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Antimicrobial Properties of Ca, Fe, Mg, and Zn / Sodalite Composites on Human Foodborne Pathogens

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ABSTRACT

In the current study, sodalite was prepared from natural material (Um Had feldspar), Quseir area, Red Sea, Egypt via hydrothermal processes. It was modified by adding (Ca, Mg, Fe and Zn) ions to improve their antimicrobial properties. The prepared and modified samples were examined using Fourier-Transform Infrared (FT-IR), X-ray diffraction (XRD), surface area, and scanning electron microscopy (SEM). The sodalite composites were applied to study their antimicrobial effects on different human foodborne pathogenic bacteria (five strains) and food spoilage yeasts (seven strains). The inhibition zone's diameter for the composite samples against pathogenic bacteria, it turns out Zn-sodalite was the best one against the Gram-positive bacteria *Staphylococcus* and the Gram-negative bacteria *Escherichia coli* and *Salmonella* sp., while Ca-sodalite works better against the Gram-positive bacteria *Bacillus cereus*. Mg-sodalite and Ca-sodalite were recorded the highest inhibition zone 14±2 against *Listeria monocytogenes* while Fe-sodalite is the lowest one against all pathogenic bacteria. Concerning the pathogenic yeasts, Zn-sodalite with its different concentrations possessed the highest antifungal activity against *Candida parapsilosis* AUMC 10217 giving inhibition zone diameter ranging from 19±0.71 to 23±0.82 mm, followed by Fe-sodalite, while Ca-sodalite and Mg-sodalite exhibited the lowest effect. Fe-sodalite gave a powerful effect on *Candida albicans* AUMC 10216 followed by Zn-sodalite (10:90%). Fe-sodalite and Mg-sodalite had a weak antifungal effect on the yeast *Rhodotorula mucilaginosa* AUMC 10731. The study demonstrates that sodalite composites are promising sustainable, cost effective and eco-friendly alternative antimicrobial agents.

1. INTRODUCTION

Zeolites are porous aluminosilicate minerals. It distinguishes with high adsorption capacity and huge ion exchange with heavy metals like Zn^{2+}, Cu^{2+}, Fe^{2+} and Ag^{+}. Their properties make them used as antiviral, antibacterial and antifungal agents [1–4].

Because of its high adsorption surface area and its high ability for cation exchange capacity, zeolites are attractive host materials for developing nano-composites [5]. The nano-composites can be prepared on two types of zeolites: zeolites-organic and zeolites-inorganic. The latter which include metals or metal oxides supported on zeolites are considered the most popular, due to the fact that they possess unique properties, molecular selectively and hyper-functional activity [6].

Voluminous articles were conducted since the work of Hagiwara [7] concerning the utilization of zeolites in the biological and medical fields (antibacterial activity).Cu^{2+}, Ag^{+}, $Zn^{2+}$$Mg^{2+}$and Fe^{2+} are the most common metals used in the antibacterial field because they have the highest antibacterial activity [8,9]. These metals are also applied for the treatment of ocular and oral infection and burns [10,11].

A foodborne disease episode can be caused by biological organisms known as foodborne pathogens [12]. When a pathogen is consumed with food, it either establishes itself and grows in the human host or generates a toxin that the host then consumes, resulting in foodborne disease. Hospitalizations, diseases, deaths, and financial losses are caused by eating food tainted with microbial toxins and foodborne pathogenic bacteria. Foodborne diseases (FBD), particularly gastrointestinal illnesses, are prevalent and detrimental to human health [13].

Candida species are human pathogens that cause superficial nail infections [14,15], mucocutaneous overgrowth, and bloodstream infections in patients with compromised immune systems [16]. They are also associated with the normal microbiota of an individual's mucosal oral cavity, gastrointestinal tract, and vagina as commensals in healthy humans [17]. *Candida albicans, Candida parapsilosis, Candida glabrata, Candida tropicalis,* and *Candida krusei* are responsible for over 90% of invasive infections [18–21]. Numerous investigations have demonstrated that a variety of dairy products include yeast species that are medically significant, such as *Trichosporon*, *Geotrichum*, and *Candida* [22–25].

Our study aims to synthesize zeolites by using Um Had feldspars, Quseir area, Red Sea, Egypt. Consequently, sodalite composites (Ca, Fe, Mg and Zn) were prepared and checked for their antibacterial and antifungal activities.

2. MATERIALS AND METHODS

2.1 Synthesis of zeolite (Sodalite)

Um Had feldspars (placers) were used in the synthesis of sodalite sample by crashed and sieved it at 250 mesh. Calcination of the sample at 800^{0} C for 2 h was achieved. The calcination sample was mixed with NaOH pellets with a ratio of 1:1.2, the mixed sample was heated again at 650 ^{0} C for 2 h. Subsequently, the mixed sample was transferred into the Teflon with 50 ml distilled water. Stirring it for 2 h and then leaving it at room temperature overnight. Transferred the Teflon to the oven at 160 ^{0} C for 48 h was carried out. Finally, washed, filtered and dried at 60 ^{0} C for 24 h [26].

2.2 Preparation of composite zeolite

Sodalite samples; $CaSO_4$, $FeSO_4$, $MgSO_4$ and $ZnSO_4$ were used to prepare the composite samples with a ratio (10:90 %, 5:95 %and 2.5:97.5 %) of salts and zeolite respectively. Salts were dissolved in distilled water and stirring it for 30 min then zeolite was added to the solution and left in the stirrer for 2 h. The samples were put in the oven for 24 h at 60 ^{0} C to increase the ion exchange capacity. Then it was washed with distilled water and calcinated for 2 h at 500 ^{0} C [27].

2.3 Characterization

To characterize the synthesized sample, using Fourier-Transform Infrared (FTIR), X-ray diffraction (XRD), surface area, and scanning electron microscopy (SEM) were applied at Assiut University, Egypt.

Crystalline structure of the sodalite was identified using a Philips X-ray diffractometer model PW 1710 (Philips, Amsterdam, the Netherlands) with CuKα radiation (λ =1.5405 Å), at an operating applied voltage of 40 kV and 30 mA current, equipped with a graphite monochromator and automatic divergence slit. Measurements were swapped from 2θ =35° to 2θ =80° with a scanning speed of 0.06°/min [26].

SEM micrograph of the samples was taken using JEOL scanning electron microscope, model JSM-5400LV (JEOL, Tokyo, Japan) at the Electron Microscopy Unit, Assiut University, Egypt.

To measure the surface area of the sample, the nitrogen adsorption-desorption isotherms were measured using liquid nitrogen at -196^{o}C by the Quantachrome Instrument Corporation, USA (Model Nova 3200).

FT-IR was used to determine the functional groups of the sodalite. FT-IR spectrum of the sodalite was recorded in the 4000–400 cm^{-1} region using Nicolet IS 10 FT-IR spectrophotometer [26].

2.4 Antimicrobial Screening

2.4.1 Microbial strains and growth conditions

Several bacterial and yeast species were used to test the metal zeolites composites' antibacterial properties. Five types of foodborne pathogenic bacteria were used to test the antibacterial activity. These included Gram-negative bacteria like *Salmonella* sp. and *Escherichia coli*, as well as Gram-positive bacteria like *Bacillus cereus, Listeria monocytogenes*, and *Staphylococcus aureus*. Yousef et al. (2021) recovered these bacterial strains from fermented meat items and molecularly characterized them [11]. Antifungal activities were tested using seven pathogenic and food spoilage yeast strains (Table 1): *Candida albicans*, *C. parapsilosis*, *Geotrichum candidum*, *Rhodotorula mucilaginosa*, and

Trichosporonasahii. By combining the bacterial or yeast cells from fresh cultures, suspensions of 0.5 McFarland turbidity were made from the pure cultures in Sabouraud dextrose broth for yeast and Nutrient broth for bacteria.

Table 1: Assiut University Moubasher Mycological Centre accession number (AUMC) of yeast strains with accession GenBank numbers and isolation source [15].

Species	AUMC No.	Accession GenBank No.	Isolation source
Candida albicans	10216	KU200444	Fingernail onychomycosis
Candida parapsilosis	10217	KU200449	Tineacapitis
Candida parapsilosis	10715	KX376269	Raw buffalo milk
Geotrichum candidum	10737	KX376262	Butter
Rhodotorula mucilaginosa	10731	KX385852	Kareish cheese
Trichosporonasahii	10195	KU095859	Fingernail onychomycosis
Trichosporonasahii	10724	KX376272	Kareish cheese

2.4.2 Determination of antimicrobial activity detection by agar well diffusion method

Agar well diffusion method was used for screening the antibacterial and antifungal activities of different sodalite composite samples with $CaSO_4$, $FeSO_4$, $MgSO_4$ and $ZnSO_4$ with ratio (10:90 %, 5:95 % and 2.5:97.5 %) of salts and sodalite respectively [13]. one ml of freshly made bacterial or yeast suspension were pipettes in sterilized Petri dishes. The Petri dish holding the inoculum was then filled with molten nutrient agar (NA) for bacteria and Sabouraud dextrose agar (SDA) for yeast strains, and thoroughly mixed. Following solidification, a sterile cork borer (5 mm in diameter) was used to create wells in agar plates. The wells were then filled with 10 mg of each zeolite composite sample. All experiments were performed in triplicate, and the plates were then incubated for 48 hours at 28 °C. The zone of inhibition, which included the well diameter, that developed during the incubation period was measured in order to assess the antimicrobial activity. Salt-free sodalite was used as a negative control [11].

3. RESULTS and Discussion

3.1 X-ray diffraction (XRD)

X-ray diffraction equipment was used to identify the synthesized sample which scanned between 4-60^0 2θ. The result shows that the intense main peak well illustrated at 24.65^0 2θ and secondary peaks have appeared at 14.16^0 2θ, 35.13^0 2θ and 31.99^0 2θ (Fig.1). The obtained result was reflecting that the synthesized materials are sodalite [29].

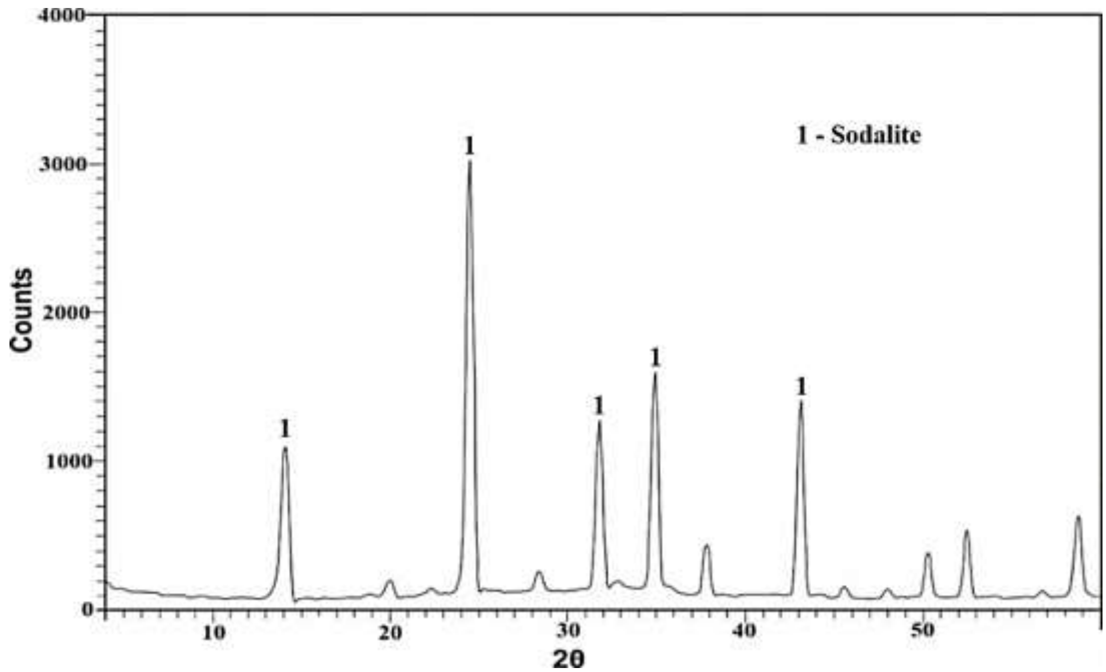

Fig.1. XRD diffractogram of the synthesized sodalite

3.2 Scanning Electron Microscope (SEM)

The synthesized material was investigated by (SEM) and study the morphology of the synthesized sodalite sample. Figure 2 illustrates that sodalite is represented by thread-ball-like and armadillo-like particles. The obtained results are similar to those mentioned by Hums, 2017 [30]. Figure 3 illustrates the pervasiveness of Zn on the surface of sodalitematerial.

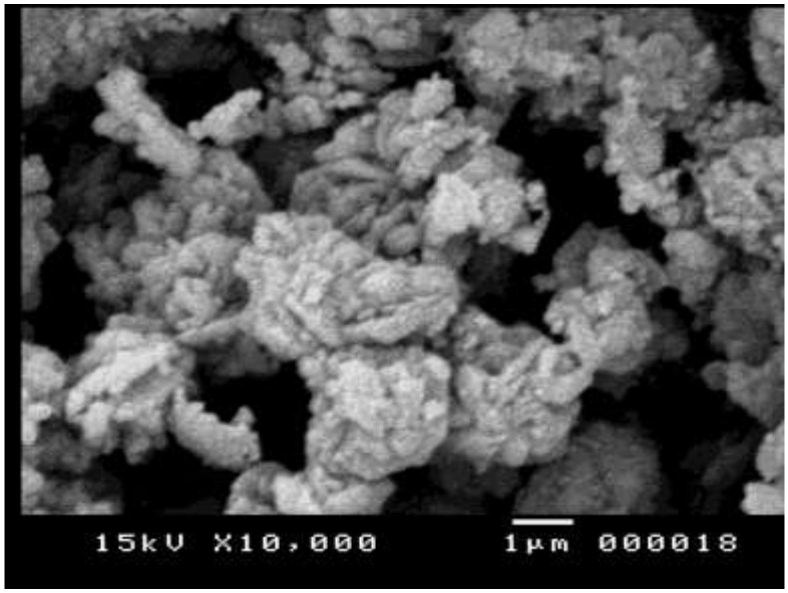

Fig.2. Thread – ball-like and armadillo-like particles of sodalite

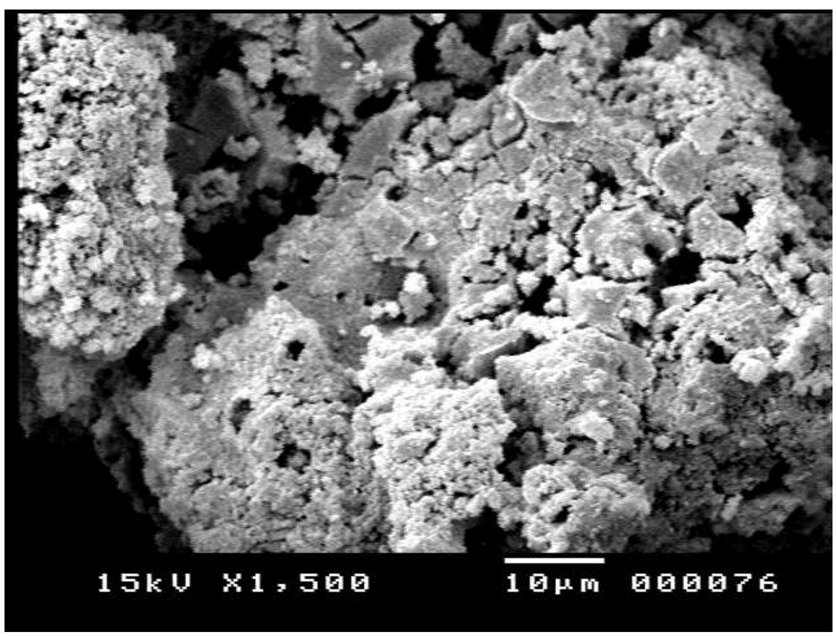

Fig.3. SEM image shows Zn nanoparticle on sodalite surface

3.3 Surface area properties

The obtained result has revealed that the surface area of sodalite minerals is about 70 m^2/g. this results match very well with the result of Lapari et. al 2015 [31].

3.4 Fourier – Transform Infrared Spectroscopy (FT-IR)

The synthesized sample was subjected to FT-IR examinations with a Nicolar spectrophotometer (model 6700) FT-IR equipped with a data station. FT-IR result shows well bands at 463, 665, 736, 977, 1653 and 3502 cm^{-1} (Fig.4). The obtained result agrees with the previous results reported for sodalite [32]. Also, the composite samples were examined and the results show that the bands of sodalite slightly shifted and the ions appear well at the bands 457 to 405 cm^{-1} (Fig.5).

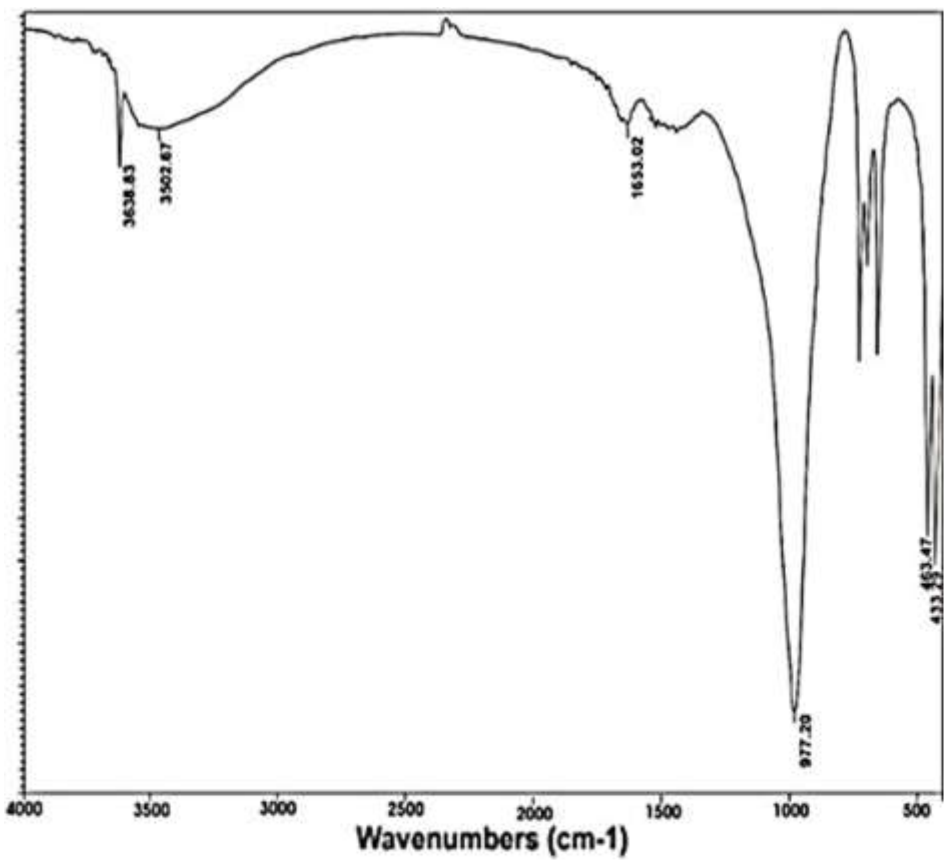

Fig.4. FT- IR of sodalite mineral

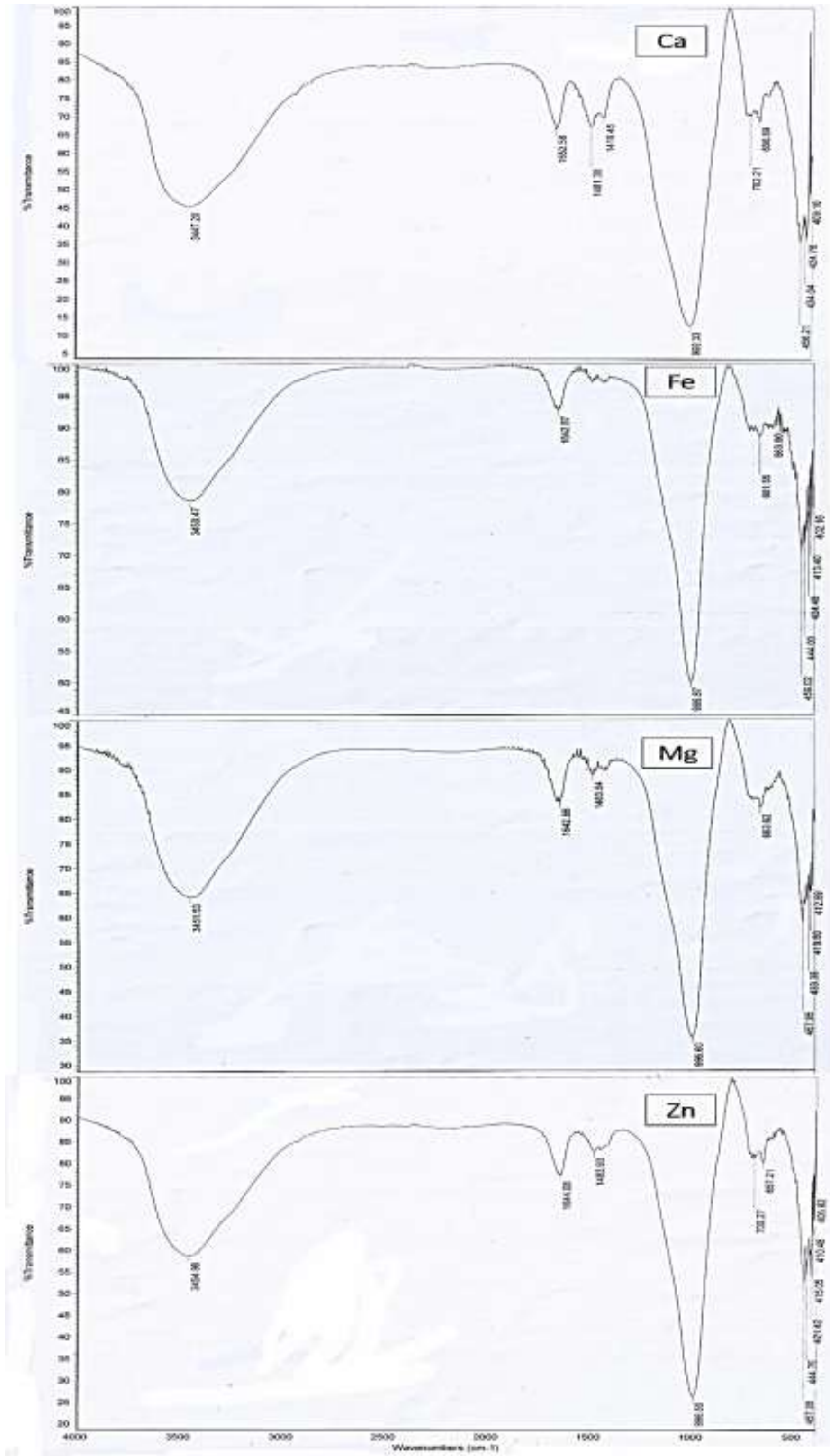

Fig.5. FT- IR of Ca, Fe, Mg and Zn/sodalitecomposite

3.5 Microbial analysis

3.5.1 Antimicrobial activity of prepared sodalites

The microorganisms tested in the investigated study were assigned to human pathogens and food spoilage microbiota. The antimicrobial activities of sodalite composite samples with Ca, Fe, Mg and Zn with ratio (10:90 %, 5:95 % and 2.5:97.5 %) of salts and sodalite respectively against the bacterial and yeast fungal species were investigated in this work, and the presence or absence of inhibitory zones and zone diameter were used to qualitatively evaluate their potency (Table 2).

The agar-well diffusion test was conducted to determine the antimicrobial effects of sodalite composites on human and foodborne pathogens (Table 2). The inhibition zone; diameter of individual treatment with zeolite composite were determined. The results indicate that zinc sodalite composite had greater antimicrobial effects against all foodborne pathogenic bacteria. Based on the results Gram-positive bacteria were more sensitive to antimicrobial agents.

Controlling the release of certain compounds and boosting their antibacterial action are the primary goals of employing sodalite as a carrier. Zn-sodalite is the most effective against both Gram-positive Staphylococcus and Gram-negative bacteria, including *Salmonella spp*. and *Escherichia coli*, while Ca-sodalite and Mg-sodaliteare the better against the Gram-positive bacteria *Listeria monocytogene*and *Bacillus cereus*. Ca-sodalite was recorded the highest inhibition zone 14±2 against *Listeriamonocytogene*, while Fe-sodalite is the lowest one against all pathogenic bacteria.

In fact, nanoparticles alter cellular macromolecules, including DNA and proteins, by interfering with the permeability of the cell wall and membrane. As well as prevent DNA replication and protein biosynthesis [33].

The combination of Zn and sodalite with its different concentrations possessed the most antifungal activity against the pathogenic yeast *Candida parapsilosis* AUMC 10217 giving inhibition zone diameter ranging from 19±0.71 to 23±0.82 mm, followed by Fe-sodalite composite (14±0.41 to 18±0.82 mm), while Ca- and Mg-sodalite composite exhibited the lowest effect (13±0.41 to 16±0.82 mm). Fe-sodalite gave a powerful effect on the pathogenic yeast *Candida albicans* AUMC 10216 with inhibition zone (12±0.41 to 22±0.41 mm), while only the highest concentration of Zn-sodalite composite had a weak effect, but calcium and magnesium did not have any antifungal activity. The Fe- and Mg-sodalite composites had a weak antifungal effect on the yeast *Rhodotorulamucilaginosa* AUMC 10731 possessed an inhibition zone from 10±0.71 to 14±0.71 mm. All metal sodalite composites did not affect the yeast, *C. parapsilosis* AUMC 10715,*Geotrichum candidum* 10737, *Tricho sporonasahii* AUMC 10195, and 10724.

Over the last three decades, *Candida parapsilosis* has been considered an emerging fungal pathogen [32] and has been placed second in various studies [33–36] after *Candida albicans*. With 46.02% of all cases in the Assiut Governorate involving superficial mycosis, yeasts were the most frequently isolated fungi. Candida was the most common yeast fungus, affecting 23.01% of patients, primarily *C. parapsilosis*, followed by *C. albicans*, *Rhodotorula mucilaginosa*, and *Tricho sporonasahii* [15]. Fungal growth was significantly inhibited by the Zn^{2+}- and Cu^{2+}-embedded zeolites in relation to Ag-zeolites 2 [4,37,38]. While certain heavy metals, including iron and zinc, are necessary for the metabolic processes of fungal species, they can also be poisonous at slightly higher concentrations than necessary. Consequently, it is possible that the ion transporter of fungal systems, where metal ions aggregate and exhibit fungicidal activity at high concentrations, may have taken up Zn and Feions.[4].

Table 2: Antimicrobial activity (inhibition zone in mm as mean ±SD) of different sodalitecomposite concentrationsusing the agar well diffusion method.

Sodalites composite	Salts: Sodalite (%)	*Escherichia coli*	*Salmonella sp*	*Listeria monocytogene*	*Staphylococcus aureus*	*Bacillus cereus*	*Candida albicans* 10216	*Candida parapsilosis* 10217	*Rhodotorula mucilaginosa* 10731
$CaSO_4$ composite	**10:90**	**7±1**	**7±0.5**	**14±2**	**9±1**	**10±2**	**0**	**16±0.82**	**0**
	5:95	**6±0.5**	**7±0.5**	**13±1.5**	**7±1**	**11±1**	**0**	**16±1.6**	**0**
	2.5:97.5	**7±0.5**	**9±1**	**14±2**	**8±2**	**9±1**	**0**	**14±0.41**	**0**
$FeSO_4$ composite	**10:90**	**7±0.5**	**7±0.5**	**10±2**	**8±1**	**8±2**	**22±0.82**	**18±0.82**	**1٤±0.71**
	5:95	**7±1**	**7±1**	**8±1**	**7±1**	**7±1**	**22±0.41**	**18±0.82**	**12±0.82**
	2.5:97.5	**7±1**	**7±0.5**	**9±2**	**9±1**	**7±1**	**12±0.41**	**14±0.41**	**0**
$MgSO_4$ composite	**10:90**	**8±1**	**7±1**	**13±2**	**8±1**	**10±1**	**0**	**16±0.71**	**11±0.41**
	5:95	**9±1**	**7±0.5**	**12±2**	**7±1**	**10±2**	**0**	**16±1.6**	**10±0.71**
	2.5:97.5	**7±1**	**7±0.5**	**14±2**	**7±1**	**8±2**	**0**	**13±0.41**	**10±0.71**
$ZnSO_4$ composite	**10:90**	**9±1**	**9±1**	**9±1**	**10±1**	**9±1**	**12±1.4**	**23±0.82**	**0**
	5:95	**8±1**	**9±0.5**	**10±2**	**9±0.5**	**9±0.5**	**0**	**23±0.82**	**0**
	2.5:97.5	**8±1**	**11±1**	**9±1**	**8±0.5**	**9±0.5**	**0**	**19±0.71**	**0**
Control	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

4. CONCLUSION

It was illustrated thatsodalite was synthesized from natural material (Um Had feldspar) that was collected from the Quseir area, the Red Sea, Egypt via hydrothermal processes. the synthesized material was modified by adding Ca^{+2}, Mg^{+2}, Fe^{+2} and Zn^{+2} ions from sulfate salts to increase their activity against positive and negative Gram bacteria also their microbial activity against yeasts. The results show variablesin diameter of inhibition zone against all bacteria that used. Ca-sodalite is the perfect one against Gram-negative bacteria (*E.coli* and *Sallamonella* sp.) and good against Gram-positive bacteria*Staphylococcus aureus*. While Mg-sodalite and Ca-sodalite are recorded the largest inhibition zone 14±2 against *Listeria monocytogene*Gram-positive bacteria. The Zn-sodalite with its different concentrations possessed the most antifungal effective against the pathogenic yeast *Candida parapsilosis* AUMC 10217 followed by Fe-sodalite, while Ca-sodalite and Mg-sodalite composites exhibited the lowest effect. Fe-sodalite composite gave a powerful effect on the pathogenic yeast *Candida albicans* AUMC 10216. Fe-sodalite and Mg-sodalite composites had a weak antifungal effect on the yeast *Rhodotorulamucilaginosa*.

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