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Evaluation of the antioxidant and inhibitory activity of fermented *Aloe vera* extract against the pathogenic bacteria isolated from diarrhoea infections

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ABSTRACT

This study aimed to isolate bacterial species from people suffering from diarrhoea and diagnose them. In this study, 43.3% of the aqueous extract of the *Aloe vera* plant was fermented by the locally isolated *Lactobacillus plantarum* bacteria, and the presence of phenolic compounds before and after fermentation was determined. The antioxidant activity was estimated using the ferric reducing antioxidant power (FRAP) method, and the inhibitory activity against bacterial isolates causing diarrhoea was also determined. The results showed that *Salmonella* bacteria are the most frequently isolated species, and the pathogenic bacterial species causing diarrhoea were *Salmonella*, *E. coli*, *shigella*, and *Proteus*. The lactobacillus bacteria isolated and diagnosed from local dairy samples were *Lactobacillus plantarum*, used to ferment the *Aloe vera* plant extract. The phenolic compounds identified and estimated in *Aloe Vera* extract were Chlorogenic acid, Caffiec acid, Cinnamic acid, Gallic acid, Coumaric acid, Vitexin and Syringic acid. The highest antioxidant activity of the *Aloe Vera* extract after fermentation was at 44.11%, compared to the non-fermented quote, which appeared at 22.17%**.** It was also shown that the inhibitory effect of fermented *Aloe vera* extract was higher against pathogenic isolates than the non-fermented extract.

تقييم الفعاليّة المضادّة للأكسدة والتُثبيّطية لمستخلص نبات صبّار الأوليفيرا (*Aloe vera*) المتخمر باستعمال بكتريا *Lactobacillus plantarum* ضد البكتريا الممرضة المعزولة من اخماج الاسهال

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الخلاصة

هدفت الدراسة الحالية الى عزل أنواع البكتريا من الاشخاص المصابين بالاسهال وتشخيصها, اذا تم عزل 43.3% من المستخلص المائي لنبات الصبار وتخميره بواسطة بكتريا *Lactobacillus plantarum* المعزولة محلياً وتحديد وجود المركبات الفينولية قبل وبعد التخمير، كما قدرت الفعالية المضادة للأكسدة بطريقة ferric reducing/antioxidant power method والفعالية التثبيطية ضد العزلات البكتيرية المسببة للاسهال. تبين من النتائج ان الأنواع البكتيرية المسببة للاسهال كانت من الأنواع الممرضة Salmonella ، E.coli، shigella ، Proteus كما تم عزل وتشخيص نوع بكتريا حامض اللاكتيك من عينات الالبان المحلية وكانت من النوع *Lactobacillus. plantarum* التي استعملت في تخمير مستخلص الالوفيرا . كانت أنواع مركبات الفينولات المشخصة والمقدرة في مستخلص الالوفيرا من الأنواع Chloro genic acid و Caffiec acid وCinnamic acid و Gallic acid و Coumaric acid و Vitexin و Syringic acid . كما ان الفعالية المضادة للأكسدة تبين بانها الأعلى فاعلية بعد التخمير التي كانت عند قيمة 44.11%. مقارنة مع قيمتها قبل التخمير التي كانت عند 22.17%. كما تبين ان الفعالية التثبيطية لمستخلص الالوفيرا المتخمر كان ذا كفاءة تثبيطية اعلى ضد العزلات المرضية من المستخلص غير المتخمر.

الكلمات الافتتاحية : اخماج الاسهال, الصبار، الفينولات, بكتريا حامض اللاكتيك، البكتريا الممرضة.

INTRODUCTION

Prebiotics include plant extracts that have a beneficial effect on human health and their role in inhibiting species of pathogenic microbes, and the use of the *Aloe vera* plant in this direction is considered the preferred option. Its extracts have active substances to treat cases of diarrhoea due to their effectiveness in inhibiting most types of pathogenic microbes and their efficacy in increasing water absorption in the intestines, which helps improve diarrhoea and reduce liquid secretions. It also includes the effectiveness of *Aloe vera* as an antioxidant since it contains many compounds such as Vitamin C, Vitamin E, Anthraquinones, Flavonoids, Tocopherols, and Polyphenols (Rawat and Saxena, 2023). diarrhoea can be defined as a condition characterised by increased bowel movement frequencies, liquid faecal secretions, and loss of large amounts of water and mineral salts. It is a prevalent disease worldwide, especially in developing countries, where children and

immunocompromised people have a high risk of infection (Dhimal *et al*., 2022; Moshiree, Heidelbaugh and Sayuk, 2022). As a result of the development of resistance of microorganisms to antibiotics, it has become necessary to find alternative materials that have antibacterial inhibitory effects. Probiotics, especially bacterial species from Lactobacillus or Bifid bacterium, are considered the most important and preferred options for treating diarrhoea due to their ability to improve intestinal function (Jassim, 2021), strengthen the immune system to fight infections, and improve digestion and nutrient absorption. It may also reduce intestinal irritation and inflammation, 2022; Johnson *et al*., 2023; Mohammed, 2022).

Therefore, this study aimed to isolate and identify the bacterial species that cause diarrhoea, as well as to isolate the Lactobacillus plantarum species to be used in fermenting Aloe vera plant extract, identifying its activated components and testing its ability to inhibit microbial infections against bacteria isolated from diarrhoea cases.

MATERIALS AND METHODS

Materials

Purified water extract from galls of *Aloe vera* was used as a starting material in this experiment. Ferric chloride ($FeCl_3$) was purchased from BDH Sigma Aldrich**.** Deionised distilled water was used throughout the experiment for all steps. Media: MacConkey agar, Blood agar, EMB agar and S.S. agar were obtained from BD Diagnostic, Le Pont de Claix, France. Instrument: Isolates incubated anaerobically using an anaerobic jar, Rodwell, England), Incubator from (Jepetech, Korea), Centrifuge from (Kokusan, Japan), and rotary from (Shimadzu, Japan).

Sample collection

One hundred twenty stool samples between 2–3 g weight were collected in clean, sterile tubes from multiple laboratories from people suffering from diarrhoea who did not take antibiotics. The stool samples were transferred to the laboratory.

Sample Culturing

Samples were cultured and incubated aerobically on suitable media, including MacConkey agar, Blood agar, EMB agar and S.S. agar by streaking method, and incubated aerobically at 37°C for 24 hours.

Identification of Bacterial Isolates

Diagnosis of Gram-negative bacteria was based on morphologic, microscopic and biochemical characteristics (Prescott *et al*. 2008, Alfred *et al*. 2005). The bacteria were

diagnosed based on their phenotypic and biochemical characteristics, and the Vitek-2 compact system was used to confirm the diagnosis.

Isolation and Diagnosis of Lactobacilli Isolates

The lactobacilli species (*Lactobacillus plantarum)* were isolated and diagnosed from local dairy samples, then cultured and incubated anaerobically using (Anaerobic jar, Rodwell, England) on MRS agar (Oxoid) at 37°C for 48 hours in the incubator from (Jepetech, Korea). The diagnosis was based on morphologic, microscopic, and biochemical characteristics (Prescott *et al*. 2008, Alfred *et al*. 2005), and the Vitek-2 compact system was used to confirm the diagnosis.

Preparation of *Aloe vera* Gel Aqueous Extract

The aqueous extract was prepared according to the method described by (Hassan *et al*. 2023), which included washing the plant leaves with distilled water; then, the leaves were cut into pieces and dried. After that, 20 g of the leaves were weighed and transferred to a one-litre glass beaker, then 100 ml of distilled water was added, and the mixture was left for 24 hours in a shaking incubator at 35°C. The solution was then filtered using a gauze and centrifuged at 3000 rpm for 10 minutes. The filtrate was concentrated using a rotary evaporator at 45°C to obtain the dry powder, which was stored in dark bottles in the refrigerator until use.

Preparing *Lactobacillus plantarum* bacteria for fermentation of *Aloe vera* extract

The bacterial suspension was prepared from *Lactobacillus plantarum* bacteria after culturing in liquid MRS medium to obtain a cell count of 1.5×10^{8} cells/ml compared to a McFarland tube at 0.5 concentration. *Lactobacillus plantarum* bacterial cells were added to the prepared *Aloe vera* juice according to the method described by (Burrowes and Hekmat 2023).

Estimation of phenols in *Aloe vera* powder

The concentration of phenolic compounds in *Aloe vera* powder was estimated by weighing 2 g of dry plant powder. After grinding the plant, 1 g was weighed and added to 5 ml of a 0.5 M NaOH solution, then incubated at 65°C in the ultrasonic water bath for 12 hours. After centrifugation at 8000 rpm for 10 minutes, the filtrate was mixed with an equal amount of ethyl acetate solution. Then, the solution was mixed using a Vortex shaker and left to be separated into two layers. A layer of the ethyl acetate was dried and suspended with 2 ml of methanol solvent to be ready for analysis using an HPLC device (Comas-Serra *et al.,* 2023).

The amount of each phenolic compound including Coumaric acid, Syringic acid, Vitexin, Chlorogenic acid, Fierce acid and Gallic acid was estimated in fermented and non-fermented

Aloe vera samples using the separation column C18 and the mobile phase of Acetic acid 0.5%: Acetonitrile at (30:70) volume ratio and a flow rate of 1ml/minute at three wavelengths of 272, 320 and 280 nm. The test was done by taking 1 ml of the extract after dissolving 1g of the extract in 10 ml of ethyl alcohol, adding 1 ml of the mobile phase and mixing thoroughly by the vortex. Then, five microliters of this mixture were injected into the system, and the retention time was compared with the appearance of the standard solution, as shown by (Gonçalves *et al*. 2023).

The following equation was used to calculate the compounds:

Compound concentration (ppm)=Area of sample band/Area of standard band X concentration of standard X dilution times.

Antioxidant assay using the FRAP method (ferric reducing/antioxidant activity):

A reaction mixture containing 1 ml of 0.05% O-phenanthroline in methanol, 2 ml of 200 M ferric chloride ($FeCl_3$) and 2 ml of different concentrations from 10 to 1000 g was incubated at room temperature for 10 minutes, and measurement was made at a wavelength of 510 nm. EDTA was used as an iron chelator. The experiment was performed in three replicates. The iron oxidative reducing activity in *Aloe vera* extract was estimated based on the method (Aboelsoued *et al*. 2019).

The FRAP reagent was prepared by mixing 50 ml of 0.3 M acetate solution at pH 3.6 with 5 ml of (PTZ T) TRI-Rydil Triazine solution. Then, ten mM was dissolved in 40 mM HCl and 5 mL of ferric chloride solution ($FeCl_3$). After that, 2 ml of fresh FRAP reagent was added to 10 L of the extract. Then, the absorbance was measured at 593 nm. A standard curve was prepared using serial concentrations of Trolox. The result was determined as Trolox equivalent in 100 g/mg of Aloe vera units.

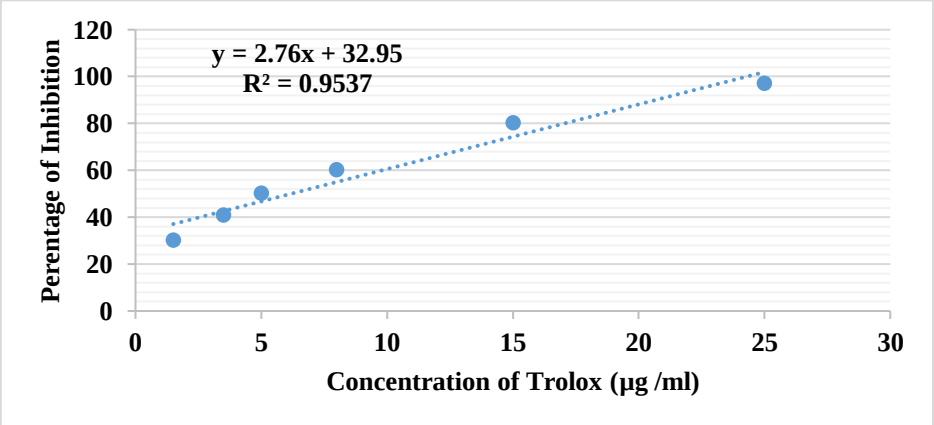

Figure 1. *Aloe vera* extract's antioxidant curve as determined by the FRAP technique

Estimation of the minimum inhibitory concentration of *Aloe vera* extract

Different concentrations were prepared for each aqueous extract to be tested for their effect on in vitro isolated bacteria. 10, 20, and 30 mg/ml concentrations were adopted. The different extract concentrations were filtered using bacterial filters at 0.22 microns for sterilisation. The lowest concentration in which the inhibitory ability of the extract was shown against bacterial isolates used for testing was adopted.

Testing the inhibitory activity of plant extract fermented with lactobacilli bacteria against pathogenic bacteria.

The agar well diffusion method was used to test the sensitivity of bacteria isolated at different concentrations from *Aloe vera* aqueous extract and the extract fermented by *Lactobacillus plantarum* bacteria**.** The technique included making four holes of equal dimensions in the solid Mueller-Hinton medium with a diameter of 6 mm using a cork borer to contain plant solutions of 0.05 ml per hole after diffusing (0.1) ml of bacterial suspension containing 1.5×10^{8} cells/ml on the medium. Then, the plates were left in the refrigerator for 1 hour to diffuse the extraction solutions. The plates were incubated at 37ºC for 24 hours. The results were obtained by measuring the diameter of the inhibition zone in millimetres, and the experiment was conducted in three replicates (Eweys, Zhao and Darwesh).

Statistical analysis

The biological experiment was implemented according to the randomised complete design CRD, and the analysis of variance was carried out using the General Linear Model within the SAS (Hefny, Awad and Youssef 2023) program. When there were significant differences between the means, Duncan's test (Johnson *et al*. 2023) was used to determine the significance of the differences between the different means at a probability level of 0.05.

RESULTS AND DISCUSSION

The results of special tests to diagnose the intestinal bacterial species showed that members of this group showed colonies with different morphology on MacConkey agar, blood agar, S-S agar and EMB agar. When these different colonies were purified in special plates, they all appeared as short, Gram-negative bacilli. It was also found that all isolates could grow at 37°C, and some could grow at 42°C, as shown in table (3). The isolates of *E. coli* appeared smooth, small and pink on MacConkey agar (figure 2). They seemed black with a metallic green sheen on Eosin Methylene blue (EMB) agar. When stained with Gram stain, they appeared as short bacilli, which were not spore-forming and were negative for Gram

stain. As for its response to the IMVIC tests, it was positive for Indole due to the decomposition of the amino acid tryptophan by the tryptophan enzyme and the Methyl red test. It was positive for the Voges-Proskauer test. It was also harmful to the Catalase but positive for the oxidase test, indicating the absence of the enzyme that converts citrates to oxidation compounds. The characteristics of this isolate suggest that it was *E. coli* isolates, as shown in table (1).

Table 1. Culture characteristics of bacteria isolated from diarrhoea

Bacterial	**Bacterial species isolate**			
Media	***E.coli***	***Salmonella spp.***	***Shigella spp.***	***Proteus spp.***
MacConkey agar	Pink colonies	Colorless	Colorless	Pale
E.M.B	Green colonies Shiny	Colorless	Colorless	Transparent
Blood agar	Milky white colonies	Gray to white	Colorless	It forms scented ripples. It smells like fish Rotting.
S.S. agar	-	Transparent with black spot	Colorless	-

- **(mean not cultured).**

However, Salmonella isolates appeared on MacConkey agar as pale, large, smooth, convex circles, as indicated in the figure (2), while on EMB agar, it appeared colourless because it is a non-lactose fermenter; these results were in agreement with (Nazari Moghadam *et al*. 2023). The Gram staining of the bacterial isolates showed them as short bacilli, non-spore-forming, and Gram-negative. It also revealed that it does not cause blood hemolysis when grown on blood agar, and it was non-motile at 25°C and appeared on the S.S. agar medium as transparent with a black spot, as seen in Figure (2). Its response to the IMViC tests showed that it was positive for Indole, oxidase and Voges Proskauer tests but negative for Methyl Red and Catalase tests, as illustrated in Table (2).

The diagnostic results of the bacterial isolates also showed that one species belonged to a species of the genus *Proteus*. This is because the colonies on the MacConkey agar medium were pale, indicating that it cannot ferment lactose. It showed a swarming phenomenon and a smell of rotten fish when grown on a blood agar medium. This swarming did not appear on MacConkey agar due to the presence of bile salts that limit its spread. The isolates appeared to consume peptone, which is a source of nitrogen.

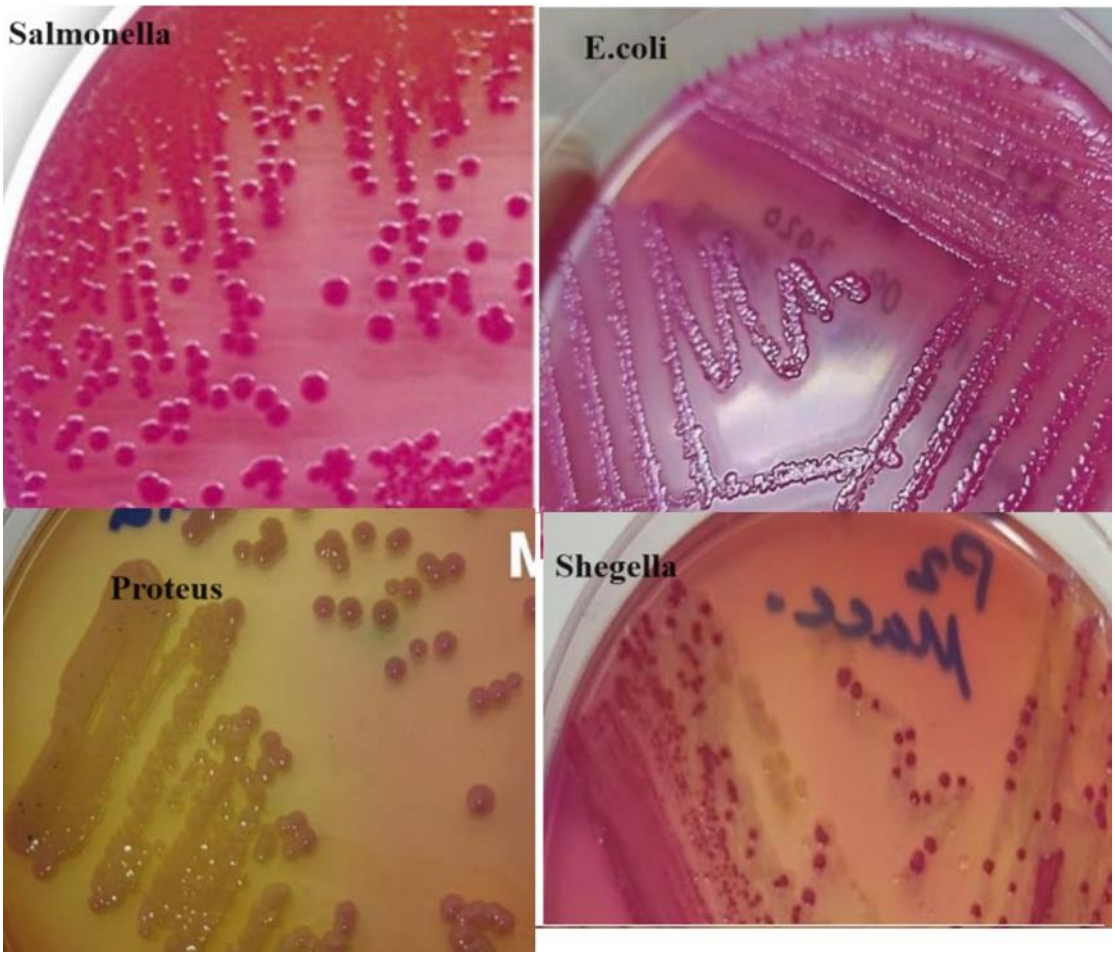

Figure 2. Bacterial kinds isolated on MacConkey medium

The pH value of the medium affected the neutral red reagent's colour and made the colonies' colour pale. However, this characteristic is common in other bacterial genera, confirmed by the swarming phenomenon of *Proteus* bacteria; Ali, 2020).

Table 2. Biochemical tests for Gram-negative bacteria isolated from diarrhoea

Types of tests	Bacterial isolates diagnosed			
	Salmonella	***E.coli***	***Shigella***	***Proteus***
Catalase	**-**	**-**	_	**-**
Oxidase	**-**	+	+	
Indole	+	+	+	+
Methyl red	**-**	**-**	**-**	**-**
Vox Proskauer	+	+	+	+
Motility	**-**	**-**	**-**	**-**
Gram stain	**-**	**-**	**-**	+
Citrate	**+**	**-**	**-**	**-**

(+): Positive results, (–) : Negative results

The results of the rest of the bacterial isolates are shown in Table (4), which shows that *Salmonella* bacteria are the most frequently isolated species. There was a 43.3% isolation rate. This could indicate a significant health issue with food safety or water transmission.

The diagnosis of the bacterial isolate of *Lactobacillus plantarum* was confirmed by growing it anaerobically at 37°C for 48 hours on MRS agar. The bacteria appeared as short, Gram-positive bacilli and non-spore-forming with creamy colour on MRS agar in the biochemical examinations (Da Silva *et al.* 2018), and the use of the Vitek-2 compact diagnostic system to confirm that the isolate is *Lactobacillus plantarum* species as shown in figure (3).

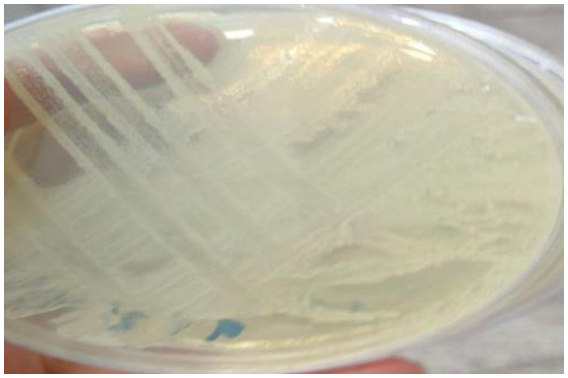

Figure 3. *Lactobacillus plantarum* isolated on MRS agar

The results in Table (3) showed a significant decrease ($P<0.05$) in the concentration of total phenolic compounds after fermentation of *Aloe vera* extract obtained from *Lactobacillus plantarum* bacteria at 37°C for 24 hours under anaerobic conditions. The concentration of phenolic compounds in the extract before fermentation contained Caffein acid, Chlorogenic acid, Cinnamic acid, Gallic acid, Syringic acid, Vitexin and Coumaric acid at 4.63, 3.98, 10.88, 2.93, 3.25, 7.19 and 3.93 mg/ml concentrations respectively. It decreased significantly after fermentation with lactobacilli bacteria. This indicates that lactobacilli bacteria affected the concentration of these phenolic compounds in the extract. This change in the concentrations of phenolic compounds indicates that lactobacilli bacteria have consumed those compounds during the fermentation process (Metzger, Krug and Eisenächer 2018).

Table 3. Estimation of total phenols in *Aloe vera* extract before and after fermentation with *Lactobacillus plantarum* bacteria

Phenolic Compounds Type	Extracts types (mg/ml)	
	***Aloe vera* Extract**	***Alo vera* fermented with *L. plantarum***
Chloro genic acid	3.98 ±0.10 [a]	0.12±0.01 [b]
Caffeine acid	4.63±0.22 [a]	0.45±0.01 [b]
Cinnamic acid	10.88±1.04 [a]	1.82±0.01 [b]
Gallic acid	2.93±0.42 [a]	0.35±0.01 [b]
Coumaric acid	3.93±0.71 [a]	0.27±0.01 [b]
Vitexin	7.19±1.05 [a]	00.1±0.01 [b]
Syringic acid	3.25481±0.71 [a]	0.218 ±0.01 [b]

The letters in each row indicate significant differences between the means at the probability 0.05. ± SD

The results were inconsistent with what was found by (De Montijo-Prieto *et al*., 2023), who found an increase in the concentration of these phenolic compounds after fermentation using lactobacilli bacteria. The results also agreed with the finding of (Xiao *et al.* 2019), who indicated a decrease in some phenolic compounds, the disappearance of some, and the appearance of new phenolic compounds in *Aloe vera* extract fermented using Lactobacilli bacteria. Nonspecific components can appear when fibre and phenolic compounds are fermented in the intestine. It was reported by (Gade and Kumar 2023) that some bacterial phenolic metabolites were modified in colonic fermentation because intestinal bacteria metabolise phenolic compounds to different extents and produce different aromatic metabolites. Changes in intestinal phenolic levels may affect bacterial composition. Phenolic compounds are likely to be helpful to the host by inhibiting the growth of pathogens and regulating commensal bacteria, including probiotics, and thus may be considered prebiotics. Previous studies indicate that some strains of *Lactobacillus, especially Lactobacillus plantarum, have a high ability to bio-convert multiple phenolic compounds into simpler compounds (Akbari et al., 2023) (Razola-Díaz et al.,*2023).

The *Aloe Vera* extract activity as an antioxidant both before and after fermentation using *Lactobacillus plantarum* is shown in table (4). The ability of the fermented extract to function as an antioxidant was found to be significantly increased ($P<0.05$) by fermentation, reaching 44.11% compared to the extract's pre-fermentation antioxidant value of 22.17%. The results of increasing the activity of the extract as an antioxidant were consistent with the findings of (Eweys *et al.* 2022), which were similar to what happens in the digestive system and the role of microorganisms in the fermentation process and converting phenolic compounds into other active compounds as antioxidants. The results were also consistent with (Xiao *et al*. 2020).

who indicated increased antioxidant activities of substances fermented by *Lactobacillus plantarum* bacteria.

Table 4. Effect of *Lactobacillus plantarum* fermentation of *Aloe vera* extract on antioxidant activity

Extract types	**Antioxidants activity**	
	Non-fermentation	***Aloe vera* fermented with *Lactobacillus plantarum***
***Aloe vera* extract**	22.17 ±3.41 b	44.11 ±5.27 a

The letters in each row indicate significant differences between the means at the probability 0.05. ± SD

The agar diffusion method by wells was used to test the isolated bacteria's sensitivity to different *Aloe vera* extract concentrations at 10, 20 and 30 mg/ml. The results are shown in table (5) and figure (4). The aqueous *Aloe vera* extract at 10 mg/ml positively inhibits their growth, as the diameter of the inhibition zone (mm) increased with increasing concentration of *Aloe vera* extract. It was found that the highest inhibition rate of the aqueous extract was recorded against Salmonella bacteria with an inhibition diameter greater than 8.1 mm at a concentration of 30 mg/ml, then against *E. coli* and *Shigella* bacteria with an inhibition diameter ranging between 6-8 mm with the same concentration and the least inhibitory effect was against *Proteus* bacteria with an inhibition diameter between 1-5 mm.

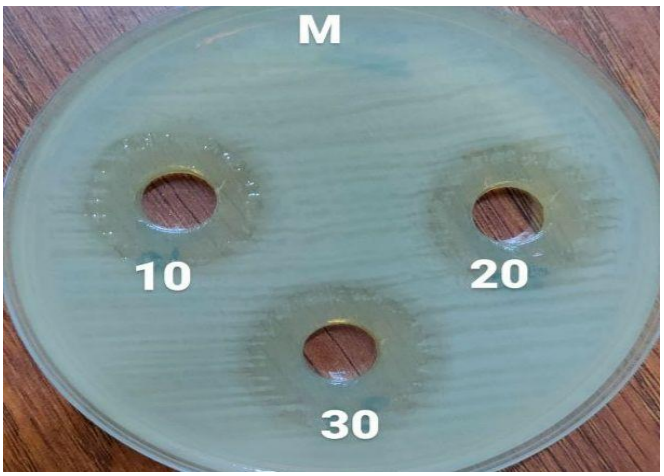

Figure 4. The Inhibition zone of *Aloe vera* extract against *Salmonella* bacteria

The results were in agreement with (Renowati, Enlita and Wahyuni, 2019) and *(Haq et al*. 2020), who stated that aqueous *Aloe vera* plant extract effectively inhibited the growth of *Staphylococcus aureus* and *E.coli* bacteria. Also, this study showed that the use of fermented *Aloe vera* extracts with lactobacilli can cause a practical inhibitory effect on the growth of

specific pathogenic bacteria, suggesting a possible use of these extracts in health and medical applications as natural supplements to antibiotics (Viviana Aparicio-Salcedo *et al*. 2023).

Table 5. Determination of the MIC of *Aloe vera* extract against pathogenic bacteria

Microbial types	*Aloe vera* extract concentration (mg/ml)		
	10	20	30
Salmonella	+	++	+++
E.coli	+	++	++
Shigella	+	++	++
Proteus	+	++	+

+= 1-5 mm, ++=6- 8-mm, +++= 8.1= ≤ mm

The results also agreed with the findings of (Gao *et al*. 2022), who stated that the activity of the extracts decreases when fermented with LAB bacteria when using fermented extracts of lactobacilli against *S. aureus*, *Shigella* and *Salmonella*. One of the causes of inhibiting pathogenic microorganisms when utilising the extract fermented with Probiotics is the production of organic acids and bacteriocins by those probiotics and hydrogen peroxide, which causes inhibitory effects on the pathogenic bacteria.

The agar well diffusion method was used to study the inhibitory effect of *Aloe vera* extract fermented with *Lactobacillus plantarum* bacteria against the bacteria isolated from children's diarrheic stool samples infected with *Salmonella, Proteus, Shigella* and *E. coli.*

Table (6) and Figure (5) showed that all pathogenic bacterial species were sensitive to the Lactobacillus plantarum --fermented extract but with different inhibition diameters.

The diameter of the inhibition zone using the aqueous *Aloe vera* extract ranged from (1-5) mm, while the sensitivity of the isolates began to increase against the *Lactobacillus plantarum* -fermented extract, as the diameter of the inhibition zone for the pathogenic bacteria against the *Aloe vera* extract fermented with *Lactobacillus plantarum* bacteria ranged from (6-8) mm. These results represent a positive effect of *Aloe vera* extracts fermented with lactobacilli on inhibiting the growth of pathogenic bacteria, i.e. the diameter of the inhibition zone increased when the inhibition effect on the bacteria increased. In general, this inhibitory effect could help reduce the growth and spread of pathogenic bacteria, suggesting the potential benefits of using *Aloe vera* extracts as antibacterial agents. This study showed that using *Aloe vera* extracts fermented with *Lactobacillus plantarum* bacteria could have a practical inhibitory effect on the growth of specific pathogenic bacteria, suggesting the potential of using these extracts in health and medical applications as natural

supplements to antibiotics. These results were consistent with (Lee *et al*. 2021), who found that the activity of the extracts increased when fermented with LAB bacteria when using extracts fermented with probiotics against *Salmonella, Shigella and S. aureus* bacteria. One of the reasons for the inhibition of pathogenic microorganisms when using fermented extract with probiotic bacteria is the production of organic acids and bacteriocins by that probiotic and the presence of hydrogen peroxide, which hurts these pathogenic bacteria. Our results agreed with (Ross *et al*. 2022), who reported that the activity of *Aloe vera* extract increased when fermented with lactobacilli with increased antioxidant activity. *Aloe vera* is an excellent natural source for probiotics and a substrate for lactobacilli fermentation. Therefore, a symbiotic drink that uses *Aloe vera* as a main constituent and lactobacilli as a probiotic with great benefits for human health may represent a promising product for development that includes the mechanism of synergistic action between the active substances of the *Aloe vera* extract with lactobacilli, which leads to increased effectiveness in inhibiting pathogenic bacteria and promoting the growth of probiotics (Cuvas-Limón *et al*. 2015).

Table 6. The effect of aqueous extract of *Aloe vera* leaves fermented with lactic acid bacteria against *Salmonella*

Bacteria Treatment	**Control**	***Aloe vera* extract**	***Aloe vera* extract+ *L.Plantrum*.**
Salmonella	-	+	++
E.coli	-	+	++
Shigella	-	+	++
Proteus	-	+	++

Diameter of inhibition zone +(1-5)mm, ++(6-8) mm

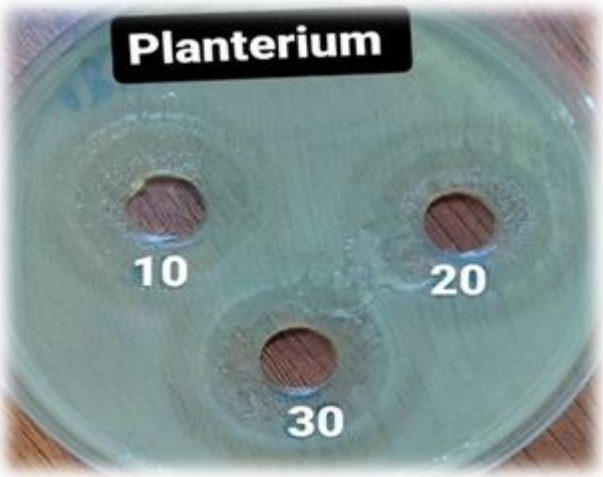

Figure 5. Inhibitory effect of *Aloe vera* extract fermented by *Lactobacillus planetarium* on *Salmonella*

CONCLUSION

It can be concluded from this study that the most frequent pathogenic bacterial species from diarrhoea patients were *Salmonella* bacteria. It was found that there was a decrease in the concentration of total phenolic compounds after *Aloe vera* extract fermentation with *Lactobacillus planetarum* bacteria, and it was shown that the aqueous extract of the *Aloe vera* plant contains active compounds and highly effective antioxidants. It was also demonstrated that the inhibitory effect of fermented *Aloe vera* extract was higher against pathogenic isolates than the non-fermented extract.

CONFLICT OF INTEREST

The authors declare that no conflict of interest is associated with this manuscript.

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