



## The Activity of Medical Plant Extracts with Al<sub>2</sub>O<sub>3</sub> Nanoparticles on the Vitality of Bacteria and their Genomes

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### ABSTRACT

This study was conducted to investigate the activity of crude plants extracts and alumina nanoparticles against *Escherichia coli* and *Staphylococcus aureus* by using two methods: agar well diffusion and DNA binding. FT-IR was used to compare the different peaks of the organic group in plant extracts and the plant extracts with an alumina nanoparticles (mix). The results showed that the extracts from three plants had antimicrobial effects against *Escherichia coli* and *Staphylococcus aureus* isolates. The mixture of the crude plant extract plus the alumina NP suspension revealed a different effect that ranged between a synergistic and an antagonistic effect. The gel electrophoreses results showed that most of the plant extracts and the mix cleaved DNA with the exception of the alumina nanoparticles alone, which did not have any clear effect.

**Key words:** bacteria, nanoparticles, Al<sub>2</sub>O<sub>3</sub>, plant extract, DNA Binding

### INTRODUCTION

Nanoparticles (NPs) are generally defined as particles that have a diameter of less than 100 nm [1]. Many studies have demonstrated potential problems if nanomaterials are released into the environment, especially considering their toxicity to microorganisms, plants, and animals [2]. The antimicrobial activities of aluminum oxide, silver nanoparticles, gold nanoparticles and iron oxide nanoparticles have been previously reported [3- 5]. Nanomaterials have proved toxic to human tissue and cell cultures, resulting in increased oxidative stress, inflammatory cytokine production and cell death [6]. Studies demonstrate the potential for nanomaterials to cause DNA mutations [7]. Aluminum oxide (Al<sub>2</sub>O<sub>3</sub>) NPs have a wide range of applications in industrial as well as personal care products [8]. Aluminum oxide NPs are important applications in the ceramics industry [9] and can be used as an abrasive material, in heterogeneous catalysis, as an absorbent, as a bio-material, and as reinforcements of metal-matrix composites [10, 11]. Previous researchers have investigated the cytotoxic effects of metal oxide particles, such as, aluminum oxide (Al<sub>2</sub>O<sub>3</sub>), titanium dioxide (TiO<sub>2</sub>), and zirconium oxide (ZrO<sub>2</sub>), on murine fibroblasts and murine monocyte macrophages [12]. Other studies revealed the toxicity range of metal oxide NPs, including Al<sub>2</sub>O<sub>3</sub>, which is 500-3000 nm in diameter, in human fibroblast cells [13]. Generally, the toxic effects of the Al<sub>2</sub>O<sub>3</sub> nanoparticles are time dependent [14].

For centuries, plants have been used as remedies and to treat diseases [15]. Medicinal plants contain some organic compounds that provide a definite physiological action on the human body, and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [16, 17]. *Matricaria chamomile* flowers mostly contain phenolic compounds and glycosides [18]. Aqueous and alcoholic extracts of *Chamomilla* flower powder are used in therapeutics to treat skin infections caused by pathogenic bacteria, as a therapy for mouth injuries, and in therapeutics to treat respiratory system infections and digestive disorders [19]. *Olea europaea* leaves contain

polyphenols that have beneficial effects on health due to their anti-hypertensive, anti-diabetic, anti-carcinogenic, anti-atherosclerotic, anti-inflammatory, and antimicrobial activities [20-25]. The genus *Origanum majorana L.* is an aromatic, and it has been used as a flavoring and herbal spice since ancient times and is also used as an antistomachic, an antihelminthic, and an alexipharmic and is useful in diseases of the heart and blood as well as for fevers, leucoderma and inflammation [26].

Our present study is focused on the development of a model to evaluate the activity of plant extracts and nanoparticles on living cells and DNA.

## EXPERIMENTAL SECTION

**Specimens** Three different dry medicinal plants were purchased from a local market. *Matricaria chamomile* flowers, *Olea europaea* leaf and *Origanum majorana* leaf.

**Preparation of plant extract** Twenty grams of dry plant was submerged in 180 ml of distilled water or 60% ethanol and was extracted for 3 hr in a Soxhlet at 60-80 °C, and then, the extractions were filtered and dried in 40°C. The residue was stored at 4° C for further analysis [27].

**Microorganisms** Pure cultures of *Escherichia coli* and *Staphylococcus aureus* isolated from wound samples were obtained from the Al-Nu'man General Hospital in Baghdad City.

**Antibiotic sensitivity test** The antibiotic susceptibility profiles of the *Escherichia coli* and *Staphylococcus aureus* isolates were determined by the standard Kirby-Bauer disk diffusion method [28]. These antibiotics, with their respective disk concentrations, are Amoxicillin-clavulanic (30µg), Carbanicillin (50µg), Cefazidime (10µg), Chloramphenicol (30µg), Gentamycin (10µg), Impenem (10µg), Methicillin (10µg), Norfloxacin (10µg), Penicillin G (10 Units), and Rifampin (5µg). Bacterial culture suspensions equivalent to 0.5 tube McFarland turbidity standards were spread on Muller-Hinton agar plates using sterile swabs and incubated aerobically at 37°C for 24 hours. The inhibition zone diameters around the antibiotic disks were measured [29].

**Characterization of gamma Al<sub>2</sub>O<sub>3</sub>** Gamma alumina oxide nanoparticles (NPs) were produced from EPRUI-Nanoparticles & Microspheres (made in China), and the suppliers' data can be summarized as follows: crystalline; white; and a particle size of 20 nm.

**Preparation of the nanoparticle dispersion** The particles (1 mg/ml) were suspended in sterile deionized water and sonicated by an ultrasonic cleaner (Lab. Tech. Model: LUC-40S/410/420) at 40 KHZ for 30 minutes at a temperature of 30-35°C [8]. The aqueous plant extract was mixed with the alumina NP solution at a ratio of 1:1 and was incubated in a water bath at 60-80°C for 1 hour until a color change was observed. The suspension was sonicated for 15 minutes before it was used.

**The agar well diffusion method** One hundred microliters (18 h cultures) of the pathogenic species were spread on sterile Nutrient agar plates (all of the tests were performed in triplicate). The wells were punched in the plates using a sterile cork borer (6-mm diameter). Then, the wells were filled with the plant extract with and without the NPs under aseptic conditions. The plates were then incubated at 36±1°C for 24 h. The results were recorded and analyzed in terms of the zones of inhibition formed around each well [30].

**FT-IR** The plant extract solution, with and without alumina NPs, was subjected to Fourier transform-infrared (Shimadzu) spectrometer at a wavelength of 400-4000 [8].

**Total DNA isolation** A Promega Genomic DNA Purification Kit (A1120) was used for the extraction of total DNA from the bacteria (*Escherichia coli* and *Staphylococcus aureus*) according to the kit manual.

**Plant extract and alumina NP interaction with the DNA** [31]: One volume of extracted DNA was mixed with either an equal volume of plant extract alone, plant extract with the alumina NPs, or alumina NPs alone and a DNA rehydration solution.

RESULTS AND DISCUSSION

Table 1 shows the resistance pattern of the *Escherichia coli* and *Staphylococcus aureus* isolates. Each isolate showed resistance to at least six antibiotics.

The mixing of the medical plant extract with the aqueous solution of the alumina NPs showed a change in color, indicating the formation a new complex (Figure 1.A). The results of the antimicrobial assessment by the agar well diffusion method showed that the crude plant extract and the mixture of the crude plant extract plus the alumina NP suspension had a different inhibitory effect against *S. aureus* and *E. coli* (Table 2). The water plant extract was more effective than the 60% ethanol plant extract. The same result was recorded previously [32, 33]. The Al<sub>2</sub>O<sub>3</sub> NPs had no effect on bacterial growth. Other research recorded that a concentration of 1000 µg/mL was moderately inhibitory for bacteria [8].

Table 1: Sensitivity test for *Escherichia coli* and *Staphylococcus aureus*

organism	Resistance	Sensitive	organism	Resistance	Sensitive
<i>Escherichia coli</i>	Amoxicillin-clavulanic	Rifampin	<i>Staphylococcus aureus</i>	Amoxicillin-clavulanic	Gentamycin Norfloxacin Rifampin
	Carbanicillin			Carbanicillin	
	Ceftazidime			Ceftazidime	
	Chloramphenicol			Methicillin	
	Gentamycin			Penicillin G	
Impenem					
PenicillinG					
Norfloxacin					



Figure 1: A. Plant extract color change after mixing with Al<sub>2</sub>O<sub>3</sub>. B. Inhibition zone for the plant extract and the plant extract with Al<sub>2</sub>O<sub>3</sub>

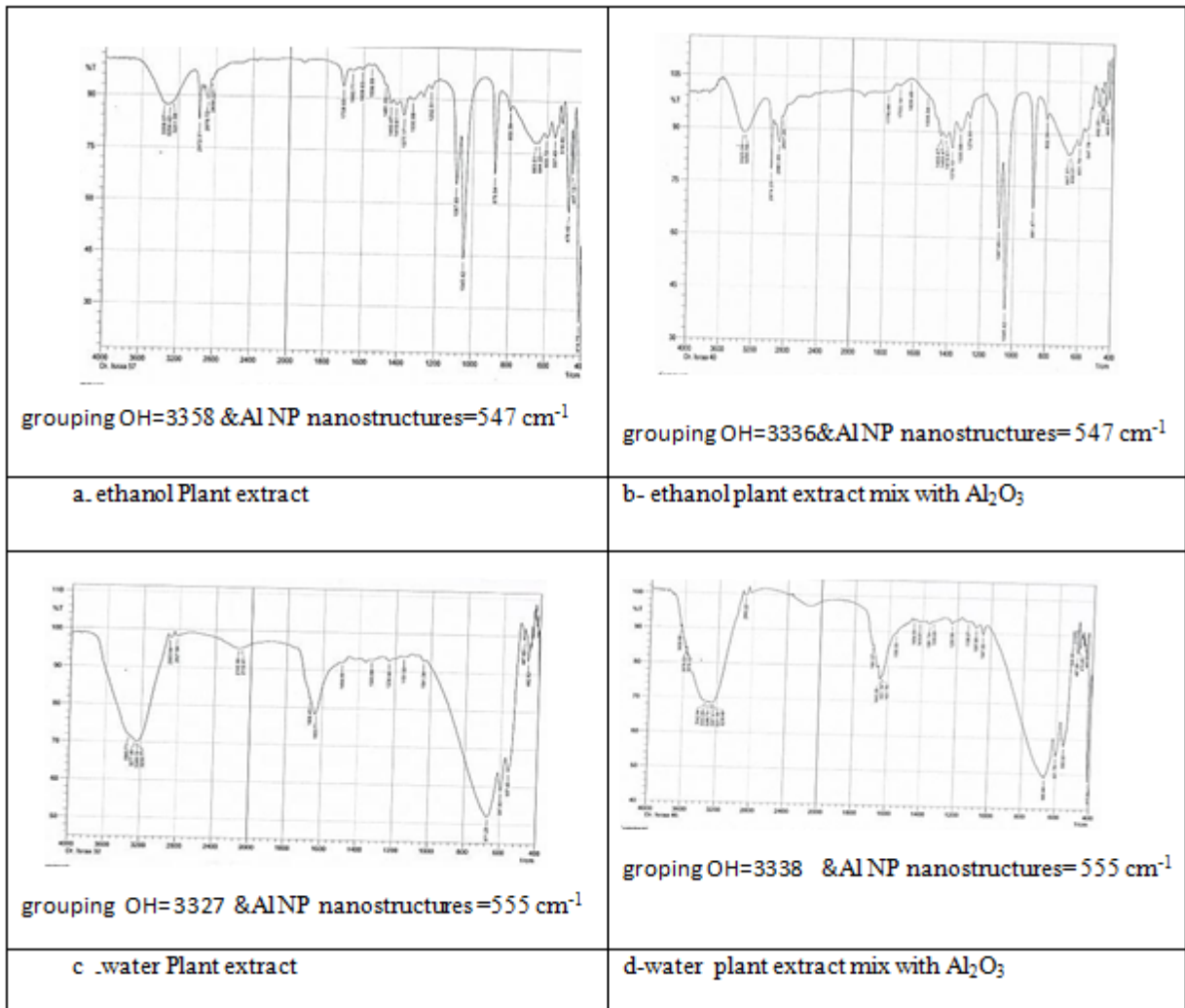
Table 2: Antimicrobial activity of medicinal plants

No.	alumina <sup>a</sup> NPs	<i>Origanum majorana</i>				<i>Olea europaea</i>				<i>Matricaria chamomilla</i>			
		Ethanol		water		Ethanol		water		Ethanol		water	
		<sup>c</sup> P.	<sup>d</sup> M.	P.	M.	P.	M.	P.	M.	P.	M.	P.	M.
<i>Escherichia coli</i>	<sup>b</sup> 0	14	18	12	16	22	18	15	10	18	0	16	20
	alumina NP	<i>Origanum majorana</i>				<i>Olea europaea</i>				<i>Matricaria chamomilla</i>			
		Ethanol		water		Ethanol		water		Ethanol		water	
		P.	M.	P.	M.	P.	M.	P.	M.	P.	M.	P.	M.
<i>Staphylococcus aureus</i>	0	14	8	12	15	10	15	15	25	15	25	25	10

a.NP (nanoparticles) b. mm (millimeter) c. P(plant extract) d. M mix(plant extract and alumina NPs)

The synergistic effect of the alumina NPs with the *Olea europaea* leaf extract was clear against *S. aureus*; however, it had an antagonistic effect with same extract against *E. coli*.

The absorption spectra in the infrared region showed characteristic bands of the crude plant extract and the crude plant extract with alumina NPs, such as the stretching bands of the OH grouping and the alumina NP nanostructures. These results are in agreement with the report by Sutradhar et al. [34] (figures 3-5).



Figures 2: The bio-functionalized (FTIR) peaks of the *Matricaria chamomilla* extract

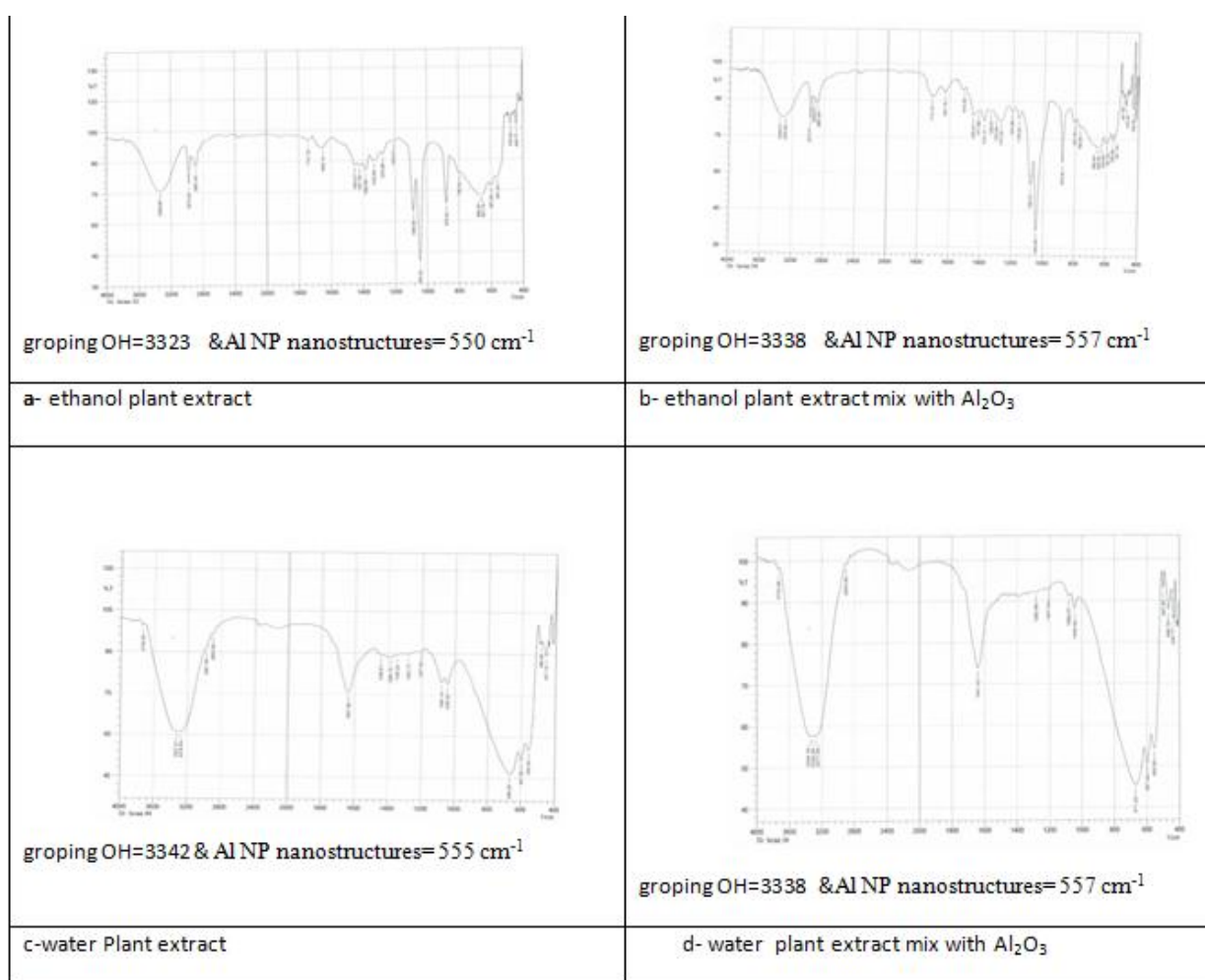


Figure3: The bio-functionalized (FTIR) peaks of the *Olea europaea* extract

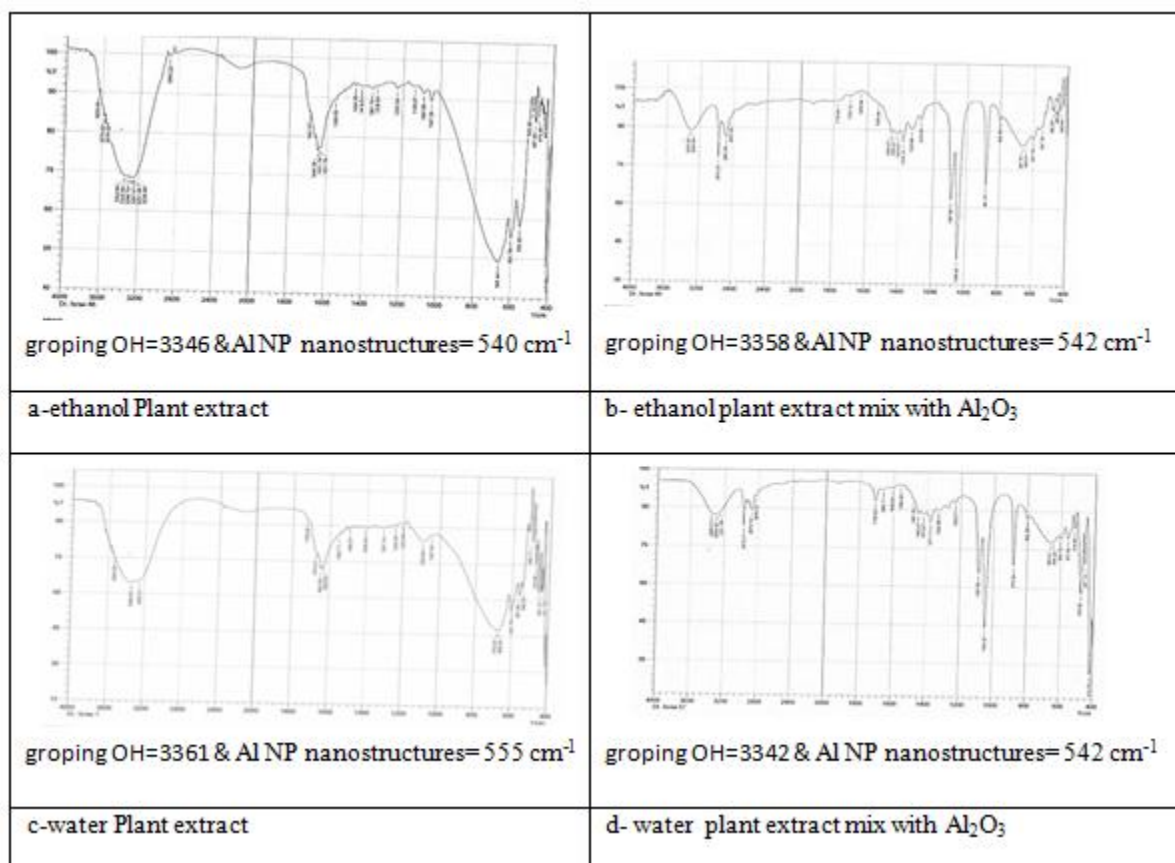


Figure4: The bio-functionalized (FTIR) peaks of the *Origanum majorana* extract

These results can be attributed to several factors, including the metal type and solubility. The compound with the N and O donor system might have inhibited enzyme production, because the enzyme, which requires a free –OH group for its activity, appears to be especially susceptible to deactivation by aluminum ions.

Both the crude plant extract and the alumina NPs showed a strand-breaking effect on bacterial DNA (Figure 5, 6, 7). [35, 36] Research by Vahdati and Sadeghi showed that silver and silica nano-particles cause severe strand-breaking effects on plasmids. The DNA strand-breaking activity in agarose showed clear results for the crude plant extraction and the crude plant extraction with alumina NPs. Exposure to alumina NPs may produce various direct and indirect gene toxic effects, such as DNA strand breaks. In this study, an unsuspected result appeared in the DNA and the plant extract interaction in both water and ethanol solvents, which might be because certain organic materials are responsible for this hydrogen bond interaction.

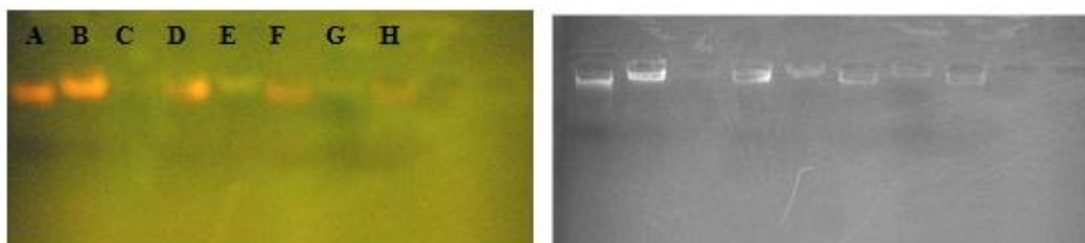


Figure 5: Gel electrophoresis diagram of the experiments using *E.coli* and the ethanol plant extract. A. DNA, B. DNA+ Al NP., C. DNA+ *Origanum majorana*, D. DNA+ *Origanum majorana* with Al NP., E. DNA+ *Oleaeuropaea* F. DNA+ *Oleaeuropaea* with Al NP., G.DNA+ *Matricaria chamomilla* and H. DNA+ *Matricaria chamomilla* with AlNP

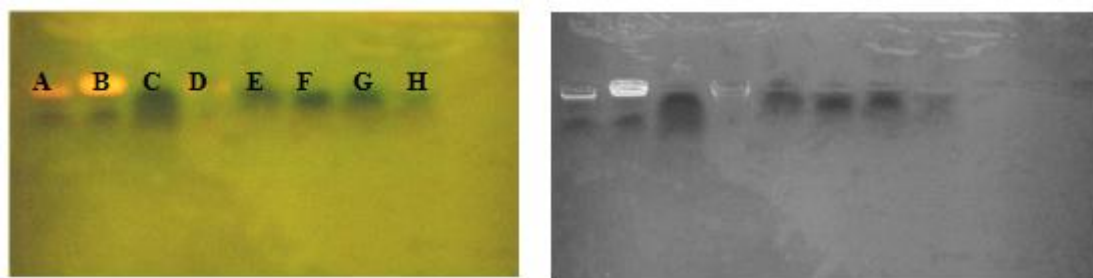


Figure 6: Gel electrophoresis diagram of the experiments using *E.coli* and the water plant extract. A. DNA, B. DNA+ AI NP., C. DNA+ *Origanum majorana*, D. DNA+ *Origanum majorana* with AI NP., E. DNA+ *Oleaeuropaea* F. DNA+ *Oleaeuropaea* with AI NP., G. DNA+ *Matricaria chamomilla* and H. DNA+ *Matricaria chamomilla* with AINP

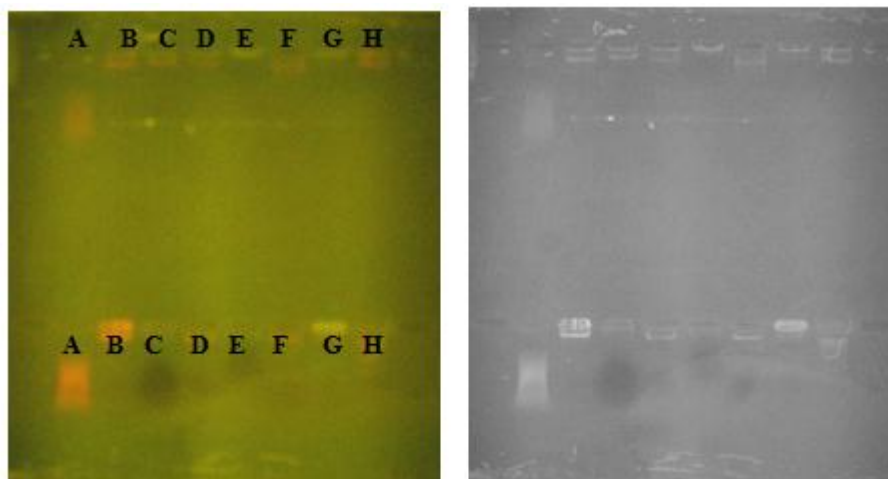


Figure 7: Gel electrophoresis diagram of the experiments using *Staphylococcus aureus* and the ethanol plant extract and the water plant extract A. DNA, B. DNA+ AI NP., C. DNA+ *Origanum majorana*, D. DNA+ *Origanum majorana* with AI NP., E. DNA+ *Oleaeuropaea* F. DNA+ *Oleaeuropaea* with AI NP., G. DNA+ *Matricaria chamomilla* and H. DNA+ *Matricaria chamomilla* with AINP

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