Kurdistan Regional Government-Iraq
Ministry of Higher Education & Scientific Research
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Antibacterial Activity of plants Extracted from Traditional Kurdish medicinal

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Abstract:

Emerging evidence of plant compound effects on micropathogen growth inhibition has opened up new avenues for assessing plant treatment properties. The purpose of this study was to investigate the in vitro antioxidant and antibacterial properties of secondary metabolites isolated from various extracts of Calendula Officials, Rhus Coriaror and antibiotics (Cephalexin). Antibacterial activity against pathogenic bacteria was assessed using the well and disk diffusion method. According to the study, the Rhus Coriaror plant inhibited microorganism growth more effectively than Calendula and the antibiotics we used. During study Gram-positive (*Bacillus*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria were examined. *Pseudomonas aeruginosa* had a 34-mm zone of inhibition, while *Escherichia coli* had a 33-mm zone. This suggests that the plant extract contains compounds that effectively inhibit the growth of *Pseudomonas aeruginosa* and *Escherichia coli*. Calendula plant demonstrated the lowest antibacterial activity against *Pseudomonas aeruginosa* 7 mm and *Bacillus cerus* 8 mm. While inhibition zone diameters indicate antimicrobial activity, additional research, such as minimum inhibitory concentration (MIC) assays, is required to determine the concentration needed for effective inhibition. This work confirms the antimicrobial activity of assayed plants and suggests further examination to identify the chemical structure of their antimicrobial compounds.

Keywords: Rhus Coriaror, Calendula Officinals, antioxidant, antibacterial, Bacillus.

1. Introduction

Nearly a quarter of the world's drugs are botanically derived, either directly extracted from plants or synthesized based on herbal compounds. Due to the involvement of oxidative stress in a variety of disorders, aging and cancer, the evaluation of novel herbal antioxidant drugs to prevent or reduce the risk of diseases has received increasing attention (Noguchi and Niki 2000).

Plant secondary metabolites such as phenolics and flavonoids, which are present in all parts of plants, have the potential to neutralize free radicals Hence, due to the high prevalence of chronic diseases, using of plants to provide essential antioxidants, especially plants with high content of phenol and flavonoids is warranted (Mathew and Abraham 2006).

On the other hand, infectious diseases are one of the most important causes of mortality, and a large number of outbreaks are associated with these infections such as avian flu influenza A, tuberculosis, Emergomyces canadensis and diarrheal diseases, which require antibiotic treatment (Michaud 2009). The application of synthetic antibiotics has led to the emergence of resistant bacteria, especially, *Escherichia coli, Pseudomonas, Bacillus* (Mangione-Smith, *et al.,* 2006). So antibiotic resistance is an account for the treatment failure of infectious diseases all over the world in recent years (Watkins, 2018).

Decreasing the clinical efficacy of many antibiotics with the emergence of increasing antibiotic resistance by microbial infectious agents has raised the screening of herbs with antimicrobial activity in the treatment of infectious diseases (Lanteri, *et al.,* 2019). Herbal medicines are widely prescribed due to their natural sources, fewer side effects, relatively low costs and more efficacies.

Hence, researchers have focused on medicinal plants and traditional medicine to find more effic.ient drugs against microbial infections (Kabra and Lodha, 2013). In recent research, aqueous and organic extracts of medicinal herbs were studied against the bacterial species including, pseudomonas, Bacillus subtilis, resistant strains of Escherichia coli. They showed substantial antimicrobial properties, the highest antibacterial activity was related to Calendula official's, Rhus coriaria (Essawi, and Srour, 2000).

Moreover, the effect of 45 species from 30 plant families that were applied in Kurdistan traditional medicine for the treatment of infectious diseases and burns were evaluated on different bacterial strains including Bacillus cereus, *Escherichia coli* and *Pseudomonas*. One of the herbal remedies in traditional medicine is the Asteraceae as a medication for the heart, focus on the senses (Hosseinzadeh *et al.,* 2003).

Calendula officinal with the scientific name of Asteraceae, contains flavones and polyphenols, as well as resin aromatic compounds (cinnamic acid, benzoic acid, phenyl ether benzoate and hydro benzoic acid) (Bucur *et al.,* 2008). Until now, about 42 compounds with anti-inflammatory and anti-infectious properties and anticancer and antidiabetic are known in the Calendula flowers such as limonene hydrocarbons, squalene, monoterpene and tertiary hydrocarbons (Ielpo *et al.,* 2000).

Rhus coriaria a member of family Anacardiaceae this diverse family of flowering plants boasts over 800 species found in tropical and subtropical regions around the world, for the treatment of various diseases such as wound healing, antioxidant, antimicrobial activity (Havasian *et al.*, 2013).

The aim of studying the antibacterial activity of medicinal plants:
1.Identify antibacterial compounds in plants.
2.Evaluate their effectiveness against bacteria.
3.Understand their mechanisms of action.
4. Explore synergies with existing antibiotics.
5. Assess safety and toxicity.
6.Contribute to the development of new antibacterial therapies.

2. **Materials and Methods**:

2.1. Plant material:

2.1.1. Preparation of plant material

The fresh leaves, inflorescence and shoots of (Calendula and Rhus) (Figure 2.1), the fresh leaves, cones and shoots were harvested, rinsed with tap water and air dried under shade and reduced to coarse powder and then micronized to fine powder using the electric blender. The powder was stored in an airtight paper bag until required.

Figure 2.1: A/ Calendula Official’s, B/ Rhus Coriaria.

2.1.2. Preparation of the ethanolic extracts

The preparation of the different parts extracts was performed following the methods described by (Verpoorte, *et al.*,1982). 20 grams of the powder were extracted with 200 ml of solvent (ethanol) contained in a 500 ml sterile conical flask and covered with cotton wool plug and wrapped with aluminum foil. Extraction was allowed to proceed for 48 h in cooler. The extract was filtered using a clean muslin cloth and then Whatman No. 1 filter paper. The filtrate was then evaporated to dryness using a rotary evaporation attached to a vacuum pump.

2.2. Antibacterial evaluation:

2.2.1. Tested microorganism:

Bacteria which were used in the process of investigation, Bacterial strains include one gram positive bacteria (*Bacillus cerus)* and two gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). The bacterial samples are frozen at -4C° in cooled incubator, later reactivated before it's used.

2.2.2. Method of antibacterial evaluation:

The antibacterial activity of the two types of plant extracts were tested against two strains of bacteria using agar- well diffusion method (Turkoglu *et al.*, 2007). From the frozen bacteria inoculation was done into nutrient agar media and Blood agar, and incubated at 37C for 24hr.

Table 2.1: Scientific classification of Selected plants.

***Calendula official's* (Gandolfo, *et al.*, 2011)**	***Rhus coriaria (* Harrington, and Gadek 2010)**
Kingdome: plantae	Kingdome: plantae
Class: Magnoliopsida	Calss: Magnoliopsida
Order: Asterales	Order: Sapindales
Family: Asteraceae	Family: Anacardiaceae
Genus: Calendula	Genus: Rhus

3. Results and Discussion:

The results suggest that Rhus extracts possess stronger antimicrobial properties compared to Calendula extracts. This difference may be attributed to variations in the chemical composition of the two plant species, leading to differential antimicrobial activity.

Of ethanol extracts tested, the Rhus extract showed the highest antibacterial activity against some strains, such as *Pseudomonas aeruginosa* (34 mm), and *Escherichia coli* (33 mm) (Table 3.1). The studied results similar with research by (Kadi *et al.,* 2011), the aqueous macerate extract showed the highest antibacterial activity against some strains, such as Pseudomonas aeruginosa (24.4mm), and Escherichia coli (23.3mm; 23.85 mm) respectively for aqueous and ethanol macerates. Further investigation into the active compounds present in Rhus extracts and their mechanisms of action could provide insights into its efficacy against bacteria.

Therefore, the minimum antibacterial activity showed in Calendula plant, such as *Pseudomonas aeruginosa* (7 mm) and *Bacillus cerus* (8 mm), this may be related to the strong activity of microorganisms in Calendula plant (Table 3.1).

The disc diffusion bioassay showed that Rhus extracts have the highest activity against all Gram-positive bacteria and they also showed good activity against Gram-negative bacteria. The reason for different sensitivity between Gram positive and Gram-negative bacteria could be ascribed to the morphological differences between these microorganisms. Gram negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while prions constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da (Nikaido and Vaara, 1985). The Gram-positive bacteria should be more susceptible since they have only an outer peptidoglycan layer which is not an effective permeability barrier (Scherrer and Gerhardt, 1971).

In other hands, the antibacterial activity of medicinal antibiotics was no inhibited, it means all three bacteria having high activity growth during using antibiotics discs. According to the results, it means traditional medicine having higher antibacterial activity than industrial medicine.

According to Figure 3.1, plate A showed that the clear circle created by Rhus coriaria inhibited the *Pseudomonas* bacteria, but in contrast, plate B did not show any antibiotic circles because of the fast growth of *Pseudomonas* bacteria and the low antibacterial activity of the type of antibiotics we used (Kabra and Lodha, 2013).

The inhibition zone observed in Figure 3.2 Plate A and B, indicates the effectiveness of Rhus coriaria and Calendula plants against E. coli bacteria. The inhibition zone refers to the area around the plant extract where bacterial growth is inhibited or reduced, typically due to the antimicrobial properties of the plant compounds.

In Figure 3.2, it's noted that the inhibition zone created by Rhus coriaria against E. coli bacteria is very clear. This suggests that the plant extract contains compounds that are highly effective at inhibiting the growth of E. coli. The clarity of the inhibition zone indicates a strong antimicrobial activity, which could be attributed to the presence of bioactive compounds such as tannins, flavonoids, or other secondary metabolites known for their antimicrobial properties (Kadi *et al.*, 2011).

On the other hand, the inhibition zone observed with Calendula plants against E. coli bacteria is not as clear. This suggests that the antimicrobial activity of Calendula against E. coli might be less potent compared to Rhus coriaria. The lack of a well-defined inhibition zone could indicate a weaker effect or possibly a lower concentration of antimicrobial compounds in the Calendula extract.

Several factors could contribute to the variation in antimicrobial activity between different plant extracts. These include variations in the concentration and types of bioactive compounds present, as well as differences in the extraction methods used to isolate these compounds from the plants.

In Figure 3.3, Plate A and B, the inhibition zones created by Rhus coriaria against Bacillus bacteria are notably clear, indicating a potent antimicrobial effect. This suggests that the plant extract contains compounds that are highly effective at inhibiting the growth of Bacillus bacteria. The clarity of the inhibition zone signifies strong antimicrobial activity, likely due to the presence of bioactive compounds such as tannins, flavonoids, or other secondary metabolites known for their antimicrobial properties.

Comparatively, the effectiveness of antibiotics against *Bacillus* bacteria, as depicted in Figure 3.3 (Plate B) and with activity zones ranged from 19, 20 and 18 mm, result in reduced bacterial growth. This could imply that the antibiotics used in the experiment might be effective against the specific strain of Bacillus bacteria tested.

While the inhibition zone diameters provide an indication of antimicrobial activity, additional studies, such as minimum inhibitory concentration (MIC) assays, are necessary to determine the concentration required for effective inhibition. Furthermore, assessing the cytotoxicity of these extracts on mammalian cells would be crucial for evaluating their safety and potential therapeutic applications (Watkins, 2018).

The obtained results might be considered sufficient to further studies for the isolation and identification of the active principles and to the evaluation of possible synergism among extract components for their antimicrobial activity. Investigations are in progress to determine the degree of toxicity of these extracts.

Table 3.1: Antibacterial activity of the Calendula, Rhus coriaria and Antibiotics.

Plant Name	**Zone of inhibition (mm)**		
Bacteria Species	***Bacillus***	***Pseudomonas***	***E. coil***
Rhus coriaria	30	34	33
	19	–	–
	–	16	19
Calendula	8	–	9
	–	–	–
	–	7	–
Antibiotics	19	–	–
	20	–	–
	18	–	–

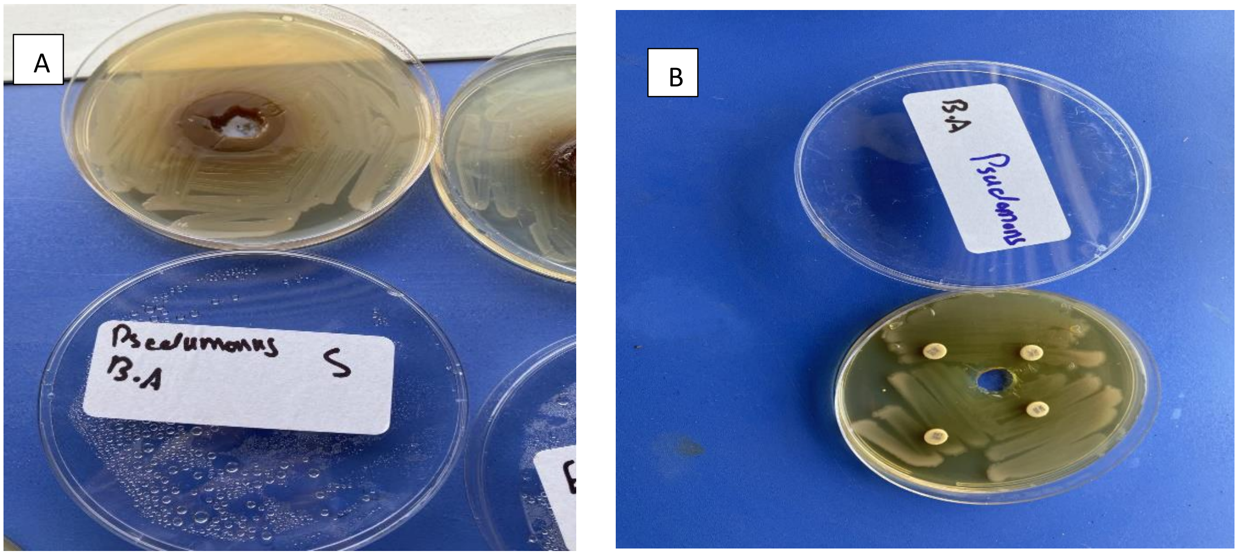

Figure 3.1: **A/** Antibacterial activity of Rhus coriaria inhibited by *Pseudomonas*. **B/** Antibacterial activity of Antibiotics inhibited by *Pseudomonas.*

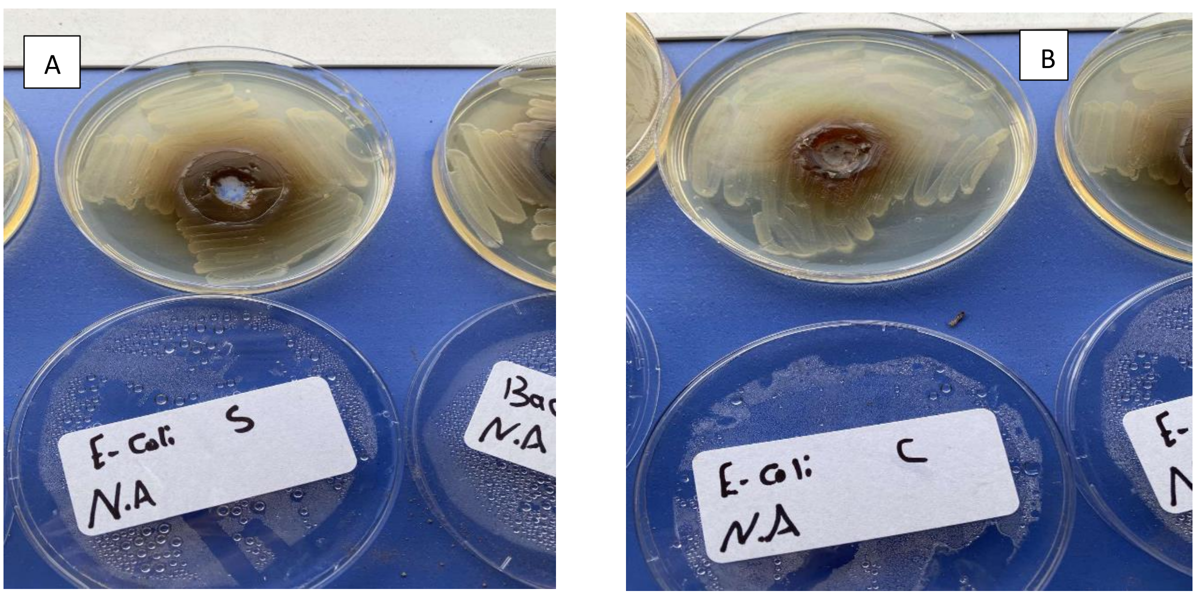

Figure 3.2: **A/** Antibacterial activity of Rhus coriaria inhibited by *E. coli*. **B/** Antibacterial activity of Calendula inhibited by *E. coli*.

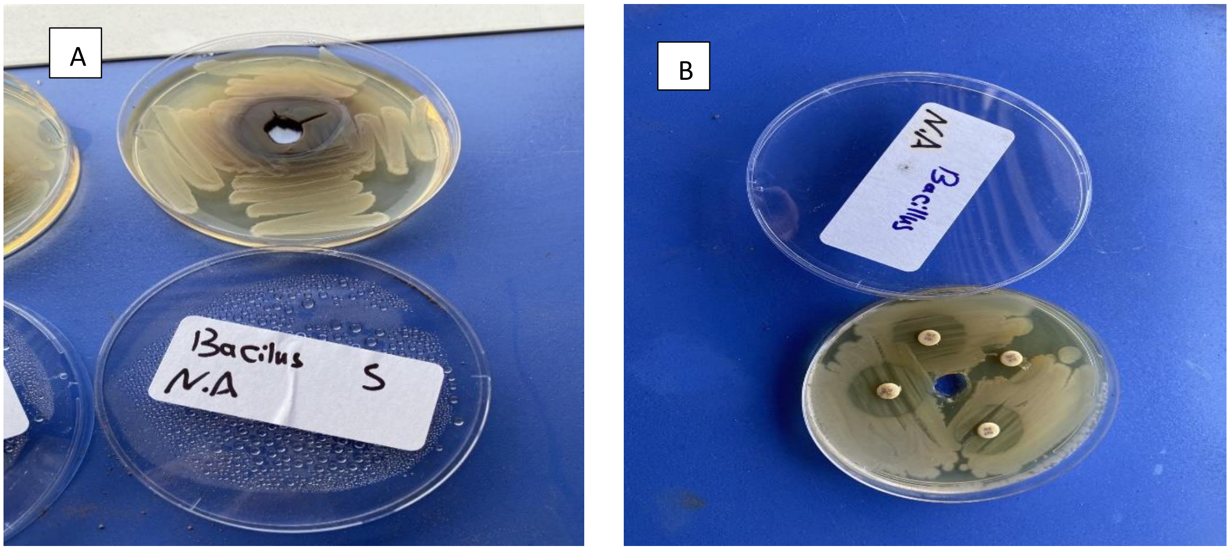

Figure 3.3: A/ Antibacterial activity of Rhus coriaria inhibited by *Bacillus.* **B/** Antibacterial activity of Antibiotics inhibited by *Bacillus.*

Conclusion:

In conclusion, the study demonstrates varying degrees of antimicrobial activity between Calendula and Rhus extracts against Bacillus, *Pseudomonas*, and *E. coli*. Rhus extracts exhibit significantly stronger inhibition zones compared to Calendula, particularly against *E. coli*. Further research is warranted to elucidate the underlying mechanisms and potential therapeutic applications of these plant extracts in combating bacterial infections. The fact that the studied plants possesses many medicinal factor makes it a very useful plants, and the extracts could be useful in therapeutic treatment, but this has to be substantiated by in vivo experiment.

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