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GREEN SYNTHESIS NANOMAGNETIC $MnFe_2O_4$ USING BREADFRUIT LEAF EXTRACT (*Artocarpus altilis*) And ANTI-BACTERIAL ACTIVITY TEST AGAINST *E.coli* And *S. aureus*

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ABSTRACT
In this research, breadfruit (*Artocarpus altilis*) leaf extract has been used to synthesize nanomagnetic $MnFe_2O_4$. The resulting nanomagnetic characterization uses a Vibrating Sample Magnetometer (VSM) to determine magnetic properties, phase type and crystal size using analytical X-Ray Diffraction (XRD), morphology and element composition using Scanning Electron Dispersive X-ray Spectroscopy (SEM-EDX) and functional groups using Fourier Transform Infra Red (FTIR). $MnFe_2O_4$ obtained was tested for antibacterial activity against the gram-positive bacteria *E. coli* and *S.aureus.* The result of the VSM characterization has magnetic property of 47.76 emu/g, the nanomagnetic XRD characterization was successfully synthesized with a peak appearing at an angle 2θ = 30.02°, 35.28°, 44.53°, 56.65°, dan 62.25°, which corresponds to the crystal plane (220), (311), (400), (511) and (440) showed an average crystal size of 25.82 nm, SEM results with various magnifications showed the formation of aggregates or chunks of unequal size, as well as the presence of Mn-O and Fe-O groups in FTIR analysis. This research showed that the nanomagnetic compound $MnFe_2O_4$ obtained from green synthesis has antibacterial properties against gram-positive bacteria E. coli and S. aureus with a Minimum Inhibitory Capability (MIC) value of 2000 ppm. These results indicated that nanomagnetic compounds using the green synthesis method have the potential to be applied to gram-positive bacteria *E.coli* and *S.aureus.*

Key words: Nanomagnetics, green synthesis, breadfruit leaves, antibacterial.

INTRODUCTION

Inorganic and organic materials can be modified with magnetic nanoparticles to produce magnetic materials. Ferrite type magnetic nanoparticles have a structure representing cubic

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divalent metal ions which have unique medium size magnetization properties, a single dominant effect, superparamagnetization (Zipare *et al*., 2015). Spinel ferrite magnetic nanoparticles have the structural formula MFe_2O_4 (M= Mn^{2+}, Co^{+2}, Fe^{2+}, Ni^{2+}, Mg^{2+}, Zn^{2+}) with a cubic spinel crystal structure which has a large surface area ratio and unique magnetic properties such as high conversion, moderate magnetization, spin filtration, and single domain effects (Iranmanesh et al., 2017). Nanomagnetic ferrite has broad spectrum antimicrobial properties that can significantly inhibit the growth and reproduction of contaminants. One of the nanoparticles that can promote antibacterial and biomedical activity is $MnFe_2O_4$ particles because the synergistic effect of Manganese Ferrite between Mn^{2+} and Fe^{2+} can significantly increase the efficiency of electron transfer in the $MnFe_2O_4$ system (Chen et al., 2023).

Magnetic nanoparticles also have a fairly large surface area, are easy and cheap to synthesize (Hariani & Riyanti, 2014). It is known that the synthesis method has an important influence on the composition, structure and morphology, implicitly on the properties of spinel ferrite nanoparticles (Puspitasari, et al., 2017). The use of magnetic nanoparticles has the advantage because it can easily be carried out magnetically, liquid waste apart from containing chemical pollutants such as heavy metals and dyes, also contains biological pollutants, namely *S. aureus* and *E. coli* bacteria, which are bacteria that are easy to carry in the environment (Tawainella et al., 2014).

Several plants are currently being developed for nanomagnetic metal biosynthesis, one of which is breadfruit leaves. Breadfruit leaves are leaves that have powerful properties in curing many diseases including hepatitis, spleenitis and diabetes because they contain phenols, steroids and flavonoids. These compounds act as capping agents or stabilizers during nanomagnetic synthesis to prevent agglomeration and have antibacterial effects against various microbes. (Ahmed et al., 2015). Nanomagnetic $MnFe_2O_4$ preparation has been carried out using methods including solid state reaction, plant extract (Green Synthesis), hydrothermal, electrospinning, and coprecipitation (Martinise et al., 2018). Among these methods, synthesis with plant extracts is an environmentally friendly alternative synthesis method.

The magnetic susceptibility of $MnFe_2O_4$ is higher than other ferrites such as $CoFe_2O_4$, and $NiFe_2O_4$ with a spin of 5 µb. This is because the magnetic moment of Mn ferrite is in accordance with the coupling scheme (Lee et al., 2007). $MnFe_2O_4$ has antibacterial properties that are useful in the industrial and medicinal domains (Icten et al., 2022). $MnFe_2O_4$ shows

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antibacterial activity against gram-positive bacteria Staphylococcus aureus and Escheria Coli (Elhaddad & Al-Fawwas, 2023). Based on this description, this research synthesizes nanomagnetic $MnFe_2O_4$ with breadfruit leaf extract, where $MnFe_2O_4$ is the magnetic core. $MnFe_2O_4$ nanomagnetic is utilized as an antibacterial against *S. aureus* and *E. coli*. The resulting $MnFe_2O_4$ nanomagnets were characterized using X-Ray Diffraction (XRD) to determine the 2θ angle value, phase type, and crystallinity. Scanning Electron Microscopy Energy Dispersive X-Ray (SEM-EDX) to observe the morphology and elements that make up the material. The saturation magnetization value was determined using a Vibrating Sample Magnetometer (VSM). energy band gap value, and Fourier Transform Infra Red (FTIR) to determine functional groups.

METHODS

Tools and materials

The instruments used in this research were FTIR (Thermo scientific Nicolet iS-10), X-Ray Diffraction (Shimadzu model XD-3H), SEM-EDX (JSM 6510 LA) and VSM (Oxford Type 1.2 H). Commercial chemicals used consist of anhydrous manganese sulfate $MnSO_4.7H_2O$ (Merck), technical ethanol (C_2H_5OH) 96%, bacteria (*S. aureus* and *E. coli*), muller hiton agar (MHA) media (Merck), macconkey agar (MCA) (Merck), Mc Farland Standard (Merck), Amoxilin (Merck), DMSO (Merck), NaCl (Merck).

Procedures

Breadfruit Leaf Sample Preparation

Samples of breadfruit leaves (Artocarfus altilis) were obtained from Perumnas, Sekayu District, Musi Banyuasin Regency, South Sumatra. Fresh samples were then collected and washed until clean, then dried at room temperature until dry. After drying, the sample was ground with a blender until powder formed.

Sample Extraction

Breadfruit leaf powder (Artocarfus Altilis) was put into a maceration bottle of 250 g. The samples were soaked with 96% technical ethanol. Maceration was carried out 2x24 hours with repetition 3 times and stored in a place protected from sunlight every 12 hours the sample was stirred. The sample was filtered using Whatman 42 filter paper, to separate the extract from the residue so that a residue-free extract was produced. The macerated sample extract is then concentrated using a rotary evaporator set at the boiling point of the solvent used. After getting the concentrated extract, it is then dried at room temperature.

Synthesis of Nanomagnetic

Synthesis of $MnFe_2O_4$ particles uses the green synthesis method by mixing $MnSO_4.7H_2O$ and $FeCl_3.6H_2O$ as providers of Mn^{2+} and Fe^{3+} ions with a coefficient ratio of 1:2. 10 mmol (2.77 g) of $MnSO_4.7H_2O$ and 20 mmol (5.41 g) of $FeCl_3.6H_2O$ were dissolved in 45 mL of distilled water, added with 5 mL of breadfruit leaf extract, stirred using a magnetic stirrer at room temperature at 550 rpm for 120 minutes. The resulting mixture was added to 2 M NaOH gradually until pH 10. The precipitate was filtered and washed repeatedly using distilled water until pH 7 was reached. The precipitate was dried using an oven at a temperature of 80 °C, then calcined in a furnace until dry at a control temperature of 400 °C for ± 4 hours. The resulting black $MnFe_2O_4$ powder was produced.

Characterization

With the use of a tube CuKα (wavelength 1.54056 Å) and X-ray diffraction (XRD), the structure of $MnFe_2O_4$ was determined, over the measuring angle range 10^o until 80^o. Calculate the diameter of the crystal size using the *Debye-Scherer* formula:

$$D = \frac{K\lambda}{\beta \; Cos\,\theta}$$

The D value is the crystal diameter, K is the Debye-Scherer Constant with a constant (0.5 nm), β is the Full Width Half Maximum (FWHM) value and θ is the diffraction peak angle. Morphology of the $MnFe_2O_4$ element using a scanning electron microscope with Scanning Electron Microscopy Energy Dispersive X-Ray (SEM-EDX) with a low vacuum of 50 psi and energy of 20 kV. The magnetic characteristics of substances are assessed using a Vibration Sample Magnetometer (VSM). The saturation magnetization value is obtained by applying and vibrating the sample magnetometer. Confirmation to determine the success of the coating was characterized using Fourier Transform Infra Red (FTIR) with conventional and sonochemical methods synthesized without ultrasonic waves producing sharp absorption at a wave number of 594.08 cm^{-1}. KBr pellets were used to prepare the samples, and the 400–4000 cm^{-1} wave number range was used for analysis.

Bacterial Suspension Culture

Bacterial suspension culture was carried out by taking 1 loop needle and then inoculating it by scratching the surface of the media so that it was tilted and incubated for 24 hours at 37 ^{o}C. Bacterial suspension culture where the test bacteria are taken from 1 rejuvenated needle, then suspended in 5 mL of Nutrient Both (NB) and stirred. The turbidity of the bacterial suspension was compared to standard McFarland solution.

Antibacterial Activity Test

Bacterial activity testing was carried out using the Kirby-Bauer disc diffusion method, using paper discs with a diameter of 5 mm with the test bacteria *S. aureus* and *E. coli*. The activity test was carried out with three repetitions. The paper discs were soaked in a nanomagnetic solution with a concentration of 1000–5000 ppm with positive control (Amoxilin) and negative control (DMSO). Using sterile tweezers, put the paper disc on the media's surface. Petri plates were incubated for 24 hours at 37 ^{o}C until the appearance of an inhibitory region. Bacterial growth was observed by observing the inhibition zone formed with a ruler or caliper.

RESULTS AND DISCUSSION

Sample extraction

The weight of the coarse breadfruit leaf powder used was 2 kg, ground using a blender, filtered using a sieve or 100-mesh sieve, resulting in 800 g of fine breadfruit leaf powder. 300 g of breadfruit leaf powder was macerated using technical ethanol and produced dark green macerate. Maserate which was concentrated using a rotary vacuum evaporator resulted in a thick extract of breadfruit leaves of 32.385 grams with a yield of 10.795%.

Synthesis Results of $MnFe_2O_4$

Figure 1. $MnFe_2O_4$ Synthesis results and tests with an external magnet

Showed that the synthesis of $MnFe_2O_4$ using the green synthesis method had been carried out with a ratio of 1:2 MnSO4.7H2O: $FeCl_3.6H_2O$ with the addition of 5 mL of thick breadfruit leaf extract. Figure 1. Showed that $MnFe_2O_4$ has superparamagnetic properties, when it was brought close to an external magnet there is a strong interaction. This test shows that $MnFe_2O_4$ had been successfully synthesized.

Characterization Results

Characterization of $MnFe_2O_4$ with VSM

$MnFe_2O_4$ was characterized using VSM with the aim of determining its magnetic properties which are described by a hysteresis curve. The hysteresis curve showed the relationship between magnetization (M) and external magnetism (H). The VSM results are shown in Figure 2.

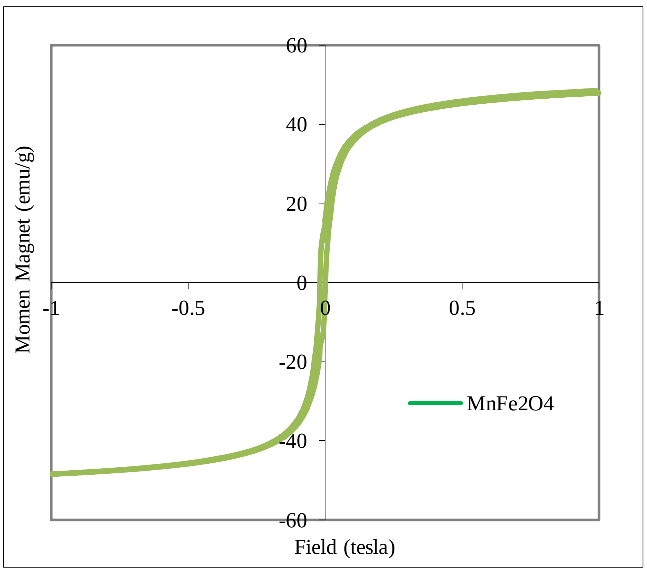

Figure 2. $MnFe_2O_4$ magnetic hysteretic loop

Based on Figure 2, it showed that the saturation magnetization value of the $MnFe_2O_4$ sample obtained in this study is 47.76 emu/g, indicating that $MnFe_2O_4$ is superparamagnetic (Tawainella et al., 2014). The uniformity of a particle's size has an impact on its magnetic characteristics (Salni et al., 2022). $MnFe_2O_4$ is included in soft magnetics and has a small crystal size so that the magnetization forms an S with a narrow hysteretic loop. The low coercivity value can facilitate demagnetization (Naaz et al., 2020).

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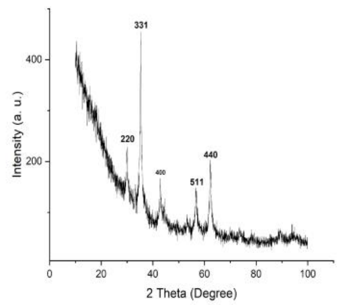
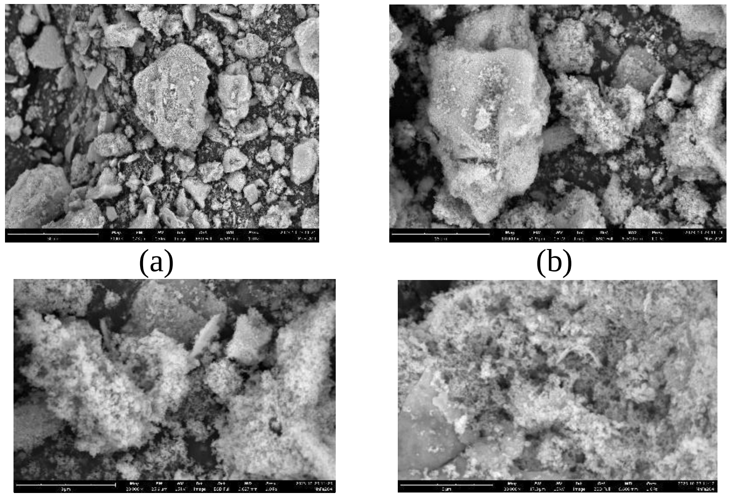


Figure 3. XRD diffractogram spectrum of $MnFe_2O_4$

Figure 3, is an XRD diffractogram of nanomagnetic $MnFe_2O_4$ with five diffraction peaks which have intensity, at an angle $2\theta =$ 30.02°, 35.28°, 44.53°, 56.65°, dan 62.25°, which correspond to the crystal planes (220), (311), (400), (511) and (440) represent $MnFe_2O_4$ with a spinel structure without impurities. The observed peaks at pattern and lattice parameters of 0.5 Å' are in good agreement with the reference structure from JCPDS data No. 10-0319. The average crystal size of $MnFe_2O_4$ calculated using the *Debye-Scherrer* equation is 25.82 nm which shows that nanomagnetics were successfully synthesized. The $MnFe_2O_4$ crystal size is included in the nanomagnetic group because it has a size of <100 nm. The Debye-Scherrer equation explains that crystal size is inversely proportional to FWHM. The greater the FWHM width value, the smaller the crystal size (Didik, 2020). The size of $MnFe_2O_4$ crystals is included in the nanomagnetic group, having a size of <100 nm.

The morphology of $MnFe_2O_4$ was identified using SEM while the element composition was determined using EDX. $MnFe_2O_4$ Shows particles at different magnifications. The SEM characterization of $MnFe_2O_4$ is shown in Figure 4. The SEM results of $MnFe_2O_4$ at various magnifications showed that it forms aggregates or chunks of unequal size, showing that nanomagnetism tends to experience agglomeration. Agglomeration occurs because the size of the nanomagnet is very small, so it has a large surface with large pores and has high surface tension, causing the nanomagnet to become unstable (Kiswanto et al., 2021).



(a) (b)

(c) (d)

Figure 4. SEM-EDX $MnFe_2O_4$ morphology with magnification (a) 3000x (b) 10,000x (c) 20,000x (d) 30,000x

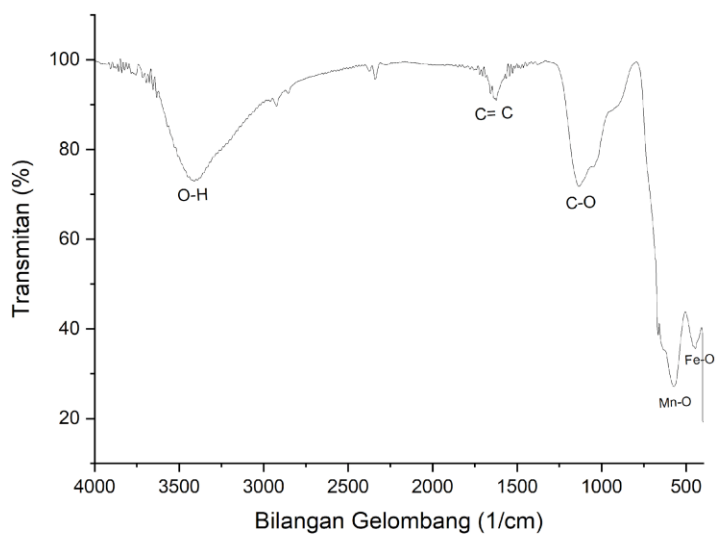


Figure 5. FTIR Spectrum $MnFe_2O_4$

In Figure 5, a typical FTIR absorption transmittance peak in the range of 400-4000 cm-1 is observed. The broad band of nanoparticles at 3600-3000 cm^{-1} is caused by OH stretching vibrations. The peak at the wave number 3410.15 cm^{-1}, the presence of the O-H group, indicated the bending, stretching and vibration of O and H, the stretching and vibration of O-H which indicates the absorption of adsorbed water molecules which showed a higher amount of OH (Jacintha et al., 2017). The C=C bending vibration shown in the wave number range 1624.06 cm^{-1} occurs due to stretching of benzene so that there is a vibration result at the wave number 1134.14 cm^{-1}, the appearance of the C-O group where the bond is from an aromatic compound.

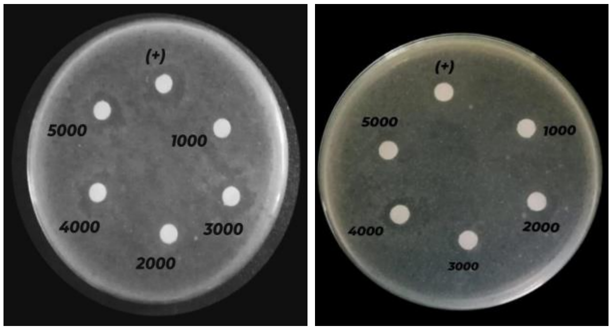
C-H bending vibration absorption peaks below 1000 cm^{-1}indicated ferrite. For the wave number 572.86 cm^{-1} the strong intensity is the vibration of manganese ferrite (Mn-O). Wave number 445.56 indicates vibration (Fe-O) which is an indication for the formation of Spinel Ferrite.

Antibacterial Test Results

Table 1. Data on the diameter of the zone of inhibition of antibacterial activity *S. Aureus* and *E. coli*

Concentration (ppm)	Inhibition zone diameter (mm)	
	S. aureus	*E.coli*
1000	0	0
2000	10,4± 0,04	10,1±0,04
3000	10,6±0,04	10,105±0,02
4000	10,615±0,28	11,105±0,04
5000	12,115±0,70	11,615±0
Antibiotik (+)	12,15±0,72	11,13±1,27

The antibacterial characteristics of the $MnFe_2O_4$ nanomagnetic compound against *S. aureus* and *E. coli* are displayed in Table 1. It is well known that the antibacterial capabilities of $MnFe_2O_4$ nanomagnetic material are dependent on both its concentration and size (Salni et al., 2022). Antibacterial activity against $MnFe_2O_4$ metal depends on size, morphology, surface area, diffusion ability of chemical molecules and also the release of metal ions. $MnFe_2O_4$ inhibits the growth of *S. aureus* and *E. coli,* where the antibacterial properties against *S. aureus* are greater than *E. coli*. Apart from that, *S. aureus* bacteria are easier to inhibit than *E. coli* because this bacteria has a structure with a high peptidoglycan content so it is difficult to penetrate the bacterial cell wall and inhibit its growth (Ashwini et al., 2017). At 2000 ppm, the research yielded a Minimum Inhibitory Concentration (MIC) value, indicating that *S. aureus* outcompeted *E. coli.* The difference in diameter of the inhibitory power of the two bacteria is different due to differences in the structure of the bacterial membrane (Hariani et al., 2020). Gram-positive bacteria are bacteria that have cell walls consisting of the main component peptidoglycan which is permeable and usually does not limit antimicrobials (Ragab et al., 2014). Using breadfruit leaf extract as a molecular capping or bridging agent between nanoparticles, it contributes to the creation of nanomagnetic $MnFe_2O_4$, which increases antibacterial activity.



(a) (b)

Figure 6. Nanomagnetic $MnFe_2O_4$'s antibacterial efficacy against (a) S. aureus and (b) E. coli

CONCLUSION

The nanomagnetic compound $MnFe_2O_4$ was successfully synthesized using the green synthesis method. The characterization results show an average crystal size of 25.82 nm, magnetic properties of 47.76 emu/g, and the presence of Mn-O and Fe-O groups. The antibacterial capabilities of $MnFe_2O_4$, a nanomagnetic compound, are demonstrated by its MIC value of 2000 ppm against *S. aureus* and *E. coli*. Because breadfruit leaf extract functions as a molecular capping or bridging agent between nanoparticles, its inclusion in the $MnFe_2O_4$ production process can enhance its antibacterial action.

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