Aflatoxin Detoxification by Biosynthesized Iron Oxide Nanoparticles Using Green and Black Tea Extracts

Hasan Gedikli,[a] Arda Akdogan,[a] Omer Karpuz,[b,*] Osman Akmese,[c] Havva N. Kobya,[a] and Cemalettin Baltaci [a]

Researchers have recently been interested in employing nanoparticles (NPs) obtained from herbal extracts through green synthesis for various applications. This study investigated the detoxification of aflatoxins, which are toxic substances produced by molds *Aspergillus flavus* and *Aspergillus parasiticus*. The present work examined the levels of aflatoxins in hazelnut and peanut puree. Turkish black tea extract (BTE), Turkish green tea extract (GTE), green synthesized black tea-based iron oxide nanoparticles (BTFeONPs), and green tea-based iron oxide nanoparticles (GTFeONPs) were produced for aflatoxin removal. Characterizations and various antioxidant and antimicrobial activities of the tea extracts and iron oxide nanoparticles (FeONPs) were investigated. The aflatoxin levels of hazelnut puree used for this study were 6.57 ± 0.06 µg/kg for aflatoxin B_1 and 13.03 ± 0.16 µg/kg for total aflatoxin, whereas the aflatoxin levels of ($AFLB_1$) peanut puree were 7.79 ± 0.15 µg/kg for $AFLB_1$ and 15.21 ± 0.12 µg/kg for total aflatoxin. Using soluble BTE resulted in a 40 to 50% decrease in aflatoxin levels in hazelnut and peanut purees, while soluble GTE led to a 30 to 45% decrease. Meanwhile, using BTFeONPs and GTFeONPs resulted in a 33 to 48% and 40 to 50% decrease, respectively, in aflatoxin levels in hazelnut and peanut purees.

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*Contact information: a: Dept. of Food Engineering, Gumushane University, 29100, Gumushane, Turkey; b: Dept. of Genetics and Bioengineering, Gumushane University, 29100, Gumushane, Turkey; c: Central Research Laboratory, Gumushane University, 29100; *Corresponding author: omerkarpuz@gmail.com*

INTRODUCTION

Aflatoxins pose a serious concern due to their high cancer-causing potential and ability to remain unaffected by metabolic processes in tissues and masses. According to the Food and Agriculture Organization of the United Nations (FAO), approximately 25% of global food sources are affected by aflatoxin contamination (Wu 2007). Consequently, aflatoxin contamination has consistently posed a global issue in the agricultural sector.

Given its substantial threat to human and animal health and the economy, numerous studies have been conducted to explore more efficient and environmentally friendly methods for detoxifying aflatoxins (Peng *et al.* 2018). Physical, chemical, and biological methods are the primary detoxification methods for degrading aflatoxins in humans, animals, food, and feed. Although each approach has limitations, they have effectively protected us from aflatoxins and mitigated substantial economic losses worldwide (Karlovsky *et al.* 2016).

Chemical methods used for aflatoxin detoxification include chlorine, hydrogen peroxide, ozone, bisulfite, ammonia, alkali, and various chemical applications. However,

these chemical methods have limitations when applied to food due to potential issues related to chemical residues. Despite these concerns, chemical degradation remains a practical method for aflatoxin-decontaminating foods (Stoloff and Trager 1965). Sodium bisulfite, a food additive widely used in the industry, is particularly effective at inactivating $AFLB_1$ in maize, even more so than ammonia and sodium hydroxide at low concentrations (Moerck *et al.* 1980). Ammonia has also been extensively studied for detoxifying aflatoxins in animal feeds, achieving over 95% degradation of aflatoxins in both gaseous and aqueous phases (Brekke *et al.* 1978).

In recent years, natural phytochemicals have emerged as promising solutions for aflatoxin inactivation, offering safety advantages over traditional techniques. For instance, the aqueous extract of ajowan (*Trachyspermum ammi*) seeds has demonstrated an 80% reduction in aflatoxin content (Hajare *et al.* 2005). Dialysed extracts of *Trachyspermum ammi* seeds effectively degraded aflatoxin G_1 ($AFLG_1$) by 90% (Velazhahan *et al.* 2010). Vasaka (*Adhatoda vasica* Nees) leaf extract has shown potential in reducing aflatoxins in both liquid media and animals, with cafestol and coffeol as the main components. Vasaka leaf extract was reported to detoxify 98% of $AFLB_1$ (Vijayanandraj *et al.* 2014). *Barleria lupulina* leaf extract has also demonstrated the ability to degrade aflatoxins, with detoxification rates of 61.1% for $AFLB_1$, 71.4% for aflatoxin B_2 ($AFLB_2$), 94.4% for $AFLG_1$, and 58.8% for aflatoxin G_2 ($AFLG_2$) (Kannan and Velazhahan 2014). *Ocimum basilicum* leaf extract achieved a 90.4% and 88.6% reduction in $AFLB_1$ and $AFLB_2$, respectively (Iram *et al.* 2016). Additionally, various flavonoids found in plants, such as tea leaves, have been shown to inhibit aflatoxin production (Tian *et al.* 2023).

Mo *et al.* (2013) investigated the inhibitory effects of tea extracts, including pu'er tea, black tea, and jasmine tea, on aflatoxin production by *Aspergillus flavus*. Most tea extracts were found to inhibit $AFLB_1$ production. Choudhary and Verma (2004) studied the protective effects of black tea against aflatoxin-induced lipid peroxidation in mouse liver. They observed that the decrease in antioxidant enzyme activities may contribute to increased lipid peroxidation during aflatoxicosis. Furthermore, coumarin in green tea extracts inhibited P450 enzyme activity in the liver and decreased $AFLB_1$-DNA insertion *in vitro* (Peng *et al.* 2018).

Moreover, developing metal NPs, through green synthesis, has gained attention as a potential solution to aflatoxin contamination. NPs, due to their unique size distribution and morphology, offer various possibilities for combating aflatoxins (Vaseeharan *et al.* 2010; Chinen *et al.* 2015). Green synthesis methods utilizing plant extracts as reducing agents have been proposed to produce metal NPs (Senthilkumar and Sivakumar 2014; Nakhjavani *et al.* 2017). Plants contain a wide range of secondary metabolites with redox capacity, making them ideal for nanoparticle biosynthesis (Sun *et al.* 2014; Abdelghany et al. 2023). Tea leaves, rich in polyphenols, alkaloids, essential oils, polysaccharides, inorganic elements, and vitamins, are well-suited for the green synthesis of NPs. Green tea contains various catechins and flavonoids, while black tea has oxidized compounds with antioxidant properties (Sharangi 2009). Green synthesis using plants offer advantages such as safety, affordability, simplicity, rapid synthesis, environmental friendliness, control over nanoparticle size and shape, suitability for large-scale production, and avoidance of cell culture maintenance (Yeltekin 2020).

NPs composed of platinum, palladium, gold, and silver exhibit remarkable catalytic activity. However, these metals are expensive. As an alternative, metal oxide NPs can be synthesized, including zinc oxide, copper oxides, nickel oxides, manganese oxides, titanium oxides, cobalt oxides, and iron oxides (Kamran *et al.* 2019). Iron, in particular,

exists in nature mainly in the form of oxides such as magnetite (Fe_3O_4), hematite (α-Fe_2O_3), maghemite (γ-Fe_2O_3), and hydroxides (Goethite and lepidocrocite, α- and γ-FeOOH), silicates, and carbonates. Iron oxide NPs are easy to synthesize, can be surface-modified, and some of them are magnetically recyclable. Thus, some can be manipulated magnetically and have specific magnetic, optical, and chemical features. As a result, these NPs have been widely explored for a variety of applications, including drug delivery, catalysis, photonics, dye removal, and environmental purposes (Laurent *et al.* 2008; Gawande *et al.* 2013; Datta *et al.* 2016). Elemental iron in the zerovalent state exists mainly in a bcc crystal structure as α-Fe and is highly prized for environmental applications (Nemecek *et al.* 2016), particularly in its nanoscale form. Magnetite is a popular choice due to its biocompatibility (Schwertmann and Cornell 2008). Maghemite (γ- Fe_2O_3), which has a spinel structure with a face-centered cubic (FCC) close-packed organization is another important class of magnetic transition metal oxide materials (O'Handley 2000). Magnetite (Fe_3O_4) is a common magnetic iron oxide with a cubic inverse spinel structure. Oxygen in this compound forms a FCC close packing, while Fe cations occupy interstitial tetrahedral and octahedral sites (Klein and Dutrow 2007). The hopping of electrons between Fe^{2+} and Fe^{3+} ions in magnetite's octahedral sites at room temperature makes it a major material type that is classified as half-metallic.

There is an increasing amount of research on using green-synthesized NPs and effective phytochemicals against aflatoxins. Asghar *et al.* (2018) examined the impact of NPs produced from green tea and black tea on aflatoxin adsorption and found that the adsorption decreased as the initial concentration of $AFLB_1$ increased. They also successfully made iron, copper, and silver NPs using the leaf extract of *Syzygium cumini* (Asghar *et al.* 2020). Silver NPs exhibited considerable antimicrobial activity, and iron NPs had a higher adsorption capacity for $AFLB_1$ than copper and silver NPs. Raesi *et al.* (2022) studied the effect of NPs, including FeONPs, and reached the result of complete removal of $AFLB_1$ under UV irradiation. Zahoor and Khan (2018) showed that nearly 90% removal of $AFLB_1$ was accomplished for magnetic carbon composites which have the presence of FeONPs on adsorbent surfaces.

Mycotoxins are likely to be present in various plant and animal-based food products consumed by humans, including rice, corn, soybeans, wheat, barley, walnuts, nuts, peanuts, spices, dried fruits, and processed items susceptible to mycotoxin-producing organisms. Aflatoxins are particularly problematic in the chocolate, pastry, and dessert industries, as well as in peanut and hazelnut puree used as raw materials in many food products.

Comparing the published reports on the properties of NPs produced by the green synthesis method and NPs synthesized by physical and chemical methods, there needs to be more information on the antibacterial and antifungal properties of metal NPs, including using aflatoxin adsorbents. In this study, the authors synthesized FeONPs using extracts from locally produced Turkish teas from the Black Sea Region through a green synthesis method. The antioxidant and antibacterial activities of the tea extracts, as well as the synthesized NPs and their ability to adsorb aflatoxins in hazelnut and pistachio puree, were investigated.

EXPERIMENTAL

The devices used are given in the analysis methods. All chemicals were purchased from Merck and Sigma Aldrich companies, Darmstadt, Germany.

Preparation of BTE and GTE and Soluble Powders of Extracts

For tea extracts, 1.5 kg black tea was mixed with 10 L water. The extracts obtained by boiling the mixtures for 2.5 hours were filtered through sieves of different sizes. The dry matter content of the filtrate was determined by a digital refractometer (Hanna HI96801, Hanna Instruments Inc., Woonsocket, RI, USA). The dry matter content was increased to 10% using a rotary evaporator at 60 °C under 150 mbar pressure. The concentrated tea extracts were subjected to drying with a spray dryer (SD-06 spray, Labplant UK (Ltd), Filey, England) at 200 °C for 4 h with a liquid flow rate of 5 mL/min. Water-soluble tea powder was obtained at the end of the spray drying process.

Preparation of BTFeONP and GTFeONP

A total of 250 mL of 0.2 M Fe^{+3} solution was prepared from $FeCl_3.6H_2O$, and 0.1 M Fe^{+2} 250 mL solution was prepared from $FeSO_4$. Equal proportions of both solutions were taken, and 250 mL solution was prepared. The pH value of 250 mL tea extracts with 10% dry matter content was adjusted to 11 using 1.0 M NaOH. The extract solutions were continuously stirred, and Fe^{+2}/Fe^{+3} solution was added 1 drop/s. The solution was stirred at 500 rpm for another 1 h. The solution turned black as Fe_3O_4 was formed. The resulting FeONP solution was centrifuged (Nuve NF 800R, Ankara, Turkey) at 7000 rpm. The precipitates were dried at 60 °C using a vacuum glass desiccator and an oven (Yusefi *et al.* 2020).

Characterization of FeONPs

Fourier-transform infrared (FTIR) spectrophotometer analysis

The presence of functional groups or the identification of chemical bonds in NPs was evaluated using FTIR analysis (Perkin-Elmer Inc., Waltham, MA, USA). The spectral study was performed in the range 400 to 4300 cm^{-1}.

Scanning electron microscopy (SEM) analysis

The morphology and size of the synthesized metal NPs were studied using SEM (SEM-Quanta FEG 250 ThermoFisher Scientific, Waltham, MA, USA).

Energy dispersive X-ray analysis (EDAX)

The elemental composition of the NPs was characterized by EDAX.

Treatment of Hazelnut and Peanut Puree with Tea Extracts and FeONPs

A total of 2.5 kg roasted hazelnuts were mixed with 1 L hazelnut oil and treated with a robot coupe R30 brand vertical type shredder and grinder at 3600 rpm for 10 min to obtain hazelnut puree. Homogenization control and aflatoxin analysis were performed to determine the amount of toxin in the obtained purees (n = 3).

Approximately 1000 g of hazelnut and pistachio puree was mixed in an Arcelik brand blender for 25 min. A total of 200 g of puree sample, 2 g BTE or GTE, and 2 g BTFeONP or GTFeONP were mixed and homogenized with a Waring brand blender for 2 min. Then, 50 mL of each sample was placed in non-sterile flat-bottomed falcon/centrifuge tubes (n = 3). The same procedure was repeated for 1.0% and 2.0% additions of BTE, GTE, BTFeONPs and GTFeONPs. According to the variable parameters specified in Table 1, the control sample was detoxified at 25, 45, and 75 °C for 2 and 4 h. At all temperature and time parameters, the samples were stirred for 2 min at every 30 min interval.

Table 1. Numbers and Abbreviations of Aflatoxin Analyses

Sample Abbreviations								Sample	Unit
BTE Peanut	BTE Haze nut	GTE Pea-nut	GTE Hazel nut	BBT-FeONP Peanut	BT-FeONP Hazel nut	GT-FeONP Pea-nut	GT-FeONP Hazel nut		
SFS1	FN1	FS1	YFN1	PSFS1	NPSFN1	NPYFS1	NPYFN1	1.0% Tea Ex. 25 °C 2 h	µg/kg
SFS2	FN2	FS2	YFN2	PSFS2	NPSFN2	NPYFS2	NPYFN2	1.0% Tea Ex. 45 °C 2 h	µg/kg
SFS3	FN3	FS3	YFN3	PSFS3	NPSFN3	NPYFS3	NPYFN3	1.0% Tea Ex. 45 °C 4 h	µg/kg
SFS4	FN4	FS4	YFN4	PSFS4	NPSFN4	NPYFS4	NPYFN4	1.0% Tea Ex. 75 °C 2 h	µg/kg
SFS5	FN5	FS5	YFN5	PSFS5	NPSFN5	NPYFS5	NPYFN5	1.0% Tea Ex. 75 °C 4 h	µg/kg
SFS6	FN6	FS6	YFN6	PSFS6	NPSFN6	NPYFS6	NPYFN6	2.0% Tea Ex. 25 °C 2 h	µg/kg
SFS7	FN7	FS7	YFN7	PSFS7	NPSFN7	NPYFS7	NPYFN7	2.0% Tea Ex. 45 °C 2 h	µg/kg
SFS8	FN8	FS8	YFN8	PSFS8	NPSFN8	NPYFS8	NPYFN8	2.0% Tea Ex. 45 °C 4 h	µg/kg
SFS9	FN9	FS9	YFN9	PSFS9	NPSFN9	NPYFS 9	NPYFN9	2.0% Tea Ex. 75 °C 2 h	µg/kg
SFS10	FN10	FS10	YFN10	PSFS10	NPSFN10	NPYFS10	NPYFN10	2.0% Tea Ex. 75 °C 4 h	µg/kg

Treatment of Aflatoxin Standards with Tea Extracts and FeONPs

The solution containing $AFLB_1$, $AFLB_2$, $AFLG_1$, and $AFLG_2$ was treated with 0.25% and 0.50% soluble black and green tea extracts and FeONPs obtained at room temperature. The solutions obtained were treated for 2 h at room temperature with occasional stirring. Afterward, aflatoxin-containing solution samples were analyzed by immunoaffinity chromatography (IAC, VICAM Aflatest, Vicam Company, Watertown, MA, USA) and high-performance liquid chromatography with fluorescence detector (HPLC-FLD; Agilent 1200, Agilent Technology, Santa Clara, CA, USA) techniques.

Analysis of Iron Content of FeONPs

A total of 0.2500 g of FeONP samples were placed in a microwave incinerator. Next, 7 mL of 65% HNO_3 and 1 mL of 37% H_2O_2 were added, and the containers were sealed and placed in the CEM Mars 5 microwave (CEM Mars 5 Mars, CEM Corp., Matthews, NC, USA) combustion unit. After the digestion process was completed, the volumes of the solutions were made up to 50 mL with distilled pure water. For the calibration curve, a 50.0 mg/L intermediate stock solution was prepared from Fe 1000 mg/L master stock solution, and standard Fe solutions at concentrations of 1.0, 2.0, 4.0, 7.5, and 10.0 mg/L were designed from this solution to prepare the calibration curve. The microwave plasma atomic emission spectrometry (MP-AES, 4200 MP-AES System, Agilent Technology, Santa Clara, CA, USA) readings were taken for calibration, and the samples were filtered through a 0.45-micron filter before reading (NMKL 170 and NMKL 161).

Aflatoxin Analysis

The method of aflatoxin analysis in nutshells includes the steps of extraction with methanol/water, clean-up with immuno-affinity column (IAC) containing monoclonal antibodies specific for $AFLB_1$, $AFLB_2$, $AFLG_1$, $AFLG_2$, elution from the column with methanol, post-column photochemical derivatization, and HPLC-FLD analysis. Working solutions of aflatoxins ($AFLB_1$, $AFLB_2$, $AFLG_1$, and $AFLG_2$) for HPLC-FLD were prepared using the master stock aflatoxin standard solution (Merck, Aflatoxin Mix (0.20 µg/mL for $AFLB_1$/ $AFLG_1$ to 0.05 µg/mL for $AFLB_2$/ $AFLG_2$) 34036-1ML-R, catalog no. 34036, Merck KGaA, Darmstadt, Germany).

Sample preparation and HPLC-FLD analysis

The 25.0 g of sample, 5 g of NaCl, and 125 mL of 70% methanol were blended at high speed for 2 min, and the extracts were filtered through the Whatman filter paper. 5 mL of the filtrate was placed in a 20-mL syringe containing 10 mL of purified water and fitted with an immunoaffinity column (IAC) (VICAM brand, Vicam Science Technology, Milford, MA, USA). The solution was passed through the IAC at a 3 mL/min flow rate. The immunoaffinity column was washed with 20 mL of deionized water at a flow rate of 5 mL/min, followed by three air passes through the immunoaffinity column. Aflatoxins bound to the column were eluted with 1.0 mL pure methanol at a 0.5 mL/min flow rate. Then, 1.0 mL of distilled water was passed through; the aflatoxin solution was collected and transferred to an amber glass vial with a volume of 1.8 mL.

The HPLC conditions comprised the isocratic mobile phase, water, methanol, and acetonitrile (6:3:2). A total of 350 µL of 4 M HNO_3 and 0.120 g KBr were added to 1.0 L of the mobile phase. The flow rate was 1.0 mL/min, and the column temperature was set to 22 °C. A 100 µL sample volume was injected. The fluorescence detector (FLD) was set to an excitation wavelength of 360 nm and an emission wavelength of 430 nm. Post-column derivatization with electrochemically generated bromine was used to increase the fluorescence intensity of aflatoxin derivatives. A 100 µA electrochemical cell was placed between the column and the fluorescence detector using a PTFE tube (30 cm) (Baltaci *et al.* 2013).

Antioxidant Activity Analyses

The total flavonoid (TFC) and phenolic contents (TPC) were determined using established methods, as described in the literature (Yuksel *et al.* 2022). The ferric-reducing antioxidant power assay (FRAP) analysis was conducted following the study by Benzie and Strain (1996). The antioxidant capacities were determined using the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay, as per the method implemented by Baltaci *et al.* (2022). The total antioxidant capacity (TAC) was measured using the phosphomolybdate assay technique described by Umamaheswary *et al.* (2007).

To evaluate the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity, 0.1 mL of the filtered sample was mixed with 3 mL of DPPH solution (10 mM) in a test tube. The mixture was vigorously vortexed and then incubated in the dark at room temperature for 30 min. The absorbance of the solution was measured at 517 nm, and the DPPH scavenging capacity was expressed as a percentage of inhibition. Trolox and ascorbic acid were used as standards (Baltaci *et al.* 2022).

Antimicrobial Activity

Microorganisms used in this study were obtained from the Gumushane University Food Engineering Department laboratories. The antimicrobial activities of tea extracts and FeONPs were determined using the disc diffusion method against ten microorganisms, including *Aeromonas hydrophila* ATCC 35654, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Shigella flexneri* ATCC 12022, *Listeria monocytogenes* ATCC 7644, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 9634, *Salmonella typhimurium* ATCC 23566, *Aspergillus flavus* ATCC 46283, *Staphylococcus aureus* ATCC 25923, *Saccharomyces cerevisiae* S288C, and *Candida albicans* ATCC 10231 (Matuschek *et al.* 2014).

The antimicrobial activity assessment was conducted in two stages: activation and cultivation of microorganisms. Bacteria were activated for 24 h at 36 °C, while yeasts were activated for 48 h at 25 °C and adjusted to 0.5 McFarlan. Sterile solid media containing 1% of the microorganisms were prepared and poured into Petri dishes, where wells of 5 mm were opened. Different concentrations of prepared samples were transferred into the wells, with dimethyl sulfoxide as a control. Petri dishes containing bacteria were then incubated for 24 h at 36 ºC, and those containing yeast and mold were incubated for 48 h at 27 ºC. The results were determined by measuring the zones around the discs after incubation (Matuschek *et al.* 2014).

Statistical Analysis

Statistical analyses were performed using Microsoft Excel software with XLSTAT (Addinsoft, Version 2020 New York, NY, USA).

RESULTS AND DISCUSSION

Aflatoxin Quantities of Natural Samples

Hazelnut and pistachio samples were homogenously pureed, and at this stage, the amount of aflatoxin in the samples (n = 3) was analyzed (Table 2). When the analysis results of the samples obtained naturally from hazelnut and pistachio purees for the study are evaluated according to the Turkish Food Codex (TGK) contaminants regulation, it was observed that the results were not appropriate.

Table 2. Analysis of Naturally Contaminated Hazelnut and Pistachio Purees

	Hazelnut Puree (µg/kg)	Peanut Puree (µg/kg)
$AFLG_2$	0.58 ± 0.01	0.58 ± 0.06
$AFLG_1$	5.21 ± 0.10	6.37 ± 0.06
$AFLB_2$	0.66 ± 0.01	0.46 ± 0.02
$AFLB_1$	6.57 ± 0.06	7.79 ± 0.15
Total Aflatoxin	13.03 ± 0.16	15.21 ± 0.12

FeONPs and Their Characterization

The most common biocompatible magnetic nanomaterials are pure iron oxides such as maghemite (γ-Fe_2O_3) and magnetite (Fe_3O_4). In this study, Fe_3O_4 NPs were obtained from Turkish black and green teas.

The color change in the reaction mixtures revealed the formation of FeONPs from both black tea and green tea. The color in the solutions of $FeCl_3$ or $FeSO_4$ with tea leaf extract changed rapidly from yellow to black. This color change indicates that FeONPs were formed, as has been shown by surface plasma resonance, indicating the reduction of metal ions by tea leaf extract (Asghar *et al.* 2018; Gebremedhn *et al.* 2019).

The formation of FeONP was confirmed by the change in the pH of the solution. The pH of the FeONP solution before reduction was set to 11.00, and the pH value after the reaction was measured as 6.30. The pH decreased during reduction in all samples and shifted to the acidic range. The studies conducted in the literature stated that the pH of the plant extract decreased with the formation of NPs after reduction (Madhavi *et al.* 2013; Asghar *et al.* 2020). Bioactive molecules, including catechins, alkaloids (such as caffeine), gallic acid, and flavonoids, in the biological matrix of black and green tea extracts were combined with Fe^{2+} and Fe^{3+} to form ferric and ferrous compounds. The addition of NaOH allows OH^- to interfere with the reaction mechanisms. Competition between ferric and ferrous compounds and OH^- bond formation with iron ions results in ferrous hydroxide and ferric hydroxide.

A shell-core structure forms where the core consists of ferrous and ferric hydroxides. Dehydration of ferric and ferrous hydroxides allows the formation of FeONPs in the form of a Fe_3O_4-magnetite structure. Fe_3O_4 nanoparticles were capped and stabilized by green and black tea leaf extracts originating organic active compounds (Awwad and Salem 2012; Ganapathe *et al.* 2020). After drying under vacuum, 29.87 g BTFeONP and 22.46 g GTFeONP were obtained. The yields were 2.99% and 2.25% for GTEs and BTEs with 10% dry matter content, respectively. The higher yield of GTFeONPs is attributed to the fact that they are richer in polyphenolic compounds than black tea.

FTIR analysis

The FTIR was used to identify possible phenolic compounds responsible for the conjugation and reduction of BTFeONPs and GTFeONPs.

The FTIR spectrum of the BTE solution is given in Fig. 1. Here, at 3242 cm^{-1}, are the O-H and N-H stretching vibrations of polyphenols. This broad absorption band is due to the hydroxyl (OH) functional groups in alcohols and phenolic compounds. C=O bond stretching in polyphenols and C=C bond stretching in aromatic ring is seen in a strong band at 1628 cm^{-1}. C-H stretching and O-H stretching in alkane and carboxylic acid are peaks at 2923 cm^{-1}, respectively. C-O stretching in an amino acid is observed in the 1031 cm^{-1} band.

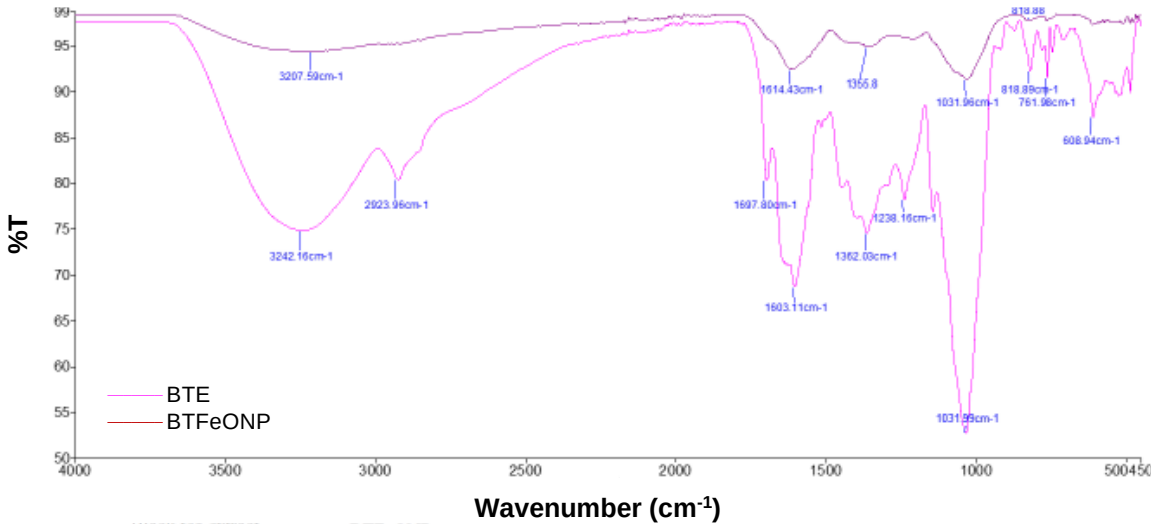

Fig. 1. FTIR spectrum of BTE and BTFeONP

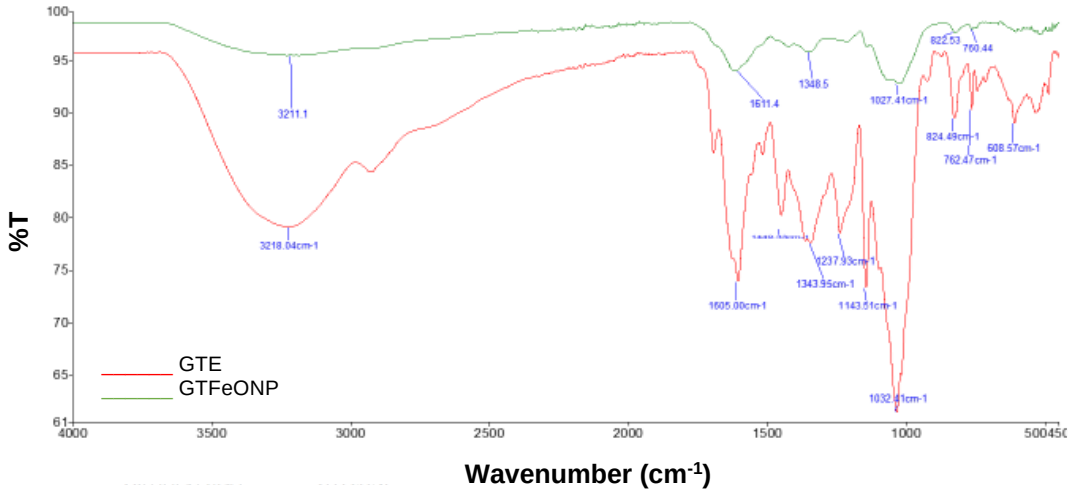

Fig. 2. FTIR spectrum of GTE and GTFeONP

In a previous study of identical FTIR bands of various tea types, such as black, oolong, and green tea, it was concluded that the FTIR bands of the tea extract solution consisted of polyphenols appearing at 3388, 1636, and 1039 cm^{-1} and were related to O-H/N-H, C=C, and C-O-C stretching, respectively (Chai *et al.* 2015). Therefore, the FTIR spectrum shows that the main functional groups in tea are polyphenols, carboxylic acid, and amino acid compounds (Senthilkumar and Sivakumar 2014).

In the IR spectrum of green tea, the band at 3218 cm^{-1} is due to stretching vibrations of O-H groups in water, alcohols, and phenols and N-H stretching in amines. The peaks at 2926 and 2864 cm^{-1} are C-H stretching in alkanes and O-H stretching in carboxylic acid; the strong band at 1605 cm^{-1} is due to C=C stretching in aromatic rings and C=O stretching in polyphenols. The band at 1343 cm^{-1} shows C-N stretching of the amide in the protein, a band at 1741 cm^{-1} shows C-O-C stretching in polysaccharides, the band at 1032 cm^{-1} shows C-O stretching in the amino acid, and finally, the weak band at 824 cm^{-1} shows out-of-plane bending of C-H.

The FTIR measurements were performed to characterize BTFEONPs and GTFeONPs and to observe the presence of polyphenols and caffeine (Fig. 2). The bands at

3600 and 2900 cm^{-1} are characterized by the O-H stretching vibration assigned to the -OH polyol group, such as catechins, C-H, and -CH_2- vibration of aliphatic hydrocarbons. The band at 1582 cm^{-1} is characterized by the presence of conjugated ketones, quinones. The bands at 1449 cm^{-1} and 1027 cm^{-1} are associated with the C-N stretching vibration and C=O stretching vibration of aromatic amines in the structure of caffeine, respectively. These results are in accordance with previous studies and indicate the presence of tea polyphenols as capping agents on the FeONP surface (Asghar *et al.* 2018).

SEM, EDAX, and MP-AES analyses of FEONPs

The elemental composition of the NPs and the relative abundance of the synthesized FeONPs were determined by EDAX, the results of which are shown in Figs. 3 and 4. The percentage of Fe metal was 7.56% in green tea NPs and 8.62% in black tea NPs. In Fe analysis with MP-AES device, it was 10.73 ± 1.51% in GTFeONP and 9.91 ± 1.67% in BTFeONPs. The results obtained from EDAX and MP-AES analyses confirm each other. Following the incineration of the sample in the acid solution by means of a microwave, the solid residue was subsequently dissolved in a nitric acid solution for MP-AES analysis. During this process, a small amount of precipitation was observed. Despite the minimal amount of precipitation, the quantity of possible missing iron content of NPs was negligible, as evidenced by the closer EDAX and MP-AES results.

Due to surface plasmon resonance, reduced FeONPs were revealed in EDAX analysis with a characteristic optical absorbance peak at 3 keV. An intense signal was observed in EDAX, indicating the reduction of ferrous ions to elemental iron. GTFeONP and BTFeONP had a composition of 49.31 and 46.85% oxygen (O), 41.97 and 40.12% carbon (C), respectively. The EDAX shows the presence of Fe along with species, such as carbon, oxygen, potassium, and small amounts of phosphorus, sulfur, and chlorine, from the tea extract, indicating that tea extracts are rich in minerals. Both BTFeONPs and GTFeONPs exhibited a negative zeta potential of -15.3 mV, suggesting that FeONPs have a sufficient surface charge for electrostatic stability to prevent excessive aggregation. The spectrum results showed that FeONPs were successfully formed.

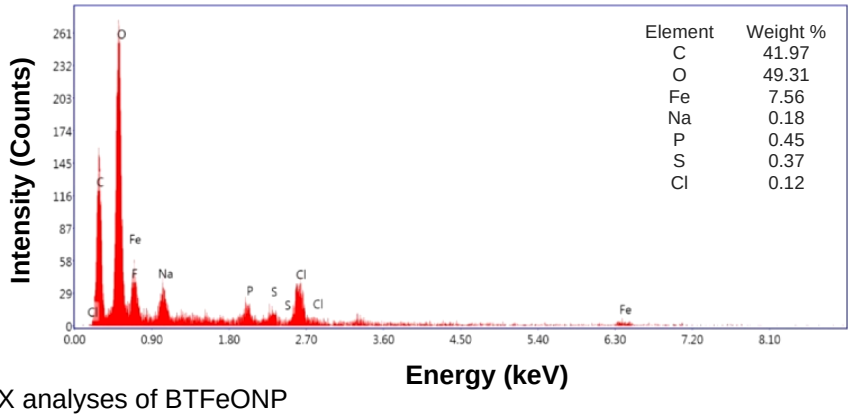

Fig. 3. EDAX analyses of BTFeONP

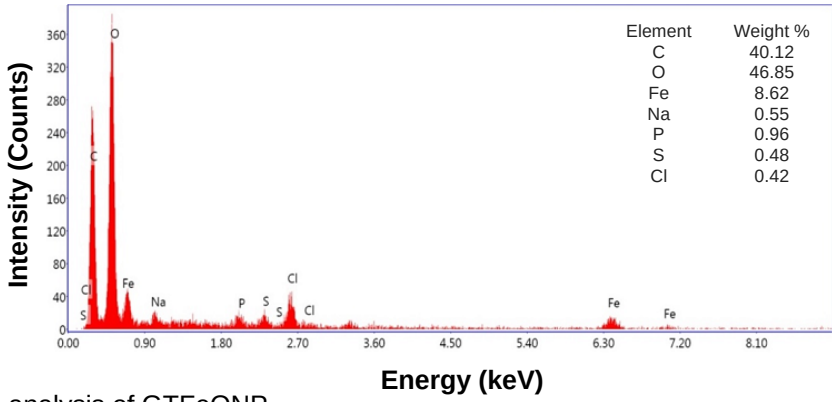

Fig. 4. EDAX analysis of GTFeONP

The SEM technique was used to visualize the size and shape of the synthesized FeONPs (Figs. 5 and 6). The SEM images confirmed that GTFeONP had a spherical shape with particle size ranging from 18 to 97 nm. BTFeONP also had a spherical shape with particle size ranging from 40 to 97 nm. Images presented the agglomeration presence among NPs, which were similar to those of previous studies in the literature as well (Asghar *et al*. 2018). The variation in FeONP size distribution is due to the various reducing properties of different naturally occurring compounds present and prevalent in GTE and BTE.

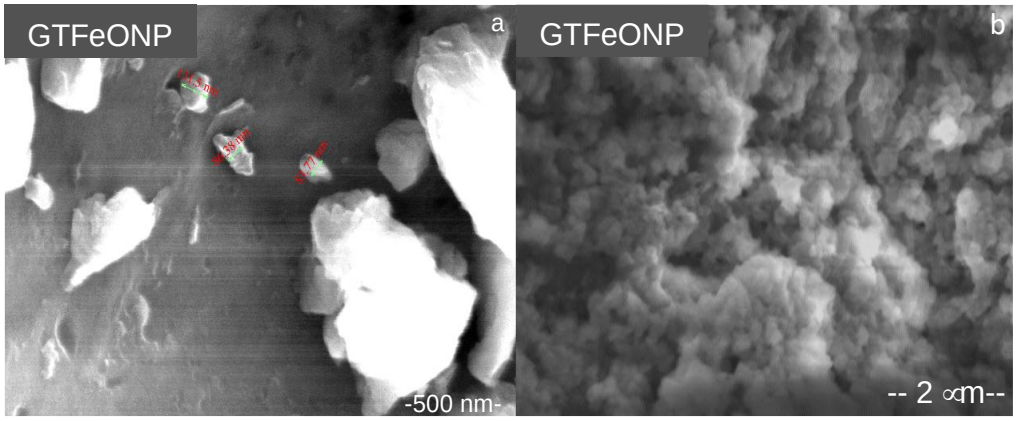

Fig. 5. SEM images of GTFeONPs at different scales a. 500 nm b. 2 μm

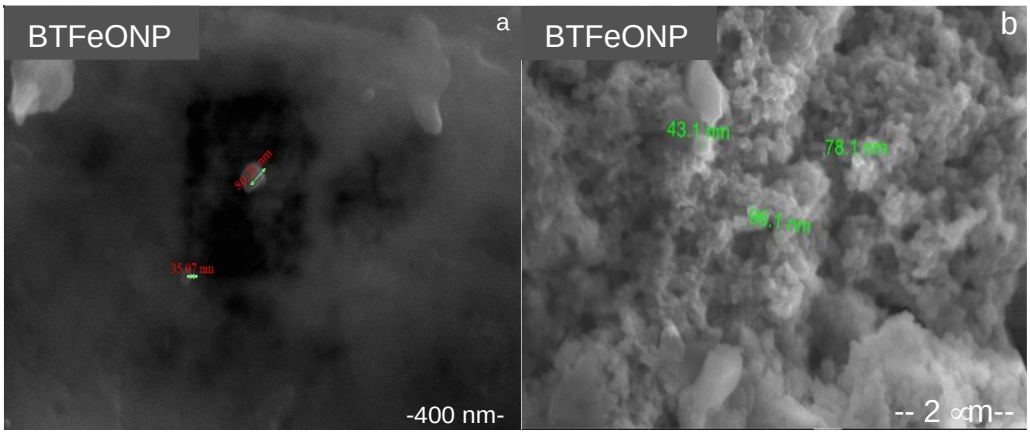

Fig. 6. SEM images of BTFeONP at different scales a. 400 nm b. 2 μm

Aflatoxin Analyses

For aflatoxin analyses, two different concentrations, three different temperatures, and two different time variables were used for each product (Table 1). Purees and solutions containing naturally occurring aflatoxins were treated with soluble tea extracts and FeONPs. Detoxification was observed to appear in all aflatoxin species. It was observed that the concentrations of tea extracts and FeONPs, temperature, and time were effective parameters in the detoxification of all aflatoxin species.

The peanut and hazelnut purees treated with powdered tea extracts

In the study of aflatoxin analysis in peanut purees treated with BTE, $AFLG_2$, $AFLG_1$, $AFLB_2$, and $AFLB_1$, the total aflatoxin detoxification percentages were 41.9, 48.6, 50.0, 41.2, and 43.7%, respectively, in the sample that had the highest temperature, time, and concentration (SFS10) (Fig. 7). The increase in percent adsorption of aflatoxins with increasing temperature increases the sorptive effect between active sites on soluble black and green tea extracts, aflatoxin species, and adjacent aflatoxin molecules in the adsorbed phase. The sorptive forces are caused by stretching with temperature (Figs. 7, 8, 9, and 10). A study found that $AFLB_1$ absorption increases with increasing temperature (Thieu and Pettersson 2008). The results also showed that the adsorption process is endothermic in nature.

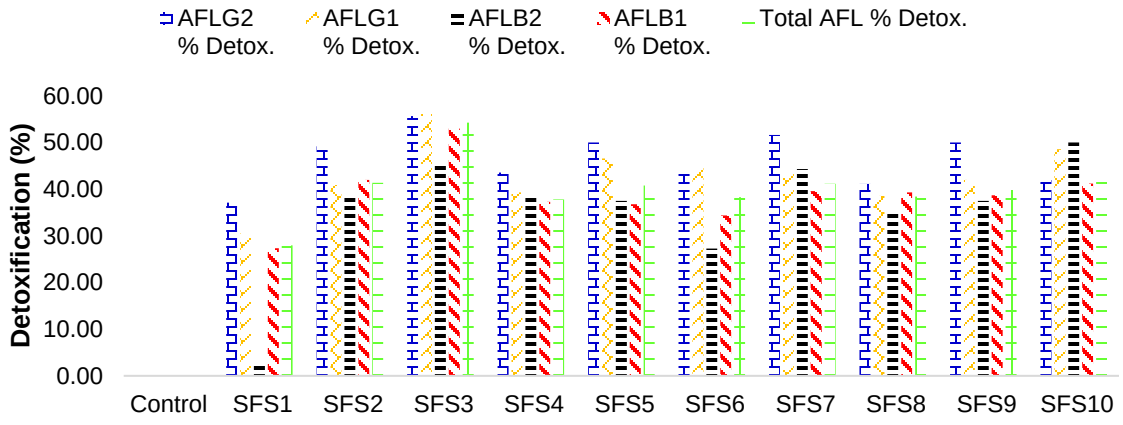

Fig. 7. Graph of % detoxification of aflatoxins in peanut puree by soluble BTE

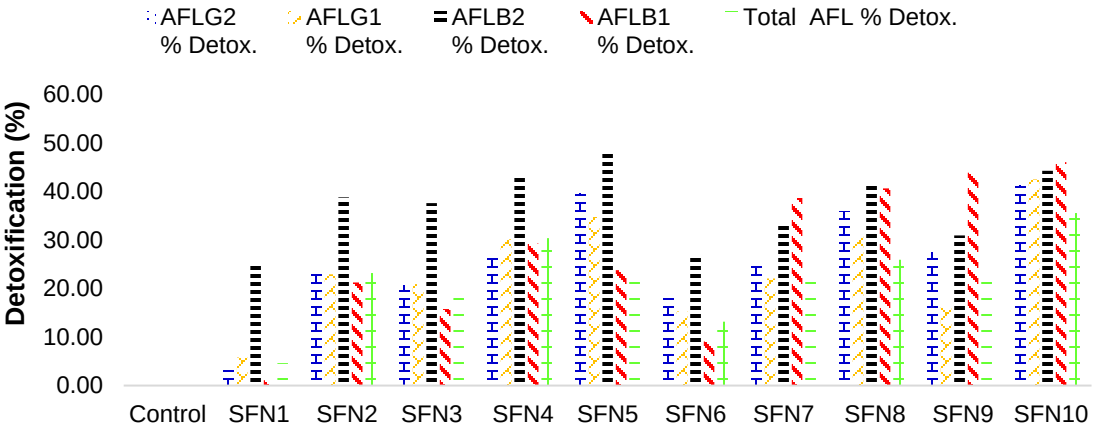

Fig. 8. Graph of % detoxification of aflatoxins in natural hazelnut puree with soluble BTE

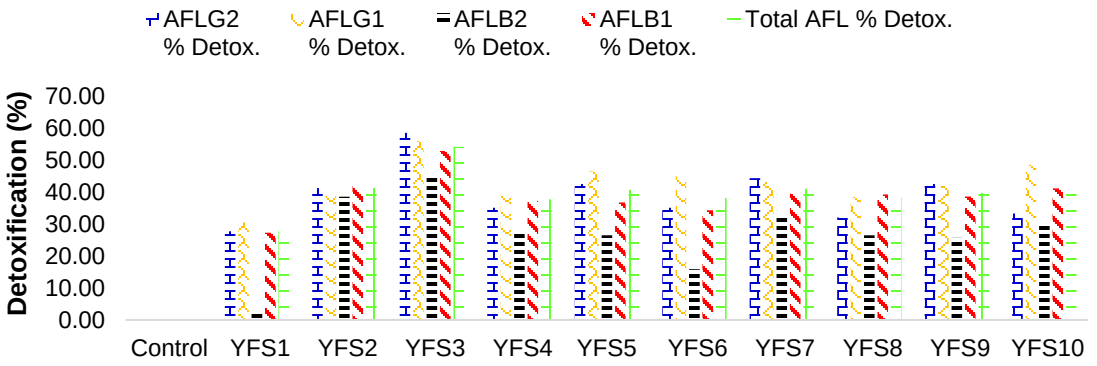

Fig. 9. Graph of % detoxification of aflatoxins in peanut puree with soluble GTE

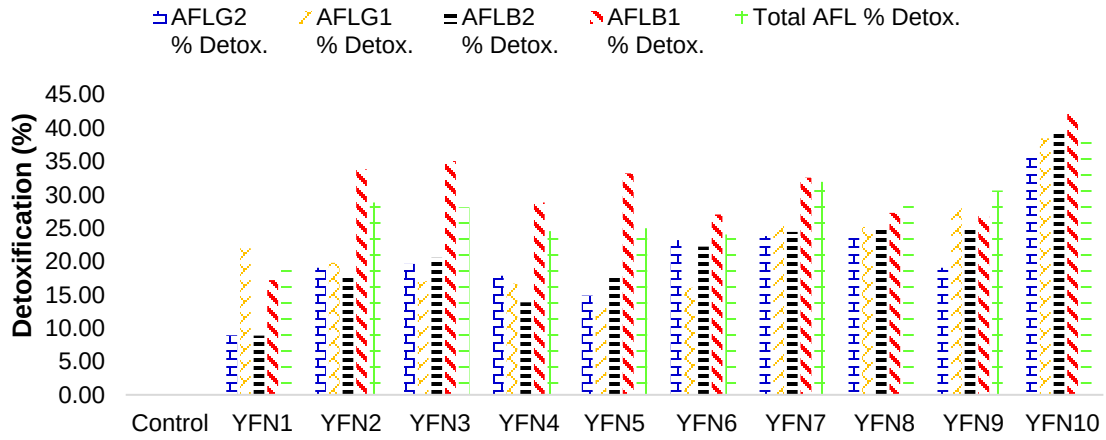

Fig. 10. Graph of % detoxification of aflatoxins in hazelnut puree with soluble GTE

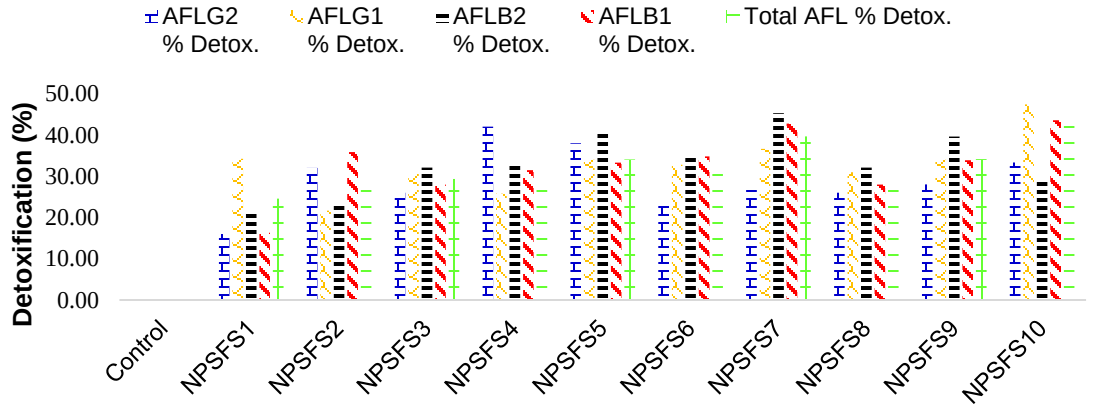

Fig. 11. Graph of % detoxification of aflatoxins in peanut puree by BTFeONP

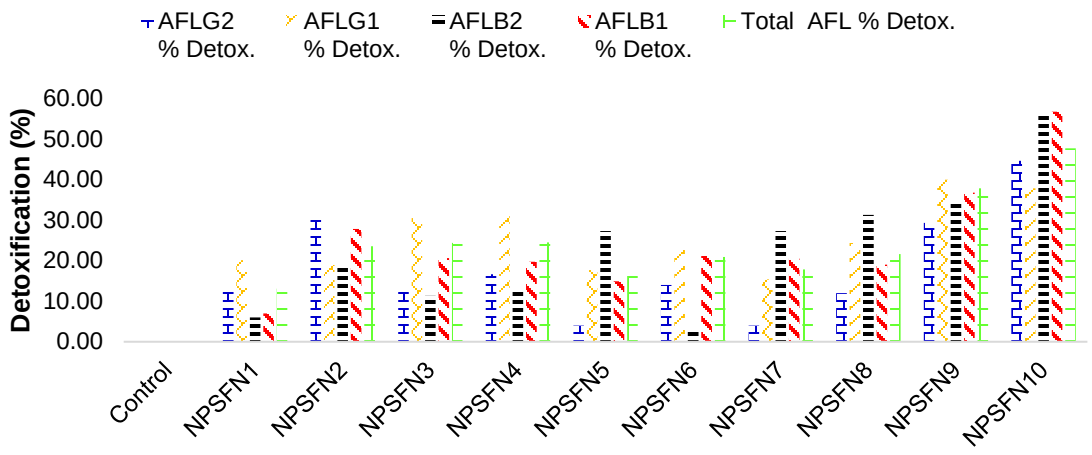

Fig. 12. Graph of % detoxification of aflatoxins in hazelnut puree by BTFeONP

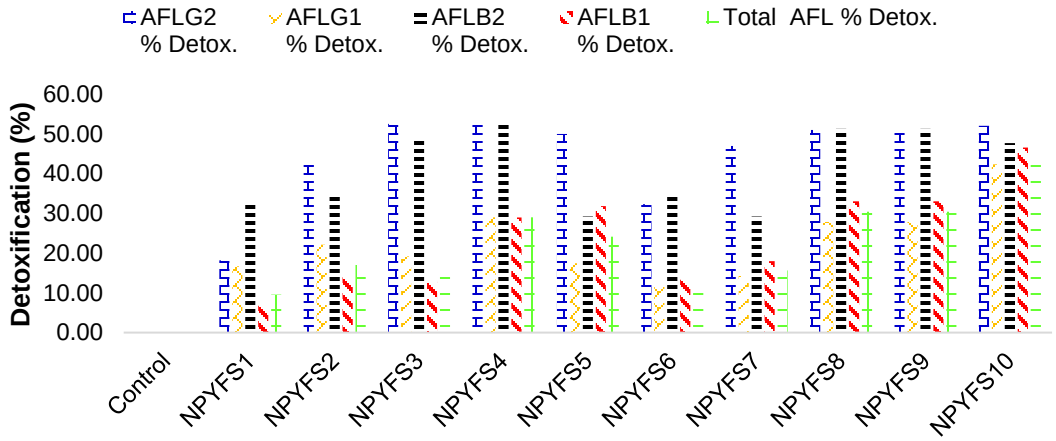

Fig. 13. Graph of % detoxification of aflatoxins in peanut puree by GTFeONP

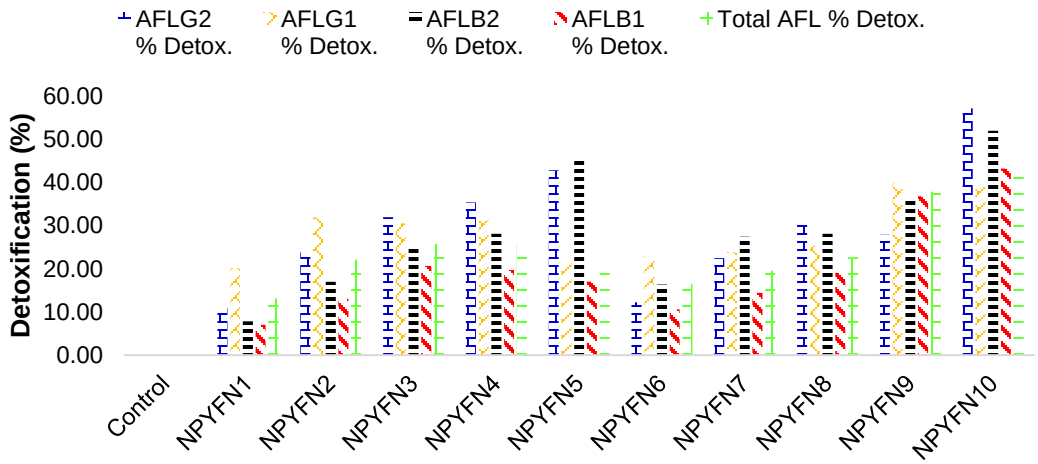

Fig. 14. Graph of % detoxification of aflatoxins in hazelnut puree with GTFeONP

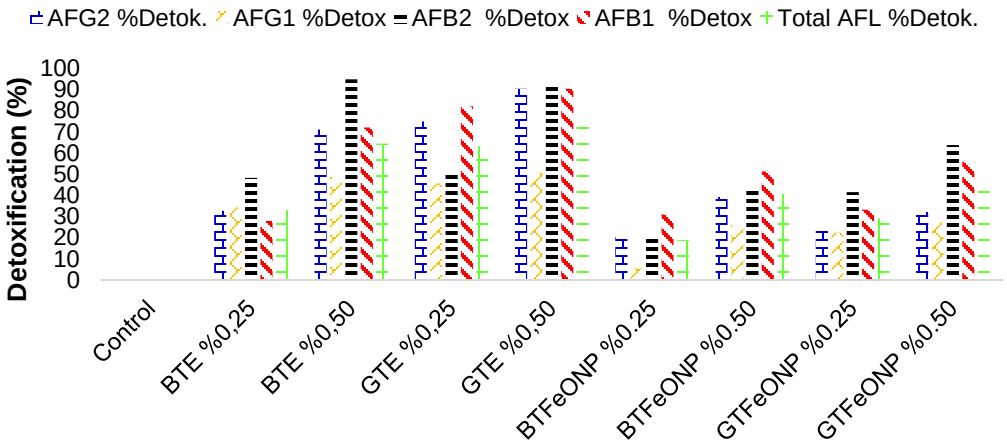

Fig. 15. Graph of % detoxification of aflatoxins in solution with BTE, GTE, BTFeONP, and GTFeONP

In the aflatoxin analysis of BTE powder-treated hazelnut purees, $AFLG_2$, $AFLG_1$, $AFLB_2$, $AFLB_1$, and total aflatoxin detoxification percentages were 41.27%, 42.52%, 44.31%, 46.09%, and 35.58%, respectively. Detoxification levels in peanut and hazelnut puree were close (Fig. 8). Although aflatoxins were in an oily environment with soluble tea extracts, they were detoxified by almost 50%.

In GTE powder-treated peanut purees, $AFLG_2$, $AFLG_1$, $AFLB_2$, $AFLB_1$, and total aflatoxin detoxification percentages were 33.33, 48.62, 29.55, 41.22, and 43.71%, respectively, in the YFS10 sample, where the temperature, time and concentration values were the highest (Fig. 9). In GTE powder-treated hazelnut purees, $AFLG_2$, $AFLG_1$, $AFLB_2$, $AFLB_1$, and total aflatoxin detoxification values were 36.31, 38.48, 39.44, 42.11, and 38.44%, respectively, in YFN10 sample, which had the highest temperature, time, and concentration values.

In recent studies with plant extracts for detoxification of aflatoxins, it has been shown that vasaka, cafestol, and kahweol leaf extracts have the potential to degrade aflatoxins in liquid media and in animals (Cavin *et al.* 2022). Vijayanandraj *et al.* (2014) measured the $AFLB_1$ adsorption capacity of aqueous extracts of various medicinal plants. In this study, *A. moschatus* Medik, *A. precatorius* (L.), *Cassia fistula* (L.), *Rhinacanthus nasutus* (L.), and *Withania somnifera* (L.) plants showed adsorption capacities of 2.5%, 41.6%, 36.4%, 40.6%, and 12.4%, respectively. Isoimperatorin (4-(3-methylbut-2-enoxy)furo[3,2-g]chromen-7-one) isolated from *Poncirus trifoliata* L. Raf. was found capable of protecting against $AFLB_1$-induced hepatotoxicity by inducing Glutathione S-transferase (GSTα) and suppressing Cytochrome P450 enzymes (CYPs) (Pokharel *et al.* 2006).

The peanut and hazelnut purees treated with BTFeONPs and GTFeONPs

It is clear from Figs. 13, 14, 15, and 16 that the detoxification increased when the temperature, time, BTFeONP, and GTFeONP concentrations increased. The detoxification percentages of $AFLG_2$, $AFLG_1$, $AFLB_2$, $AFLB_1$, and total aflatoxin detoxification values were 33.3, 47.4, 47.8, 43.6, and 44.0%, respectively, in the NPSFS10 sample, where the temperature, time, and concentration were the highest in the peanut purees treated with BTFeONP. The $AFLG_2$, $AFLG_1$, $AFLB_2$, $AFLB_1$, and total aflatoxin detoxification percentages were 44.7, 37.8, 56.7, 56.7, 56.8, and 47.8%, respectively, in the NPSFN10

sample, where temperature, time, and concentration were the highest among BTFeONP-treated hazelnut purees. The $AFLG_2$, $AFLG_1$, $AFLB_2$, $AFLB_1$, and total aflatoxin detoxification rates were 52.02, 42.34, 47.69, 46.56, and 43.2%, respectively, in the NPYFS10 sample, where temperature, time, and concentration were the highest among the peanut purees treated with GTFeONP. The detoxification percentages of $AFLG_2$, $AFLG_1$, $AFLB_2$, $AFLB_1$, and total aflatoxin were 57.1%, 39.0, 52.0, 43.2, and 42.2%, respectively, in the NPYFN10 sample, where temperature, time, and concentration were the highest among GTFeONP-treated hazelnut purees.

Asgar *et al.* (2018 and 2020) investigated parameters of time, pH, nanoparticle amount, and the temperature impact on aflatoxin removal for their iron, copper, and silver NPs synthesized from the extract of black tea, green tea, and Syzygium *cumini* leaves. Increases in temperature increased the adsorption of aflatoxin by NPs in their studies, which also showed the similarly endothermic character of FeONPs in this study. The endothermic phenomenon of NPs may be due to increased molecular movements that cause active site abundance for the adsorption procedure at greater temperatures. As discussed in the section on tea extracts and their impact on aflatoxins, it was found that the active sites on the adsorbent became stronger and were better able to bind with the aflatoxins, as well as between adjacent aflatoxin molecules in the adsorbed phase (Thieu and Pettersson 2008). In line with the results of the previous studies, $AFLG_2$, $AFLG_1$, $AFLB_2$, $AFLB_1$, and total aflatoxin detoxification increase with increasing incubation time, temperature, and concentration in the current study.

The synthesis and characterization of green NPs using extracts from mint, thyme, rosemary, and eucalyptus plants and their detoxification efficiency against $AFLB_1$ were studied. It was stated that the high removal efficiency of plant extracts should encourage the production of nanomaterials as an alternative to chemical, physical, and biological methods used to eliminate or reduce the toxicity of $AFLB_1$ (Jawad *et al.* 2022a).

The aflatoxin-added solutions treated with BTFeONP, GTFeONP, GTE, and BTE

Tea extracts and FeONPs were added to the toxin solution at 0.25% and 0.50% concentrations. After 15 min of stirring, filtration was performed. The obtained solutions were analyzed for aflatoxin. Figure 15, which shows the effect of tea extracts, shows that the detoxification percentage rate increased when the values increased, depending on the concentration. A prominent result in Fig. 15 is the detoxification of around 50% for $AFLG_1$, where detoxification increased to about 90% when green tea soluble extract was added and about 0.5% for other aflatoxins. At the same concentration, a detoxification rate of around 70% was observed in BTE except for $AFLB_2$. The GTE, which had a higher detoxification rate, can be interpreted as follows. The amount of compounds in green tea is more elevated than in black tea. In addition, depending on the concentration, detoxification rates increased as the concentration increased.

During the examination of the NP concentration effect on aflatoxin solutions, increases in the detoxification percentages were observed when there was an increase in the concentration of FeONPs. Another interesting result was that when BTFeONP was used, there was a detoxification of around 14% for $AFLG_1$, while detoxification increased to about 22% when GTFeONP was added and about 0.25% for other aflatoxins. With the exception of BTFeONP $AFLB_2$ at the same concentration, a detoxification of around 21% was observed. The higher detoxification of green tea extract and GTFeONPs is attributed to the higher compound diversity and the concentration increases.

The detoxification percentage with the addition of BTFeONP 0.25% and 0.50% was lower than with the addition of GTFeONP 0.25% and 0.50%. The detoxification percentages of BTFeONP in solution on $AFLG_2$, $AFLG_1$, $AFLB_2$, $AFLB_1$, and total aflatoxin were 39.3, 26.2, 44.0, 51.3, and 40.7%, respectively. The detoxification rates of GTFeONP in solution on $AFLG_2$, $AFLG_1$, $AFLB_2$, $AFLB_1$, and total aflatoxin were 32.1, 27.2, 63.8, 55.9, and 44.3%, respectively.

In a study where green silver NPs were synthesized from alcoholic extracts of plants, it was stated that these NPs were effective in removing or preventing the production of mycotoxins, especially $AFLB_1$ (Jawad *et al.* 2022b). Al-Rajhi *et al.* (2022) observed that the production of *F. incarnatum* mycotoxins, such as beauvericins, fusarins, moniliformin, and enniatins, was reduced by as much as 62.8%, 45.4%, 58.1%, and 55.0%, respectively, at a concentration of 400 ppm of copper oxide NPs. Research on milk magnifies the role of aflatoxin M_1 in threatening consumers' health and increases interest in the hygienic quality of milk produced. Therefore, FeONPs were used in a new method with high specificity and sensitivity for aflatoxin M_1 detoxification of milk in pasteurized milk-producing factories. The researchers claimed that their method was more feasible, faster, and cheaper than the current applications in dairy factories (Jouni *et al.* 2018). Abdelghany *et al.* (2020) investigated the synergistic effect of 50 ppm AgNPs and *Juniperus procera* stem extracts. They observed decreased $AFLB_2$ and $AFLG_2$, synthesis using various concentrations of methanolic plant extracts with AgNPs.

Antioxidant Activity

The antioxidant activity results of BTFeONP, GTFeONP, and soluble black and green tea extracts are given in Table 3. The antioxidant activities of black and green tea extracts and tea extract-based NPs were evaluated through six approaches, including FRAP, DPPH, ABTS, TFC, TPC, and TAC. The antioxidant analysis results of FeONPs produced from GTEs and BTEs consistently demonstrate a high level of antioxidant activity.

Table 3. Antioxidant Activities of BTFeONP, GTFeONP, BTE, and GTE

	TPC (mg GAE/g)	TAC (mg AAE/g)	TFC (mg QE/g)	DPPH (mg AAE/g)	DPPH (% Inhibition)	ABTS (mg AAE/g)	FRAP (mg $FeSO_4$/g)
BT FeO-NP	14523.89 ± 467.36	48213.13 ± 567.08	98948.57 ± 951.43	23214.41 ± 522.91	91.31 ± 2.03	8037.28 ± 7.60	1148.16 ± 18.56
GT FeO-NP	15262.78 ± 352.11	74845.70 ± 308.03	381205.71 ± 854.72	23466.67 ± 324.70	92.29 ± 1.26	8708.33 ± 53.18	1198.16 ± 27.06
BTE	40.86 ± 0.04	111.23 ± 0.96	65.52 ± 0.24	294.75 ± 1.37	10.23 ± 1.11	274.42 ± 0.40	92.70 ± 0.37
GTE	44.82 ± 0.08	275.13 ± 1.88	335.98 ± 1.04	511.66 ± 2.16	15.67 ± 1.32	511.79 ± 0.65	111.47 ± 0.31

The FRAP assay, including FeONPs, is commonly used to evaluate compounds' reducing capacity and antioxidant potential. In the current study, FRAP results of the BTFeONP, BTE, GTFeONP, and GTE were 1150, 92.7, 1200, and 11.5 mg $FeSO_4$/g, respectively. In a study by Machado *et al.* (2013), FeONPs synthesized from green tea and

black tea extracts exhibited FRAP values of 32 ± 2.4 μmol Fe(II)/g and 14 ± 1.6 μmol Fe(II)/g, respectively.

The DPPH assay measures the free radical scavenging ability of antioxidants, providing valuable insights into the potential of FeONPs to neutralize free radicals. IC_{50} values of ascorbic acid as a standard control was 0.094 mg/mL. DPPH analyses showed that BTFeONPs, GTFeONPs, BTE, and GTE had IC_{50} values of 0.43, 0.38, 3.83, and 3.32 mg/mL, respectively. The DPPH outcomes for BTFeONP, BTE, GTFeONP, and GTE were 91.3, 10.2, 92.3, and 15.7% in the current research, respectively. A separate investigation by Mohamed *et al.* (2021) found the highest DDPH value for green tea-based iron oxide NPs at 33% in their research. Haydar *et al.* (2022) found that DPPH scavenging activity increases with increasing nanoparticle concentration based on fresh tea leaves, where the DPPH activity was 82.5% for green tea leaves-based NPs compared to 74.5% and 94.3% in ascorbic acid standard and plant extract, respectively. Martínez-Canabas *et al.* (2021) showed the result of DDPH of GTE as a normalized value of 0.87 compared to the maximum DDPH value of 9.59 Trolox equivalent of their study.

The ABTS assay is another common method to evaluate the antioxidant capacity of substances, including FeONPs. In the present study, the ABTS results indicated that BTFeONP, BTE, GTFeONP, and GTE exhibited antioxidant activities of 8040, 274, 871, and 512 mg ascorbic acid equivalent (AAE)/g, respectively. In a study by Haydar *et al.* (2023), the ABTS assay was used to assess the antioxidant activity of FeONPs produced from green tea extracts. The study reported an ABTS value of 88.8% at the highest dose of 100 microg/mL compared to the butylated hydroxy-toluene standard (95.51%) and GTE (73.25%), indicating their noteworthy antioxidant potential.

The TPC is another critical parameter in assessing the antioxidant potential of compounds. Phenolic compounds, including catechins in green tea, contribute substantially to the antioxidant activity of FeONPs. In the current work, BTFeONP, BTE, GTFeONP, and GTE showed TPC results of 14500, 40.9, 74800, and 44.8 mg gallic acid equivalents (GAE)/g, respectively, confirming the presence of substantial phenolic compounds in the NPs. Machado *et al.* (2013) reported results for GTFeONP and BTFeONP as 1.45 ± 0.04 and 1.56 ± 0.05 (mmol GAE)/L. Another study compared the TPC result of a normalized value of 0.74, calculated according to the maximum TPC result of 8.13 mmol GAE/L (Martínez-Cabanas *et al.* 2021).

The determination of TFC provides insights into the presence of these beneficial compounds in the FeONPs. The results of the TFC were 98900, 65.5, 381000, 336 mg QE (Quercetin equivalent)/g for BTFeONP, BTE, GTFeONP, and GTE, respectively. The TAC assay comprehensively evaluates the overall antioxidant activity, considering the contributions of various antioxidant compounds present in FeONPs. The TAC outcomes were 48200, 111, 15300, and 275 mg AAE/g for BTFeONP, BTE, GTFeONP, and GTE, respectively.

NPs had quite higher antioxidant activity than extracts ($p < 0.05$). At the same time, GTE and GTFeONPs showed higher antioxidant capacity than BTE and BTFeONPs.

Antimicrobial Activity

The increased effectiveness of the NPs can be credited to the existence of unique functional groups from the plants present on their surface. These specific groups notably enhance their antimicrobial capabilities. Furthermore, the smaller size of the NPs enables them to penetrate the bacterial cell wall more easily, resulting in the eventual death of the cells. As highlighted by Kanagasubbulakshmi and Kadirvelu (2017), both the size of the

NPs and the structure of the bacterial cell wall are critical factors that determine the antimicrobial activity of the NPs.

The antimicrobial properties of BTFeONPs and GTFeONPs were investigated against ten bacterial strains, two fungal strains, and one yeast strain. The study found that increasing the amount of NPs enhanced the antimicrobial activity, as indicated in Table 4. The most effective results of the agar diffusion method values of GTFeONPs were 8.98 mm against *Bacillus cereus* (ATC 9634), 13.2 mm against *E. coli* (ATCC 25922), 10.3 mm against *E. coli* (ATCC 35150), and 15.7 mm against *Salmonella typhimurium* (ATCC 23566).

In contrast, the high inhibition zones values of BTFeONPs were 12.0 mm against *E. coli* (ATCC 25922), 18.0 mm against *E. coli* (ATCC 35150), 15.7 mm against *Salmonella typhimurium* (ATCC 23566), and 14.0 mm against *Staphylococcus aureus* (ATCC 25923). However, no antimicrobial activity was observed for five bacterial strains (*Aeromonas hydrophila* ATCC 35654, *Bacillus subtilis*, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, and *Shigella flexneri* ATCC 12022) and all tested fungal and yeast strains (*Aspergillus flavus* ATCC 46283, *Candida albicans* ATCC 10231, and *Sac. cerevisiae* S288C).

Table 4. Antimicrobial Activity Results

	GT FeO NP 400 ppm	BT FeO NP 400 ppm	GT FeO NP 200 ppm	BT FeO NP 200 ppm	GT FeO NP 100 ppm	BT FeO NP 100 ppm	GT FeO NP 50 ppm	BT FeO NP 50 ppm	
Bacteria sp.	**Diameter of Inhibition Zones (mm)**								**Penicillin G (10mg)**
Aeromonas hydrophila ATCC 35654	-		-		-		-		34.00 ±0.01
Bacillus cereus ATCC 9634	8.98 ±0.01		5.76 ±0.01		-		-		30.00 ± 0.01
Bacillus subtilis	-		-		-		-		34.00 ±0.01
Enterococcus faecalis ATCC 29212	-		-		-		-		32.00 ±0.01
Escherichia coli ATCC 25922	13.15 ±0.01	12.00 ±0.01	8.18 ±0.01	10.80 ±0.01	4.56 ±0.01	6.56 ±0.01	-	5.19 ±0.01	34.00 ±0.01
Escherichia coli O157:H7 35150	10.29 ±0.05	18.00 ±0.05	5.87 ±0.01	10.00 ±0.01	-		-		34.00 ±0.01
Listeria monocytogenes ATCC 7644	-		-		-		-		30.00 ±0.01
Salmonella typhimurium ATCC 23566	15.67 ±0.01	10.67 ±0.01	9.45 ±0.01	5.67 ±0.01	5.98 ±0.01		4.21 ±0.01		34.00 ±0.01
Shigella flexneri ATCC 12022	-		-		-		-		30.00 ±0.01
Staphylococcus aureus ATCC 25923	-	14.00 ±0.01	-	8.00 ±0.01	-	5.65 ±0.01	-		38.00 ±0.01

Fungus - Yeast	Diameter of Inhibition Zones (mm)								Nystatin 30 µg/mL
Aspergillus flavus ATCC 46283	-		-		-		-		11.00 ±0.1
Candida albicans ATCC 10231	-		-		-		-		12.00 ±0.1
Sac. cerevisiae S288C	-		-		-		-		18.00 ±0.1

Asghar *et al.* (2018) conducted a study to evaluate NPs' antibacterial properties derived from green and black tea leaf extracts. Their results indicated that the NPs' effectiveness followed Ag-NPs > Cu-NPs > Fe-NPs, where the inhibition zones exhibited by Fe-NPs, Cu-NPs, and Ag-NPs ranged from 11 to 13 mm, 14 to 16 mm, and 19 to 21 mm, respectively. These findings suggest that the synthesized Ag-NPs possessed the most potent antibacterial activity due to their smaller size than Cu-NPs and Fe-NPs.

CONCLUSIONS

1. This study introduced a straightforward, affordable, and environmentally friendly method to produce iron oxide nanoparticles (FeONPs) for industrial use. The process avoids the use of harmful reducing, capping, and dispersing agents.
2. The FeONPs produced were analyzed using Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and energy-dispersive X-ray analysis (EDAX) techniques. The study suggests that FeONPs produced by this green synthesis method can be utilized in various food safety applications.
3. The research demonstrates that FeONPs synthesized through green methods effectively detoxify aflatoxins. According to the study, hazelnut and peanut purees treated with soluble Turkish black tea extracts (BTE) and green tea extracts (GTE) showed a reduction in aflatoxin levels of 40 to 50% and 30 to 45%, respectively. Moreover, BTFeONPs and GTFeONPs also decreased the levels of aflatoxins in hazelnut and peanut purees at 33 to 48% and 40 to 50%, respectively.
4. The nanoparticles (NPs) produced in this work exhibited a greater level of antioxidant and antibacterial activities than the tea extracts. The antioxidant capacity of GTE and GTFeONPs was greater than that of BTE and BTFeONPs. The study found that increasing the amount of NPs enhanced the antimicrobial activity of several bacteria.
5. These results showed that FeONPs obtained by green methods from biological raw materials, such as tea, can be an environmentally friendly and health-friendly option for food safety applications such as aflatoxin removal.

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