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Study of the effect and antimicrobial activity of some pigmented plant extracts on the growth of Gram-positive and Gram-negative bacteria

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Abstract: Three plant extracts, roselle (Hibiscus Sabdariffa), sumac (Rhus coriaria), and Juglans regia bark, were tested for antibacterial efficacy against diverse bacteria isolates. The study's aims were to examine and evaluate the biological activity of three pigmented medicinal plants' aqueous and alcoholic crude extracts against pathogenic and non-pathogenic bacteria in vitro. as well as testing the cytotoxicity of crude extracts. A well diffusion method was used to complete the primary screening on Gram positive and Gram-negative isolates, both extracts [aqueous and alcoholic] had a broad-spectrum impact while alcoholic extracts had the greatest effect. However, both extracts had varied effects against all bacterial strains at the minimal inhibitory concentrations. The two extracts had a bactericidal effect, and the growth of all bacteria in this investigation was monitored for seven days with no growth. The extracts' cytotoxic effect on red blood cells (RBCs) was assayed, the result showed no cytotoxicity in the absence of alcohol with the only lysis occurring in the 200 mg/ml concentration tube of the alcoholic extract of sumac (Rhus coriaria). Thus, the three plants may be used as a source of natural bactericidal agent safely.

Keywords: Medicinal plants, antimicrobial effect, aqueous extract, alcoholic extract, cytotoxicity

1. INTRODUCTION

Plants that have therapeutic capabilities or have a pharmacologically helpful effect on the human body are known as medicinal plants. Alkaloids, terpenes, sterols, flavonoids, glycosides, cyanogenic, saponins, tannins, lactones, resins, volatile oils, quinines, and other secondary metabolites are synthesized and accumulated spontaneously
(Motaleb et al., 2011). Lanier (2020) referred to herbal medicine; is the use of certain herbs and the plant-based parts such as; flowers, stems, leaves, roots, etc., to the heal of various illnesses. It was the only option during the ancient times, although there are many different ways to the treatment of an illness in today's day in age.

Hibiscus sabdariffa L. (Roselle, Karkade) Family: Malvaceae, *Rhus Coriaria* L.(Sumac) Family: Anacardiaceae and *Juglans regia* L. (Walnut) Family: Juglandaceae; were considered as some of medicinal plants of Arabic region (AL-Hakeem, et al., 2012).

Roselle (*Hibiscus sabdariffa L.*), sometimes known as "karkade," is a valuable annual crop that may be produced effectively in tropical and subtropical areas. The plant's most commercially useful part is the fleshy calyx (sepals) that surrounds the fruit (capsules). The dried calyces can be soaked in water or the entire plant can be taken as a beverage. It can be converted into a refreshing cold drink or a warming hot beverage.

It has certain therapeutic qualities as well (Mohammed, et al., 2012) as well as the used in folk medicine to treat hypertension and liver disease and fever (Lusida, et al., 2017). Fullerton et al. did studies on bioactive components in *Hibiscus*, such as alkaloids, flavonoids, and terpenoids. Antibacterial properties of phenolics and biterpenoids against the bacteria *Escherichia coli*. (Fullerton, et al., 2011)

Antimicrobial activity, phytochemical constituents and cytotoxicity in the *H. sabdariffa* aqueous-methanolic extract was investigated, beside using for lethality assay of brine shrimps. Cardiac glycosides, saponins, flavonoids and alkaloids were found in the extract, and it exhibited the antibacterial activities against *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus stearothermophilus*, *Serratia mascences*, *Escherichia coli*, *Clostridium sporogenes*, *Bacillus cereus, Klebsiella pneumoniae*, *Pseudomonas fluorescence* (Olaleye, 2007).

Sumac, also known as *Rhus coriaria* Linn. (Anacardiacea), for millennia, it has been used as a spice, condiment, appetizer, and sour agent. Tannins, flavonoids, anthocyanins, organic acids, flavones, proteins, fiber, volatile oils, and nitrates, nitrites and other phytochemical components that are nutritionally and medicinally important have been detected in various portions of sumac. The plant also possesses minerals which are beneficial in the treatment of practitioners have also prescribed this plant as antimicrobial, abortifacient, and stomach tonic (Karadaş, et al., 2020).

Gabr and Alghadir (2019) reported that; in vitro, The aqueous extract of *Rhus coriaria* and its components were effective against a variety of microbes, including *P. aeruginosa*, *Staph. aureus*, and *Staph. aureus* (MRSA), and the results indicated that *Rhus coriaria* and its extract have antioxidant, antimicrobial, and anti-inflammatory activity, suggesting that they could be used in the formulation of novel anti-infection and wound-healing drugs.

In addition; The walnut, a member of the Juglandaceae plant family, is a highly prized and widely distributed tree nut around the world due to its high nutritional value (Kafkas, et al., 2020). The walnut (*Juglans regia* L.) is a medicinal tree that contains a variety of medicinally useful chemical compounds (Chebyshev, et al., 2019). Walnut tree bark which is called locally (Derum) has a variety of health benefits. It can be used as a full oral solution as well as for skin conditions such as eczema, blisters, and acute itching (also called pruritus). It's a natural blood purifier. Walnut bark includes a lot of manganese and other minerals, as well as an important subgroup of a polyphenolic substance called ellagitannins, according to studies. Ellagitannins are powerful antibacterial and antioxidant compounds. The teeth are instantly whitened when the moistened bark is used. The presence of carbonates, potassium, sodium, phosphorus, calcium, magnesium, copper, iron, manganese, and zinc ions in walnut bark causes the whitening effect. It also keeps the gums tight and robust, ensuring that the teeth live a long and healthy life. Derum can be particularly effective against oral infections, tooth decay, gingivitis, pyorrhoea, bad breath & cavities when used on a regular basis. In Iran, (Zakavi et al., 2013) investigated the antibacterial efficacy of ethanolic and aqueous extracts of *Juglans regia* bark on four oral bacteria: *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus salivarius*, and *Staphylococcus aureus*.

The Iranian bark of juglone, regiolane, exhibits antibacterial activity against major oral bacteria, and the ethanolic extract was more effective against the microorganisms tested than the aqueous extract.
Another study (Aldawood et al., 2017) evaluated and assessed the antibacterial effect of Derum (*Juglans regia* L.) bark extract at different concentrations on salivary microflora, and concluded that the study supports the use of natural products as medicines and confirms the plant's antibacterial potentials.

2. MATERIAL & METHODS

Sampling and Extraction of plants

Plant parts were purchased from the local markets, then pulverized (if it was not previously), and based on the methods of (Umeh et al., 2005; Gberikon et al., 2015), Twenty-five grams (25g) of powdered plant materials (flowers and stem bark) were weighed into clean bottles, extracted separately in tightly closed bottles using (125ml) aqueous and ethanol, and kept at room temperature for 48 hours. Suspensions were then filtered and placed in beakers with Whatman No. 1 filter paper, filtrates collected, and poured into dry Petri dishes for desiccation in warm room temperature.

Bacterial isolates preparation

Four bacterial isolates were obtained independently *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus sp*. three of them were pathogenic, provided by Al-Baian private laboratory, the others were isolated from the environment or a person normal flora. Two of the pathogenic bacteria were Vitek previously identified, the last one was identified morphologically, while the ecological isolates subjected to Gram staining in addition to some of biochemical test.

Antimicrobial activity test

The antibacterial properties of the examined extracts were assessed using the well diffusion method against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus* sp., according to the principles of (Bauer et al., 1966; Miles and Amyes, 1996). Each of the test microorganisms was cultured on Muller Hinton Agar [MHA, Merck] plates with 0.1 ml of bacterial suspension containing 3.0 x 108 CFU/ml [McFarland standard 1], then spread evenly using an L-shape spreader In the cultured MHA agar, The cork borer was used to create 6 mm diameter wells, which were then filled with 0.1 ml of each extract at a 200 mg/ml concentration. As controls, sterile distilled water and 70% ethanol were employed. The antibacterial activity was measured by measuring the mean of the inhibition zone diameters around each well after a 24-hour incubation period at 37 °C. The experiment was carried out three times. (Al-Manhel & Niamah, 2015).

The Minimum Inhibitory Concentration [MIC]

The Minimum Inhibitory Concentration [MIC] was measured using the well diffusion method, as described previously, using five concentrations of both alcoholic and aqueous extracts [200, 100, 50, 25 mg/ ml] (Paarhusip & Sitanggang, 2011).

Cytotoxicity test

Aqueous and alcoholic extracts were tested for cytotoxicity on human red blood cells (RBCs) according to (Nair et al., 1989) for maintaining osmotic pressure in RBCs. A Ringer solution

was used, and concentrations of about 25, 50, 100, and 200 mg/ml of the extracts were added to 1ml of RBCs with 19ml of Ringer in serial test tubes, and the mixtures were monitored every hour for 12 hours (Al Hawani et al., 2020).

3. RESULTS

The antibacterial activity of roselle (*Hibiscus Sabdariffa*), sumac (*Rhus coriaria*) and *Juglans regia* bark were tested on four species of Gram positive and negative bacteria using concentrations of 200, 100, 50, 25 mg/ml aqueous and alcoholic extracts. The findings revealed a broad range of activity against Gram positive and Gram-negative microorganisms. Alcoholic extracts were more effective than aqueous extracts, with a broader inhibition zone and an effect on all bacterial species. At 100,200 concentrations, the aqueous extracts had an effect on bacteria. Except for the sumac (*Rhus coriaria*) aqueous extract, which showed an effect on Gram positive bacteria at all concentrations. Table (1,2,3)

Table 1: The diameter of the inhibition zone and MIC measured in mm for both aqueous and alcoholic extraction for *Hibiscus Sabdariffa*

Concentration strain	**25 mg/ml**	**50 mg/ml**	**100 mg/ml**	**200 mg/ml**
Aqueous extracts				
Escherichia coli	-	15	18	25
Pseudomonas aeruginosa	-	-	18	25
Staphylococcus aureus	-	14.5	17	20
Bacillus sp.	-	-	17	22.5
Alcoholic extracts				
Escherichia coli	13.5	15.5	17.5	28.5
Pseudomonas aeruginosa	12	14.5	19.5	23
Staphylococcus aureus	12	13	15.5	21
Bacillus sp.	-	20	23.5	26

Table 2: The diameter of the inhibition zone and MIC measured in mm for both aqueous and alcoholic extraction for *Rhus coriaria.*

Concentration strain	**25 mg/ml**	**50 mg/ml**	**100 mg/ml**	**200 mg/ml**
Aqueous extracts				
Escherichia coli	-	-	20	22
Pseudomonas aeruginosa	-	-	22	24
Staphylococcus aureus	14.5	21	27.5	31.5
Bacillus sp.	16	19	20	25.5
Alcoholic extracts				
Escherichia coli	13	18	22	30
Pseudomonas aeruginosa	12	17	26.5	29.5
Staphylococcus aureus	20	22.5	28	29
Bacillus sp.	20.5	21	24.5	28.5

Table 3: The diameter of the inhibition zone and MIC measured in mm for both aqueous and alcoholic extraction for *Juglans regia* bark.

Concentration strain	**25 mg/ml**	**50 mg/ml**	**100 mg/ml**	**200 mg/ml**
Aqueous extracts				
Escherichia coli	-	-	-	13.5
Pseudomonas aeruginosa	-	-	-	11
Staphylococcus aureus	-	-	-	11
Bacillus sp.	-	-	-	12
Alcoholic extracts				
Escherichia coli	12.5	13.5	19	19.5
Pseudomonas aeruginosa	14	15	19	21
Staphylococcus aureus	13.5	15	21	23.5
Bacillus sp.	17	18	21.5	25

The minimum inhibition zone [MIC] assay

The minimum inhibition zone concentration for alcoholic extract of Roselle (*Hibiscus Sabdariffa*) was 25 mg/ml for *S. aureus, E. coli, and Pseudomonas* respectively, while at concentration of 50 mg/ml for *Bacillus* sp., The aqueous extract gave an MIC of 50 mg/ml for *S. aureus, E. coli* and for *Bacillus* sp. and *Pseudomonas aeruginosa* it was 100 mg/ml. Whereas the alcoholic extract of sumac (*Rhus coriaria*) showed an MIC at 25 mg/ml for all bacterium isolates, the aqueous extract showed an MIC at 25 mg/ml for Gram positive bacteria and 100 mg/ml for Gram negative bacteria. On the other hand, the MIC for both aqueous and alcoholic extracts of Juglans regia bark was 25 mg/ml for alcoholic extract and 200 mg/ml for aqueous extract for all bacterium isolates respectively. Which shown in Figure (1, 2, 3)

The cytotoxicity trails

The results of assaying the cytotoxic effect of the extracts on red blood cells [RBCs] revealed that there was no lysis for the RBCs in 25,50,100,200 mg/ml of the alcoholic extract, with the only lysis occurring in the 200 mg/ml concentration tube of the alcoholic extract of sumac (*Rhus coriaria*). The RBCs cells were examined under a microscope to ensure that the cells were raptured, but there was no lysis effect on RBCs with the control [alcohol]. In all concentrations [25,50,100, and 200 mg/ml], aqueous extract revealed no hemolysis of RBCs, and the same results were seen in the control [distil water] tubes as shown in Figure 4.

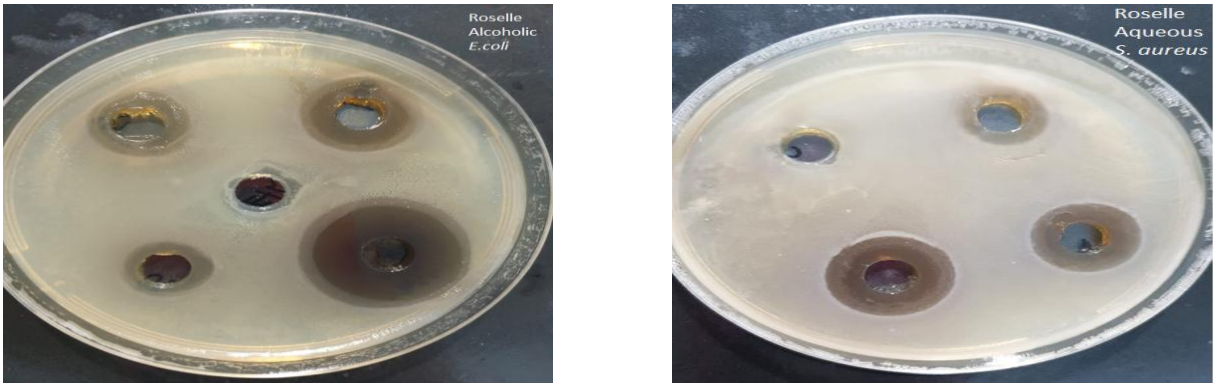

Figure 1: Roselle result sample

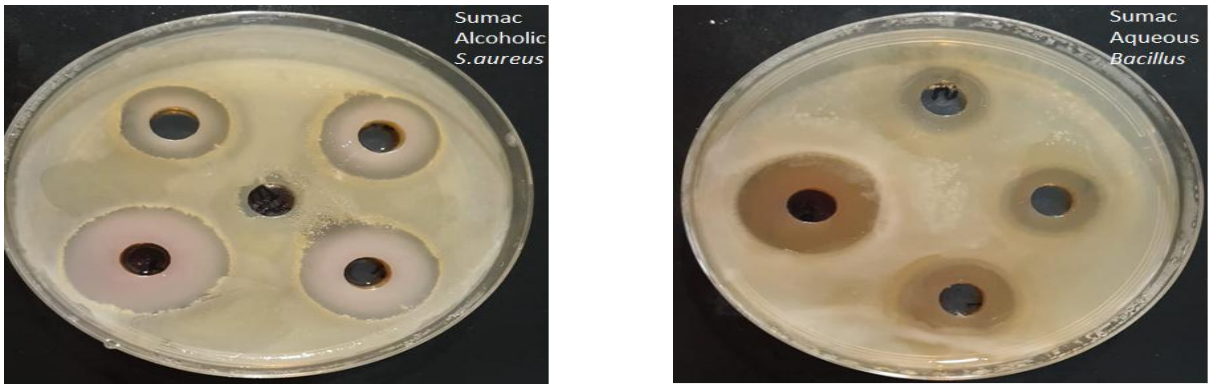

Figure 2: Sumac result sample

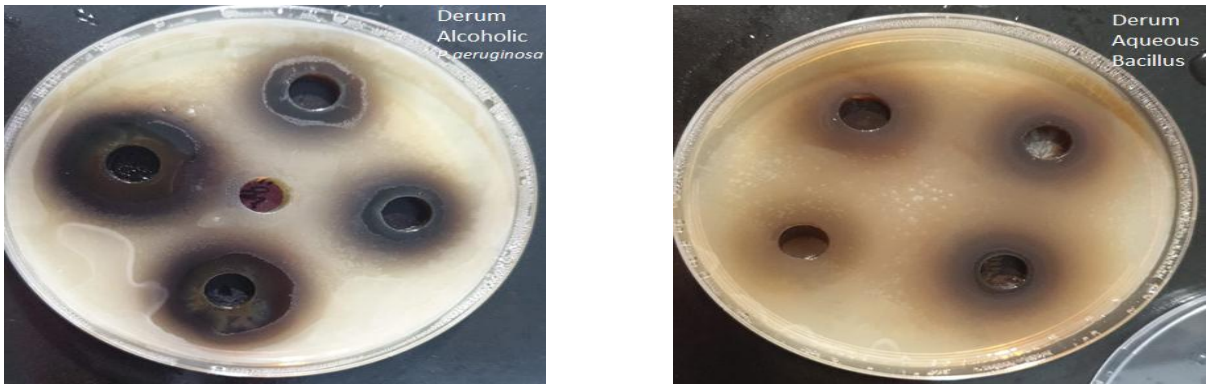

Figure 3: Derum result sample

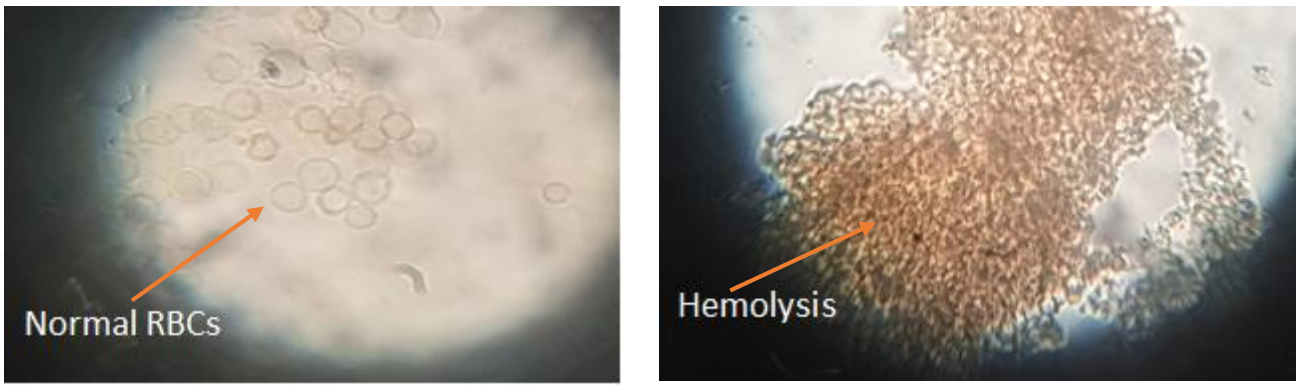

Figure 4: Cytotoxicity test show the RBCs shapes under microscope

4. DISCUSSION

The global prevalence of infectious diseases caused by bacteria is a major public health problem, and scientists have been searching for any available, low-cost, and safe-to-use medicines for years. Many attempts have been made to synthesize antibiotics that meet the above criteria, but the main roadblock has always been the side effects of any synthesis compound.

Plants were widely used to cure a variety of illnesses as anesthetics, disinfectants, and food preservatives, while some had significant negative effects. However, out of the 400,000 plant species on the planet, only a limited number of plants have been proved to exhibit antimicrobial

activity during the years of research (Varahalarao & Chandrashekhar, 2010) Medical plants are increasingly being used as natural antibacterial agents in addition to drug development.
In this study Roselle (*Hibiscus sabdariffa*) was extracted into ethanol and distilled water, and the crude extracts were used to screen Gram positive and negative bacteria for antibacterial activity. Roselle possesses antibacterial properties that are effective against both Gram positive and Gram-negative microorganisms.
, according to the findings. Gram positive bacteria are less sensitive to both alcoholic and aqueous extracts than Gram negative bacteria, and alcoholic extract is more effective against bacteria than aqueous extract, as indicated in Table 1. The minimum inhibitory concentration MIC [mg/ml] demonstrates that the alcoholic extract had an effect on *S. aureus, E. coli, and Pseudomonas aeruginosa* at concentrations of 25 mg/ml respectively, and of 50 mg/ml for *Bacillus* sp. The results were observed for the aqueous extract which showed effect at concentration of 50 mg/ml on *S. aureus* and *E. coli* respectively While the aqueous portion showed no activity with *Bacillus* sp. and *Pseudomonas aeruginosa* at concentrations of 50, 25 mg/ml, the MIC was at the concentration of 100 mg /ml. these results supported by (Karadaş et al., 2020; Alshami & Alharbi, 2014).
Antibacterial activity of an ethanol extract of *Rhus coriaria* against a variety of Gram negative and Gram-positive bacteria strains was discovered. Gram positive bacteria were more resistant to both alcoholic and aqueous extracts than Gram negative bacteria, as shown in
Table 2. The most active MIC was for *Rhus coriaria* alcoholic extract, with MIC = 25 mg/ml for all four bacterium species and the largest zone of inhibition. While the aqueous part had no activity against Gram negative bacteria at concentrations of 50 and 25 mg/ml, the MIC for Gram positive bacteria was 25 mg/ml, Earlier sumac study has shown that R. coriaria has antibacterial and antifungal activity in vitro, which is consistent with previous sumac research.(AL-Jubory et al., 2010; Shabbir, 2012)
This research was done on the *Juglans regia* bark to learn more about its antibacterial properties. Experiments on four bacterial species revealed that ethanolic bark extract has a robust antibacterial effect against Gram-positive bacteria were more affected and Gram-negative bacteria at all concentrations which demonstrate in Table 3. Regarding with the alcoholic extract effect of inner stratum of oak (Jaft) and stem bark of *Juglans regia* L. on the *Staphylococcus aureus* which isolated from the wound infection, (Khalid and Jaafar, 2019) showed the potential effect of stem bark of *Juglans regia* extract on *S. aureus* isolates, indicated it can be used in the treatment of wound infection. While the aqueous extract had no impact on bacteria except at a high concentrations of 200 mg/ml. The reason for the insensitivity to concentrations may be attributed to the fact that they were insufficient to produce the desired effect. This may be due to the decrease in the concentration of active substances in these concentrations, as it was found that the sensitivity of bacteria increases with increasing concentration. As for the sensitive bacteria, they all showed clear inhibition areas around the hole, It was noted that by leaving the dishes in the incubator for more than 24 hours, there was no change in the diameter of the inhibition areas. This contradicted with (Taha & Al-wadaan, 2011), Aqueous extract provides broad spectrum antibacterial effect against both gram-positive and gram-negative bacteria, according to the study. A minimum inhibitory concentration (MIC) of 25 mg/mL of alcoholic extract inhibited the growth of *P. aeruginosa, Bacillus sp., S. aureus, and E. coli.* (Cruz-Vega et al., 2008). (Taha & Al-wadaan, 2011) and with 200 mg/mL MIC with aqueous extract of all species, respectively. (Al-Rawi et al., 2021).
The effect of aqueous and alcoholic plant extracts on inhibition of some types of microbes that cause food spoilage was studied by Al-Manhel and Niamah (2015), who found that the alcoholic extract has much more activity on investigated fungi and bacteria than the aqueous

extract. They attributed this to the extraction method and the solvent used in the extraction process, both affecting the final product of the extraction. According to Ferreira et al. (2018), alcoholic extraction revealed a significant percentage of phenolic compounds, which are the most active products. The permeability of the cell membrane is also affected by the phenolic extract. The alcoholic extract's superiority over the aqueous extract may be attributed to the presence of the phenol component in the alcoholic extract compared to the absence of this chemical in the aqueous solution.
In our study, the cytotoxicity of ethanolic and aqueous extracts of roselle (*Hibiscus Sabdariffa*), sumac (*Rhus coriaria*), and *Juglans regia* bark extracts was investigated. The results revealed that the alcoholic extract showed no RBC hemolysis only at high concentrations [200 mg/ml] of the alcoholic extract of sumac (*Rhus coriaria*), whereas the aqueous extract showed no hemolysis at all concentrations used, in addition to the control [distil water]. Chi & Wu (1991) discovered that the leakage of the small cation K+ from cells caused hemolysis in ethanol-treated RBCs due to a disruption in osmotic pressure, and thus suggested that the formation of membrane pores might be involved in the defected cytoskeletal network of ethanol-treated RBCs. This finding supports our results.

5. CONCLUSION

The aqueous and alcoholic crude extracts of roselle (Hibiscus Sabdariffa), sumac (*Rhus coriaria*), and *Juglans regia* bark exhibit a strong antibacterial action on Gram positive and negative bacteria, there was a greater effect of the alcoholic extract on the four isolates than the aqueous extract, and the diameters of inhibition were greater around the wells containing the concentrations of the different extracts. with varying minimum inhibitor concentrations and no cytotoxicity. Plants that are used locally can be used safely as disinfectants.

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