sanjay, S., Shukla, A.K. (2021) Free radicals versus antioxidants, Potential therapeutic applications of nano-antioxidants, Springer. pp. 1-17.

Tumilaar, S.G., Hardianto, A., Dohi, H., Kurnia, D. (2024) A comprehensive review of free radicals, oxidative stress, and antioxidants: Overview, clinical applications, global perspectives, future directions, and mechanisms of antioxidant activity of flavonoid compounds. Journal of Chemistry 2024:5594386.

Uddin, G., Rauf, A. (2012) Phytochemical screening, antimicrobial and antioxidant activities of aerial parts of Quercus robur L. Middle-East journal of medicinal plants research, 1:1-4.

Xu, D.P., Li, Y., Meng, X., Zhou, T., Zhou, Y., Zheng, J., Zhang, J.J., Li, H.B. (2017) Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. International journal of molecular sciences, 18:96.

Zeppilli, D., Orian, L. (2025) Concerted proton electron transfer or hydrogen atom transfer? an unequivocal strategy to discriminate these mechanisms in model systems. Physical Chemistry Chemical Physics, 27:6312-6324.