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ORIGINAL ARTICLE

Inhibitory effect of magnetic aqueous extract of *Syzygium aromaticum* L on two types of oral pathogenic bacteria

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ABSTRACT

Aim Magnetic fields affect all living things, which is why they are the subject of extensive research around the world. The aim of this study was to investigate the effects of different levels of static magnetic field on chemical behaviours of aqueous extract of *Syzygium aromaticum* L and their effect on selective oral pathogenic bacteria.

Methods This study utilized locally manufactured dipolar static magnetic fields with strengths between 1200 and 3200 Gauss, measured by a Sylocimol Residence magnetizer. Gram positive G (+) *Streptococcus viridans* and G negative (G-) *Porphyromonas gingivalis* strains were used in studies on the antimicrobial properties of *S. aromaticum* extract. Tests were carried out using the clove essential component (eugenol) and its functionalized extract to evaluate various bacterial inhibitions and establish whether there is a synergistic effect between the extract and subjected to the magnetic field.

Results Polyphenol extraction increased with eugenol content. The analytical method's reliability was confirmed by chromatograms of eugenol and eugenol acetate standards, demonstrating good separation of compounds. The selectivity of the method was guaranteed by the purity index of the eugenol and eugenol acetate peaks. The aqueous extract of *S. aromaticu*m showed magnetic properties. The extract showed antimicrobial activity against two bacterial strains. Cell viability changed at different magnetic field intensities, and the inhibition zone for *P. gingivalis* increased significantly due to prolonged exposure.

Conclusion The magnetized extract inhibits both G (+) and G (-) bacteria when combined with clove extract.

Keywords: anti-bacterial activity, clove extract, magnetized extract, oral bacteria

INTRODUCTION

Magnetism is widely used in the fields of Physics, Medicine, Industry, and Commerce, and has notable effects on metals known for centuries; however, in the case of living organisms, its influences are a more recent discovery that has not yet been fully developed or disseminated (1). The application of electromagnetism involves a series of experimental conditions ranging from different forms of application and exposure time, the level of electromagnetic induction, frequencies, intensities, and types of electromagnetic fields, but it is necessary to establish spaces for more specific work in the area (2).

There are numerous types of living microorganisms, and each one contains trace amounts of ferromagnetic material, most commonly magnetite, which aids in the orientation of the host in the Earth's geomagnetic field (3). One technique to provide evidence that this material is present inside the cell observes how the cell reacts when a magnetic field is introduced (4).

Magnetic fields are classified into two types: static magnetic and pulsed magnetic. Both types of magnetic fields have distinct medical applications (5).

Syzygium aromaticum (L.), popularly known as Indian Clove, is a plant of the *Mirtaceae* family, perennial and arboreal in size, which produces small, aromatic, long- hermaphrodite flowers, which are sold dry even in the development phase bud, and are the main raw material for obtaining Clove essential oil (6). In phytotherapy, it is used in several ways, such as in aqueous extracts, ethanol extracts, and in the form of essential oil (7).

Clove essential oil can be extracted from relatively simple processes, steam distillation is the most widely used, as it generates an essential oil of excellent quality and allows the processing of large amounts of raw material (8). Many studies have demonstrated in vitro antimicrobial action of clove essential oil against many types of pathogenic bacteria and fungi (9)).

The antimicrobial and antioxidant properties of cloves can also be used in food industry, as a preservative condiment, increasing food durability by decreasing oxidation and inhibiting microbial development (10), carried out the extraction of the essential oil of *S. aromaticum* using the steam distillation method and analysed it using the gas chromatography technique coupled with mass spectroscopy (11).

It was found that Eugenol is the main component of clove

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essential oil, corresponding to 52.53%, followed by Caryophyllene (37.25%) (12–14).

Clove essential oil is a powerful antimicrobial and antioxidant agent. These characteristics are attributed mainly to its major compound, eugenol (15,16). Thus, we hypothesized that clove extracts have relevant antimicrobial characteristics being a potential alternative to the use of conventional antimicrobials, preservatives, and pesticides, requiring, however, further research to verify feasibility of its practical application in health and agriculture (17).

There is a particular interest in evaluating the antimicrobial activity of clove extracts and clove-based extracts, so that they could be used in the medical sector as alternatives to traditional antimicrobial agents.

The aim of this study was to investigate the effects of different levels of static magnetic field on the chemical behaviours of aqueous extract of *Syzygium aromaticum* L and their effect on selective oral pathogenic bacteria.

MATERIALS AND METHODS

Materialss and study design

The study was done at the Department of Microbiology, Ninevah College of Medicine, Ninevah University, Mosul City, IRAQ, in 2023.

In all experiments, deionized water was used as a solvent (18MΩ cm^{-1}) (Easypure deionized water systems, Thermo Fisher Scientific, Waltham, Europe). Chemical analytical reagent (AR) grade and reagents with 99% purity were used (Sigma-Aldrich, in St. Louis, Missouri, USA).

All ethical approvals for the research were obtained from the University Ethics Committee of the Faculty of Medicine.

Methods

Magnetic water preparation. To carry out antibacterial study and phytochemical analysis with magnetically treated water, a 250 L reservoir was used and a Sylocimol magnetizer (Valbonne, Alpes-Maritimes, France) 1,000 L, was added (Figure 1), which is capable of magnetizing 1,000 L/1h of water. According to the manufacturer, when subjecting water to the magnetic field of the Sylocimol magnetizer, ionization occurs that promotes the dissociation of the water molecule, H_2O into OH^- and H^+. Hydroxyl (OH^-) reacts with minerals found in water (18). To carry out the magnetized water production, a permanent magnet magnetizer with a magnetic induction of 1200 Gauss and 3200 Gauss was used. The device contains two magnets, north and south poles (Figure 1).

Particle size of ground vegetable raw material. The granulometric distribution of the ground plant was determined by the sieving technique. The plant material was ground in a MARCONI knife mill (model MA 680, Marconi Equipamentos para Laboratórios Ltda., a Brazilian company. Piracicaba, São Paulo, Brazil.). After grinding and homogenization, a representative sample of the plant material was taken and subjected to the determination of the granulometric distribution, using a set of Bertel sieves (São Paulo, Brazil.).

Extraction of bioactive compounds from Indian clove. Clove extract was prepared by dynamic maceration using dried and

Figure 1. Sylocimol Residence magnetizer (Valbonne, France)

ground *S. aromaticum* flower buds. Glass extractors coupled to a thermostat bath with a temperature set at 50 °C were used. Double distilled water and a plant mass, solvent volume ratio of 1:10 (w/v) were used as extracting solvent (12). To obtain the aqueous extract, 10 mL of magnetic water (1200 Gauss and 3200 Gauss, respectively) was added to the clove powder and mixed until homogenized. Then the mixture was transported to a centrifuge at 1,600 rpm for 10 min. at room temperature of 28 °C; after homogenization, the supernatant was transferred to a drying oven at 48 °C for 72 hours for evaporation and obtaining the gross mass of the aqueous extract. The extractive solution was concentrated in a rotary evaporator at the pressure of 600 mmHg and the temperature of 55 °C.

Quantification of eugenol by High-Performance Liquid Chromatography (HPLC). The reversed phase with Diode Array Detection (HPLC-DAD) technique was used to quantify eugenol in liquid and solid samples (13). Typical HPLC column used was a C18 (octadecylsilane, ODS) column. The chromatographic conditions used were selected based on a method previously validated in the laboratory (19).

The analyses were carried out in a LC-20A series chromatograph (Prominence Shimadzu, Kyoto, Japan), oven temperature of 30 °C, flow rate of 1 mL/min, injection volume of 20 µL and isocratic mobile phase composed of methanol: water 60:40 (v/v). Quantification was performed at a wavelength of 280 nm. The samples were diluted in methanol 60% (v/v) magnetically stirred for 15 min at 45 °C and then centrifuged at 3500 rpm for 5 min. The supernatant was filtered through a 0.45 µm membrane and 20 µL was injected into the chromatograph. Analytical eugenol curves were prepared by diluting known concentrations of the standard in a mobile phase.

Quantification of total polyphenols. The methodology for measuring total polyphenols is based on the Folin-Denis method (20), which consists of the reduction of phosphomolybdic-phosphotungstic acid by phenolic compounds, in a basic medium, producing an intense blue colour that is measured by spectrophotometry at the wavelength of 750 nm, with a reaction time of 2 minutes. Each assay was performed in triplicate and the results were expressed as equivalents of gallic acid per gram of extract using the analytical curve.

Magnetization measurements. Saturation magnetization (Ms)

measurements were performed using a Quantum Design MPMS (Magnetic Property Measurement System) magnetometer. The measurements were carried out under a variable field from 50000 to -50000 Oe, at the fixed temperature of 300 °C.

Antimicrobial activity. The antibacterial effect of plant extracts obtained by magnetic water (1200 and 3200 Guass) extraction method was analysed by the Kirby-Bauer method (disk-diffusion). For this, the bacterial strains (*Streptococcus viridans* ATCC 33399 and *Porphyromonas gingivalis* ATCC 33277 strain) were inoculated in Tryptic Soy Agar (TSA) medium and incubated until their logarithmic phase of growth (37 °C/18-20 h). A suspension of the inoculum was prepared in saline solution (0.85% w/v) adjusting its turbidity to 0.5 of the standard of McFarland ($1x10^8$ CFU/ mL at a wavelength of 625 nm). For the inoculation of the agar a sterile swab was used, which was soaked in the solution, and the excess wiped out. After 15 minutes, the Petri dish was inverted and placed in an incubator (34 °C/16-18 h). The inhibition zones were measured visually using a Vernier calipers (Mitutoyo, Kawasaki, Japan). Colonies that were too small or too faint to be seen were disregarded.

Clove extract solutions were employed in the agar well test, with sterile water serving as the negative control and amoxicillin (1 mg/mL) serving as the positive control. At the end of the time period, the inhibition values and zone widths were measured and recorded (21). The diameters of the halos were determined using a manual calipers (Mitutoyo, Kawasaki, Japan). The results were expressed in mm (millimetres). Halos were considered to have antimicrobial potential against isolates from extracts that generated halos ≥ 6 mm.

RESULTS

The extractive content is an indication of the efficiency of the extraction depending on the extraction solvent used. In water (aqueous media) it was 34.96 ± 1.42% and 35.04 ± 0.94%, respectively. The results of total phenols showed that the polyphenol extraction also increased with an increase in eugenol content.

The validation of the analytical method is necessary in order to guarantee the reliability of the results of quantification of the monitored active principles, demonstrating that it was appropriate for this application. With the chromatographic conditions validated, the chromatograms of the eugenol and eugenol acetate standards were obtained (Figure 2), with good separation of the compounds being observed. The selectivity of the method was guaranteed by the purity index of the eugenol and eugenol acetate peaks of the samples and standards.

The magnetic properties of the aqueous extract were identified through the magnetization curves (Figure 3). This characterization for the reduced sample was being provided. The saturation magnetization values obtained for magnetite were 70 emu/g and the magnetization curves for species showed the absence of hysteresis.

The magnetized extract synthesized from *S. aromaticum* aqueous extract showed activity against two bacterial strains evaluated showing growth inhibition halos in the 5-15 mm range. *Streptococcus viridans* was sensitive to magnetized extract (inhibition zone of 15.09 ± 1.37mm).

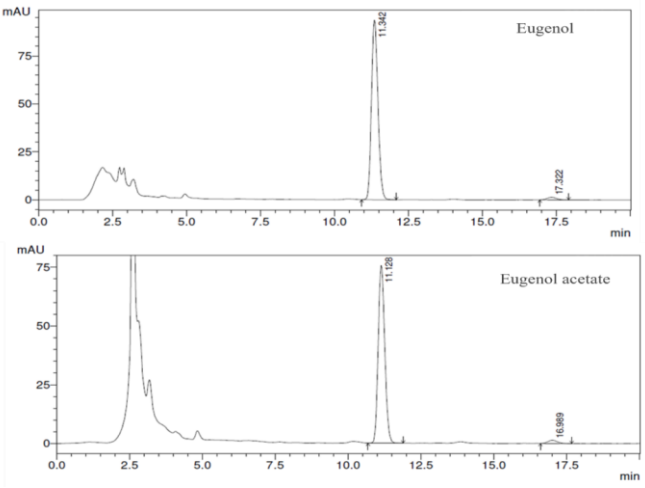

Figure 2. Chromatograms of eugenol (above) and eugenol acetate (below)

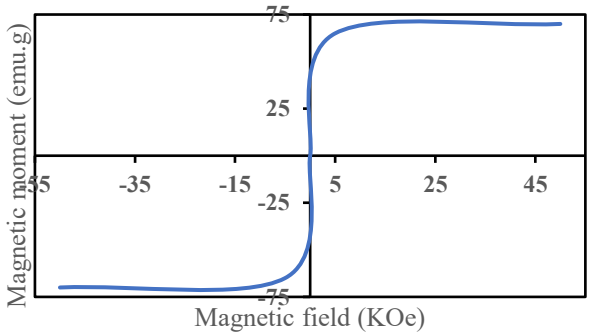

Figure 3. Magnetization measurements of aqueous extract isolated with magnetic water (3200 Guass)

Synthesized magnetized extract was tested for their antibacterial efficacy using an agar well diffusion assay. The sizes of the inhibition zones caused by clove extract alone show that neither bacterial species are affected.

In the comparison to the control group, the use of a magnetic field had a statistically significant impact on bacterial growth ($p<0.05$) (Figure 4).

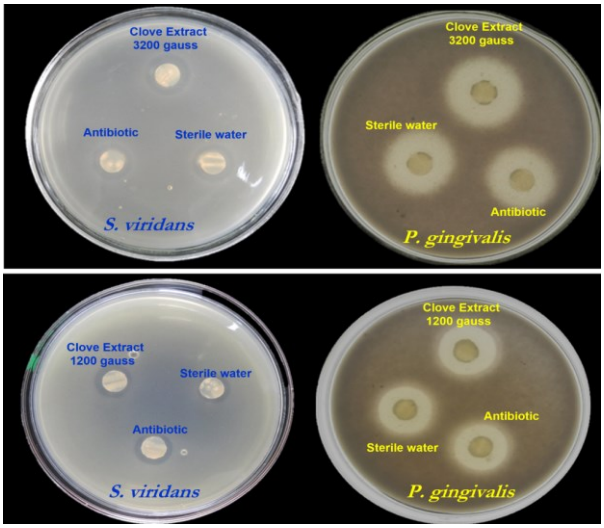

Figure 4. Agar diffusion test results of *Syzygium aromaticum* e aqueous extract isolated with different magnetic fields of magnetic water, sterile water and antibiotic (amoxicillin)

Table 1. Antibacterial activity by the Kirby-Bauer method of synthesized nanoparticles using iron magnetic nanoparticles derived from *S. aromaticum* aqueous extract as a biological reducing agent

Aqueous magnetic field (Guass)	Controls	Inhibition zone (mm)	
		Porphyromonas ingivalis	***Streptococcus viridans***
1200	Amoxicillin	24.22 ± 1.36	31.18 ± 1.91
	Negative control (sterile water)	0.00	0.00
	S. aromaticum Extract	4.94 ± 0.92	9.36 ± 0.64
3200	Amoxicillin	24.22 ± 1.36	31.18 ± 1.91
	Negative control (sterile water)	0.00	0.00
	S. aromaticum Extract	14.15 ± 1.02	15.09 ± 1.37

It is essential to highlight key points related to the effects of different magnetic field intensities (1200 and 3200 Gauss) on antibacterial activity using magnetic nanoparticles synthesized from *S. a*romaticum extract as a biological reducing agent (Table 1).

The effect on *Porphyromonas gingivalis*: When the bacteria were exposed to a 1200 Gauss magnetic field, the inhibition zone diameter was 4.94 ± 0.92 mm. This idicates that the nanoparticles had limited activity in inhibiting the growth of the bacteria. As the magnetic field intensity increased to 3200 Gauss, the inhibition zone expanded to 14.15 ± 1.02 mm. This increase in the inhibition zone size suggests enhanced antibacterial activity of the nanoparticles against *P. gingivalis* with a higher magnetic field intensity.

The effect on *Streptococcus viridans*: At 1200 Gauss, the inhibition zone for *S. viridans* was 9.36 ± 0.64 mm, showing that the nanoparticles significantly inhibited bacterial growth at this intensity. When exposed to 3200 Gauss, the inhibition zone increased to 15.09 ± 1.37 mm. This indicates that higher magnetic field intensity boosted the effectiveness of the nanoparticles on *S. viridans*.

In the amoxicillin group at both magnetic field intensities (1200 and 3200 Gauss), the effect of amoxicillin on *P. gingivalis* and *S. viridans* remained unchanged, with inhibition zones of 24.22 ± 1.36 mm and 31.18 ± 1.91 mm, respectively. This shows that amoxicillin's antibacterial effect was strong and consistent regardless of the magnetic field.

Negative control (sterile water): No effect on bacterial growth was observed with sterile water at both magnetic field intensities, as the inhibition zones were 0.00 mm. This confirms that the nanoparticles and *S. aromaticum* extract are the main factors contributing to the antibacterial effect.

DISCUSSION

The flavonoids and phenolic acids present in the flower buds of cloves have different degrees of polarity and the surfactants facilitate the extraction of both types of molecules. The presented results showed that water was the best solvent for the extraction of polyphenols, due to its high solubility in this solvent.
Our results showed the saturation magnetization values (Ms) of 70 emu/g, for magnetite, similarly with the values predicted by the literature at 85 emu/g (13), corroborating the increase in the magnetic interaction of the particles observed after the reduction procedure. The magnetization curve also provides the magnetic behaviour profile of the materials; the low values obtained in our study of the coercive field and remnant magnetization and the absence of hysteresis, confirm the paramagnetic state of the plant extracts (9).

The saturation magnetization values for magnetite in our study were measured at 70 emu/g. The magnetization curves for the species displayed no hysteresis, which confirms the paramagnetic behaviour of the particles derived from the plant extract (22).

Our study demonstrated that the analysis of essential oil produced 80% eugenol using gas chromatography, and this result is consistent with other studies that confirmed this finding (13,14). Another study showed that the eugenol content in the oil ranged between 47% and 91%. The variation in percentages compared to the current study is attributed to the different origins of the S. aromaticum buds used in the extraction process(15). We conclude that the extraction method influences the percentage of eugenol, which in turn affects the antimicrobial activity.

Natural products are considered an important source of new antibacterial agents; 80% of new drugs or semisynthetic drugs are obtained from natural sources (23).

Synthesized magnetized extract was tested for their antibacterial efficacy using an agar well disk diffusion assay. The sizes of the inhibition zones caused by clove extract alone showed that neither bacterial species are affected. Probably because unmodified carrageenan had no impact on microorganisms. When the diameters are compared, it is clear that the 0 mM group (negative control) had no effect against either strain of bacteria. Magnetized extracts were more effective against the Gram-positive group, specifically *Streptococcus viridans* bacteria, when the two groups are compared to one another. Simultaneously, *Streptococcus viridans* bacteria with the concentration of 10 mM had a maximal inhibitory diameter zone. In this light, it has been found that the synthesized magnetized extract is effective against selected microorganisms.

In general, when antimicrobial activity is examined, it is thought that magnetized extract attacks the cell walls of bacteria and disrupts the structure. Here, the surface area and electrostatic interaction of nanoparticles (NPs) played a direct role in disrupting the microorganism structure of the particles. Nanostructures with large surface areas increased the interaction (24). One of the possible causes lies in the preservation of discs fortified with plant material having a phenol component since, according to WHO recommendations (25),

this must be stored at 4 ˚C to avoid deterioration of the antibiotic. Another possible failure could have been the amount of inoculum per volume unit that was not correct.

Since magnets can kill bacteria, they could be added to the treatments for gums that are pulling away from the teeth. When the periodontal tissues are in good health, the support for the oral tissues under a partial or supradental denture is much better (26).

There is evidence that natural water can be made healthier by a magnetic field because it kills microorganisms (27).

The antibacterial mechanisms of magnetized extract have not been fully elucidated, but several studies (23) have shown that magnetized extract can adhere to the cell membrane surface, thus perturbing permeability and respiratory functions. Furthermore, magnetized extract interacts with the membrane surface and can penetrate the bacterium (28).

To use the disk diffusion method, the diffusion capacity of the compound to be evaluated must be taken into account, a characteristic that is not easy to determine because the components of soluble eugenol (plant extract) are not exactly known, and which of these bind to the surface of the nanoparticles (29).

It should also be taken into account that in many cases where the disk diffusion method is used for evaluation, a certain unspecified volume of antibiotic is usually placed on the disk at no specific concentration, and therefore the results may not be reproducible (29).

Our result is in accordance with other researchers that the polarized parts of the large magnet produce electrical potentials that are particularly harmful to bacteria (30). The bacterial cells cannot control how ions move through their membranes because ion's energy would be too great for their membrane potentials (30). Many crucial biological functions depend on ions moving through membranes (31). Bacterial cells become "very sick" when they are unable to use protein channels to control ionic currents. Magnetic fields affect various types of bacteria differently, as demonstrated by numerous researches (32).

In conclusion, it was discovered that there were substantial differences between the inhibition zone of oral bacteria and the inhibition zone after irrigation with magnetized water of various magnet strengths. Since magnetized water has the best antibacterial properties, it is suggested that it should be used frequently as an irrigant for optimum dental hygiene. It is also inexpensive, fresh, and easily accessible. According to the data presented in this study, *S. aromaticum* magnetized aqueous extract has antibacterial activity against Gram-positive and Gram-negative pathogenic microorganisms that affect both humans and animals. This study provides a green clove extract-based magnetic extract that uses clove aqueous extract as a reducing agent and is cost-effective, scalable, and environmentally friendly. Magnetic aqueous extracts were successfully separated with the aim of fabricating a material that satisfies biomedical uses.

AUTHOR CONTRIBUTIONS

Conceptualization, W.S.E. and S.A.Al-S.; methodology, R.I.Al-S.; software, R.I.Al-S.; validation, W.S.E. and R.I.Al-S.; formal analysis, S.A.Al-S.; investigation, W.S.E.; resources, S.A.Al-S.; data curation, R.I.Al-S.; writing—original draft preparation, W.S.E.; writing—review and editing, S.A.Al-S.; visualization, R.I.Al-S.; supervision, W.S.E.; project administration, W.S.E. All authors have read and agreed to the published version of the manuscript.

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TRANSPARENCY DECLARATION

Conflict of interests: None to declare.

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