Antimicrobial activity of cashew nut testa extract (*Anacardium occidentale* L.)

Phuong T. Nguyen, Luyen T. L. Nguyen, Mai T. T. Bui, Hau L. M. Nguyen, & Huan T. Phan*
Faculty of Chemical Engineering and Food Technology, Nong Lam University, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: August 27, 2024
Revised: October 10, 2024
Accepted: October 31, 2024

Keywords

Anacardium occidentale L. testa
Antibacterial activity
MIC
Phytochemical screening
Polyphenols

***Corresponding author**

Phan Tai Huan
Email: pthuan@hcmuaf.edu.vn

ABSTRACT

The cashew nut (*Anacardium occidentale* L.) testa, commonly considered as a by-product of cashew processing, is rich in polyphenols. This research investigated the antimicrobial effects of the cashew nut testa extracts prepared using a mixture of 0.22% cellulase and pectinase (1:1, v/v) with a ratio of raw material to solvent of 1:55 (v/v), an extraction temperature of 49°C and pH 4.0 for 60 min. Phytochemical screening revealed the presence of various phenolic compounds in the testa extract, including saponins, coumarins, triterpenoids, tannins, flavonoids, and alkaloids. The extract's antimicrobial efficacy was assessed against 4 bacterial strains associated with food poisoning: *Bacillus cereus, Staphylococcus aureus*, *Shigella* spp., and *Salmonella typhimurium*. Remarkably, the extract demonstrated inhibitory action against *Staphylococcus aureus*, producing an inhibition zone diameter of 1.00 mm at a concentration of 25 mg/mL and the largest diameter of 12.93 mm at 800 mg/mL. The Minimum Inhibitory Concentration (MIC) values were determined as follows: 200 mg/mL for *Salmonella typhimurium*, 100 mg/mL for both *B. cereus* and *Shigella* spp., and 25 mg/mL for *Staphylococcus aureus*.

Cited as: Nguyen, P. T., Nguyen, L. T. L., Bui, M. T. T., Nguyen, H. L. M., & Phan, H. T. (2024). Antimicrobial activity of cashew nut testa extract (*Anacardium occidentale* L.). *The Journal of Agriculture and Development* 23(Special issue 2), 155-165.

1. Introduction

Food spoilage and its etiological agents have been prevented using chemical preservatives for a long time (Mostafa et al., 2012). Although these chemical preservatives have demonstrated effectiveness in preventing foodborne diseases and controlling outbreaks, their repeated use has led to the accumulation of chemical residues in the food and feed chain, the development of microbial resistance to these chemicals, and adverse side effects on human health (Harris et al., 2018). For this reason, current food processing trends focus on using natural compounds, which are considered safe alternatives and satisfy the consumer preferences for more "green foods". Manufacturers have been searching for safer

natural alternatives like phytochemicals (such as polyphenols, including flavonoids, and essential oils rich in terpenoids, such as carotenoids) (Gutiérrez-del-Río et al., 2021).

Cashew (*Anacardium occidentale* L.) is a member of the *Anacardium* genus of the Anacardiaceae family. It is native to Brazil but is now widely grown in many tropical countries such as Mozambique, Tanzania, Kenya, Indonesia, Thailand, India, and Vietnam (Lubi & Thachil, 2000; Paramashivappa et al., 2001; Das et al., 2004). The cashew apple fruit includes two parts: the true fruit and the false fruit. The false fruit comprises 90% of the whole fruit's weight but is usually discarded during harvest. The true fruit consists of three main parts: the nutshell, testa, and kernel. The testa is considered waste in the cashew nut processing industry (Sruthi & Naidu, 2023). Currently, this by-product is often partially used as animal feed or as fuel for furnaces. Testa constitutes about 1 - 3% of the total weight of cashew nuts and contains biologically active compounds such as (+)-catechin, (-)-epicatechin, epigallocatechin, epigallocatechin gallate, gallic acid, syringic acid, and p-coumaric acid (Sruthi & Naidu, 2023). These compounds exhibit antimicrobial activity against human pathogens (Markus et al., 2017).

There are many methods to extract polyphenols, such as solid-liquid extraction (SLE), pressurized liquid extraction (PLE), ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical extraction (SCE) (Ajila et al., 2011). However, in this study, a mixture of cellulase and pectinase, an efficient and eco-friendly method, was used to extract phenolic compounds. Additionally, the disk-diffusion assay was also used in this study. It offers many advantages over other methods, including simplicity, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents, and the ease of interpreting results provided (Balouiri et al., 2016). The objective of this study was to evaluate the effect of the extraction conditions on total polyphenol content (TPC) and reveal the presence of various phenolic compounds within the cashew nut testa extract. Particularly, the evaluation of the antimicrobial activity of the extract against four bacterial strains: *Bacillus cereus*, *Staphylococcus aureus*, *Shigella* spp., and *Salmonella typhimurium*, respectively, was carried out.

2. Materials and Methods

2.1. Materials

Organic cashew nut testa (CNT) was provided by Hanfimex Group, Binh Phuoc branch. The raw testa included hard shells, broken kernels, and testa, which were products of cashew processing. The raw testa was removed from the broken kernels and hard shells, and the testa was retained. After that, the raw testa was dried at 45°C to a moisture content below 10%, pulverized, and sieved through a 0.5 mm screen to obtain a fine powder. Finally, CNT was packed in silver zip bags and stored in the fridge at 8°C for further use (Figure 1).

Figure 1. Cashew nut testa powders.

2.2. Chemicals

Folin-Ciocalteu reagents were supplied by Merck (Darmstadt, Germany). Standard gallic acid was purchased from Sigma-Aldrich (USA). Enzyme pectinase (P) (4130 U/g) and cellulase (C) (endoglucanase, 774 U/g) were purchased from Novozymes in Singapore. The buffer was a mixture of sodium phosphate monobasic dihydrate (Merck, Germany) and citric acid monohydrate (Fisher, USA). Mueller-Hilton agar (MHA, Merck, Germany) was used as the antibacterial medium. Tryptone Soya Agar (TSA, Merck, Germany) and Tryptone Soya Broth (TSB, Merck, Germany) were used as the culture medium, and Gentamicin (Merck, Germany). Qualitative chemicals, including ethanol (C_2H_5OH), ferric chloride ($FeCl_3$), sulfuric acid (H_2SO_4), chloroform ($CHCl_3$), potassium iodide (KI), and ammonium hydroxide (NH_4OH), all met analytical standards. All other chemicals used were of analytical grade. Analytical water was obtained by a Milli-Q filtration system (Millipore, Bedford, Massachusetts, USA).

2.3. Cashew nut testa extraction preparation

2.3.1. Preparation of sample for phytochemical screening

The qualitative analysis of bioactive compounds in cashew nut testa (CNT) such as saponins, coumarins, triterpenoids, tannins, flavonoids, alkaloids, and phenolic compounds was carried out using methods referenced from studies by Godghate & Sawant (2013), Iqbal et al. (2015), and Singh & Kumar (2017). Firstly, 20 g of cashew nut testa powder sample was prepared and subjected to magnetic stirring extraction using distilled water and absolute 80% ethanol as extract solvents. The extraction was carried out under a vacuum and protected from light, with each solvent having a volume of 200 mL and an extraction time of 48 h. The resulting extract was then vacuum filtered to obtain a pure liquid, which was stored in the fridge at about 4°C until further used. The qualitative results were considered positive when the basic identification reaction for each chemical group was clearly expressed and stable for a certain period. The level of positivity was determined through complementary chemical reactions.

2.3.2. Preparation of cashew nut testa extract

Cashew nut testa was extracted at a temperature of 49°C and pH = 4 for 60 min with a mixture of the C:P enzyme (ratio 1:1) with a concentration of 0.22% and a ratio of CNT: solvent was 1:55 (g/mL), (Dao, 2023). Firstly, the Erlenmeyer flask containing 54 mL of buffer solution (pH = 4) was heated up to 49°C . Then, it was added 1g of cashew nut testa powder and 1 mL of a mixture of C:P enzyme (0.22%). After that, the flask was incubated for 60 min. Then, the temperature of the flask was increased to 90°C and kept for 5 min. After inactivating the mixture of C : P enzyme, the flask was cooled in a cold-water bath (about 10°C). The extract mixture was filtered by vacuum filtration to obtain a clear amber-colored solution. The extract was stored in a dark glass bottle to protect it from light and was placed in the fridge at 8°C for further used.

2.3.3. Preparation of freeze-dried powder of CNT extract

The CNT extract at a concentration of 3.2 - 3.4° Brix was poured into the vials with the appropriate sample amount. The vial was covered with a food wrap and poked at least 5 holes to let the steam escape and limit the liquid overflow into the machine during the drying process. After that, the sample was frozen and placed in the drying rack. The freeze-drying process started when the vacuum reached below 4 mmHg. After 72 h of drying, the lyophilisates were collected and stored in silver zip bags with added silica desiccant to protect them from light as well as moisture and were placed in the fridge at about 8°C for further used.

2.4. Qualitative phytochemical analysis

2.4.1. Saponins

Five mL extract of CNT was mixed with 20 mL of distilled water and then it was agitated in a graduated cylinder for 15 min. The formation of foam indicated the presence of saponin in the sample (Godghate & Sawant, 2013).

2.4.2. Coumarins

Three mL of NaOH 10% was added to 2 mL of aqueous extract of CNT. The yellow colour was formed that showed the presence of coumarins in the sample (Godghate & Sawant, 2013).

2.4.3. Triterpenoids

One mL of the extract of CNT was treated with 1 mL of chloroform and filtered. The filtrate was added with a few drops of concentrated sulphuric acid, shaken, and allowed to stand. If the lower layer turns red, a steroid is present. A golden yellow layer at the bottom indicated the presence of triterpenoids in the sample (Singh & Kumar, 2017).

2.4.4. Tannins

One mL of CNT extract was taken and treated with 1 mL of 10% alcoholic ferric chloride solution. The formation of a blue or greenish colour showed the presence of tannins in the sample (Iqbal et al., 2015).

2.4.5. Flavonoids

The CNT extract was treated with a NaOH 10% solution. The formation of an intense yellow color was observed, indicating the presence of flavonoids in the sample (Godghate & Sawant, 2013).

2.4.6. Alkaloids

Wagner's reagent was formed from a mixture of 2 g of potassium iodide and 1.27 g of iodine, which were dissolved in distilled water to make the final volume 100 mL. Two mL of Wagner's reagent was mixed well with 2 mL of filtrate. The appearance of a brown color indicated the presence of alkaloids in the sample (Iqbal et al., 2015).

2.5. Total phenolic content (TPC)

Determination of the total phenolic content was carried out using the Folin-Ciocalteu test. Firstly, 0.1 mL of the cashew sample diluted with 1.8 mL of Folin-Ciocalteu reagent (10%, v/v) was shaken well and left for 5 min. Then add 1.2 mL of 15% Na_2CO_3 to the solution and make up to 10 mL with distilled water. The mixture was kept in the dark for 90 min, with the absorbance measured at 734 nm in a PhotoLab 6600 UV-Vis spectrophotometer (Jenway 7300, England). A standard curve was generated using different concentrations of gallic acid (R^2 = 0.9983) and distilled water was used as a control. The results were expressed in grams of gallic acid equivalents (GAE) per kilogram of dry weight (DW) (Nguyen et al., 2021).

2.6. Antimicrobial activity test

2.6.1. Disc diffusion assay

The assay was carried out using the disk diffusion method (Balachandran et al., 2013). Four strains of food bacterial pathogens *Bacillus cereus* ATCC 11178, *Staphylococcus aureus* ATCC 6538, *Salmonella typhimurium* ATCC 14028, and *Shigella* spp., were identified by the Quality Measurement Standards Technical Center 3 (QUATEST 3) in Ho Chi Minh City. The Muller-Hinton agar (MHA) plates were inoculated using a sterile swab with bacterial suspensions equal to 0.5 McFarland turbidity. The final concentration of the bacterial suspension was 1.5×10^8 CFU/mL. The lyophylisates of CNT extracts were suspended in distilled water. Standard blank

disks 6.0 mm diameter were placed on media and inoculated with 20 µL of the extract solution. The plates were incubated at 37°C for 24 h. The zone of inhibition was measured. The distilled water was used as a negative control.

2.6.2. Minimum inhibitory concentration (MICs)

The MIC was determined using the sample dilution method. The lyophilisates of cashew nut testa extract were suspended in distilled water at different concentrations of 800, 400, 200, 100, 50, and 25 mg/mL and further fractionated through a sterile membrane. The turbidity of the microbial suspension was adjusted to McFarland 0.5, equivalent to 1.5 x 10^8 CFU/mL. The bacteria were then spread onto MHA agar plates using a sterile cotton swab and allowed to dry. Next, 10 µL of each sample concentration was pipetted onto a sterile paper disc (diameter = 6 mm), dried, and placed on the agar surface with evenly spread bacteria. The paper disc was lightly pressed to secure it onto the agar surface. The positive control was the antibiotic gentamicin, while distilled water was the negative control.

2.7. Statistical analysis

Experiments were arranged with one random factor and three repetitions. The raw data was collected and processed using Microsoft Excel 2020. Statistical analysis was performed using Statgraphics Centurion XVI software (Statpoint Technologies Inc., USA), and differences among groups were analyzed using one-way ANOVA variance analysis, followed by the Multiple Range Test. The criterion for statistical significance was set at $P < 0.05$. The results were shown as mean and standard deviation (Mean ± SD), the differences in mean values were determined using one-way ANOVA at $P < 0.05$ significance level.

3. Results and Discussion

3.1. Qualitative bioactive compounds of cashew nut testa extraction

The qualitative analysis of bioactive compounds in CNT was carried out using water. The qualitative results of bioactive compounds in cashew nut testa extract are presented in Table 1.

Table 1. The results of the qualitative phytochemical compounds from extract of cashew nut testa by water and ethanol

Phytochemical compound	Chemical test	Indicator	Results
Saponins	Froth test	Foam 1 cm form that lasted for 10 min	+
Triterpenoids	Salkowski test	Appearance of red-brown colour	+
Tannins	Braemer's test	Greenish grey	+
Flavonoids	Ammonium test	A layer of yellow color-ation at the bottom	+
Alkaloids	Wagner's test	Formation of brown precipitate	+
Coumarins	Three mL of 10% NaOH was added to 2 mL of aqueous extract of CNT.	Formation of yellow colour	+

Results (+) are means of the presence of phytochemical compounds; CNT: Cashew nut testa.

According to Godghate & Sawant (2013), the presence of foam up to 1 cm above the mixture for 2 - 3 min was indicative of the presence of saponins (Figure 2a). The control sample (test tube No. 0) showed no observable phenomenon. However, the sample extracted with water (test tube No. 1) produced a thin layer of foam that lasted for 10 min. Similarly, the sample extracted with ethanol (test tube No. 2) also formed a thin layer of foam that lasted for 10 min, but it also caused turbidity due to the excess ethanol reacting with water. This indicates that saponin was present in both samples of extract, with clear positive results.

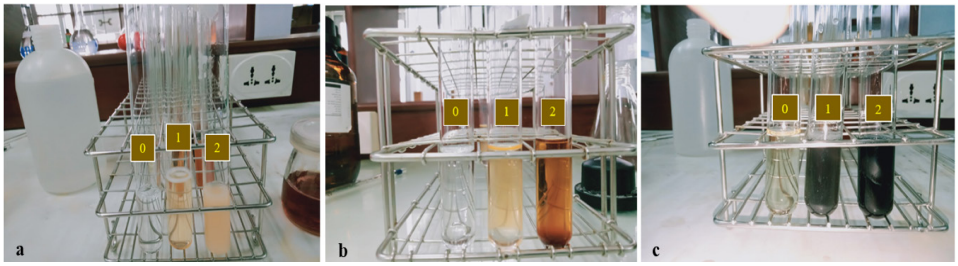


Figure 2. Results of test for saponins (a), triterpenoids (b) and tannins (c) in cashew nut testa. extract. Test tube No. 0 contained the control sample, test tube No. 1 contained the sample extracted with water, and test tube No. 2 contained the sample extracted with ethanol.

The results in Figure 2b show a separation between water and non-polar solvent (test tube No. 0), and a separation of light yellow (test tube No. 1) and brownish yellow at the bottom (test tube No. 2). These are indicators of the presence of triterpenoids in the extract.

Figure 2c showed a colour change in the control sample due to the colour of the reagent (test tube No. 0), green appeared in the sample extracted with water (test tube No. 1) and grey-blue appeared in the sample extracted with ethanol (test tube No. 2). These are indicators showing the presence of tannins in the extract. In Figure 3a, sample zero did not show any colour change, but samples 1 and 2 showed a change from clear yellow to dark yellow and from brownish yellow to dark brown, respectively. This result indicated the presence of flavonoids in both extracts.

Upon observation of Figure 3b, it was evident that sample zero contained a yellow colour, which was the colour of the reagent. Sample 1 showed a subtle colour change, while sample 2 changed from a light brown to a dark brown and became opaque. These changes indicated the presence of alkaloids in both extracts. Based on these indicators, it can be concluded that both extracts contain alkaloids.

Figure 3. Results of test for flavonoids (a), alkaloids (b) and coumarins (c) in cashew nut testa extract. Test tube No. 0 contained the control sample, test tube No. 1 contained the sample extracted with water, and test tube No. 2 contained the sample extracted with ethanol.

Figure 3c showed that sample zero did not exhibit any change, while samples 1 and 2 showed cloudiness at stage A, which gradually decreased at stage B. This indicated the presence of coumarins in both extracts.

In short, the findings indicated that all six bioactive compounds were detected in various samples of cashew nut testa extracted with either water or ethanol. The components found in CNT extracts, including alkaloids, flavonoids, tannins, saponins, triterpenoids, and coumarins, were the primary phenolic compounds that have also been found in extracts of numerous other plants.

Qualitative analysis of some bioactive components of the methanolic leaf extract of *M. citrifolia* (Aishatu et al., 2020) showed the presence of tannins, steroids, saponins, flavonoids, and alkaloids. These results agreed with the findings of preliminary phytochemical screening of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan (Gul et al., 2017). The phytochemical analysis showed that the *Ephedra intermedia* plant extract contains a mixture of phytochemicals such as reducing sugars, cardiac glycoside, phenolic compounds, flavonoids, and alkaloids (Gul et al., 2017). Additionally, a wide variety of bioactive compounds such as alkaloids, saponins, coumarins, flavonoids, terpenoids, and tannins were found to be present in the leaves of *Annona squamosa* Linn. *(custard apple)* (Nguyen et al., 2020).

The extract contained most of the polyphenols present in the CNT. These are important polyphenols in plant cells. According to Abbas et al. (2017), they play a role in fighting pathogens from microorganisms, preventing harmful oxidation reactions, and protecting plants from the effects of ultraviolet radiation. In addition, polyphenols also have a positive effect in the treatment of diseases related to the cardiovascular system, nerves, cancer, osteoporosis, and diabetes (D'Archivio et al., 2007).

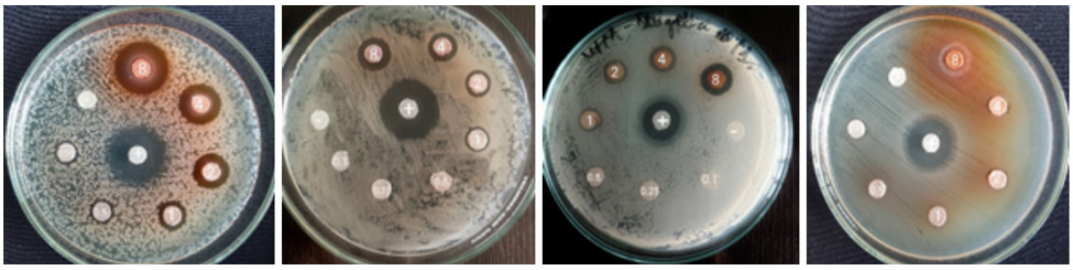
3.2. Total phenolic content of CNT extracts

In this study, the total phenolic content (TPC) of the cashew nut testa extract was determined. The results revealed a TPC of 240.06 ± 1.03 mg GAE/g DW, which was also similar to some other studies. Studies have shown that CNC (cashew nut shell) contains a significant amount of phenolic compounds, as evaluated by Mazzetto et al. (2008) in their study on ethanol extracts directly obtained from the seed coat. The concentration of total phenols in these extracts was found to be approximately 185.44 mg/gallic acid equivalent (GAE). This result is consistent with the findings of Kamath & Rajini (2007), who also used ethanol to extract phenolic compounds from the seed coat. They observed a higher total phenolic content (243 mg/GAE) when the extraction was performed under stirring at 37°C for 3 h, compared to the ethanolic extract at room temperature. These results suggest that the cashew nut shell is a rich source of phenolic compounds, which could potentially be explored for their antibacterial activity against pathogenic strains.

3.3. Antimicrobial activity

The antibacterial activity of the CNT extract was evaluated using the disc diffusion method, as indicated by the antibacterial rings around the test paper circle. Results are presented in Figure 4.

Figure 4 shows that Gentamicin has a strong antibacterial ability against 4 tested bacterial strains. Specifically, Gentamicin is more sensitive to G+ bacteria such as *S. aureus*, and *B. cereus* than to G– bacteria such as *S. typhimurium*, *Shigella* spp.



A) B) C) D)

Figure 4. Antimicrobial activity of the phenolic compounds on food-borne pathogens (A) *Shigella* spp. (B) *B. cereus* (C) *S. aureus* (D) *S. typhimurium*.

Additionally, Table 2 showed the antibacterial activity of CNT extract against *S. typhimurium, B. cereus, Shigella* spp., and *S. aureus* were found in the range of 25 - 200 mg/mL. The highest zone of inhibition was obtained for *S. aureus* and the lowest for *S. typhimurium*. Based on the antibacterial activity of the CNT extract, the minimum inhibitory concentration (MIC) values were performed using the broth dilution microplate method. The CNT extract controlled the growth of *S. aureus* with MIC of 25 mg/mL, *B. cereus* and *Shigella* spp. with MIC of 100 mg/mL, and *S. typhymurium* with MIC of 200 mg/mL. The maximum inhibition zone diameter was exhibited by CNT extract against *Staphylococcus aureus* (12.93 mm), *Bacillus cereus* (7.67 mm), *Shigella* spp. (7.08 mm), and *Salmonella typhimurium* (3.93 mm) at 800 mg/mL concentration.

Table 2. Antibacterial activities profile of cashew nut testa extract

Concentration of cashew nut testa extract (mg/mL)	*Diameter of inhibition zone (mm)*			
	Bacillus cereus	*Salmonella typhimurium*	*Staphylococcus aureus*	*Shigella* spp.
Gentamycin (1 mg/mL)	$14.17^{a} \pm 0.14$	$9.67^{a} \pm 0.01$	$15.00^{a} \pm 0.04$	$11.17^{a} \pm 0.14$
800S	$7.67^{b} \pm 0.14$	$3.93^{b} \pm 0.05$	$12.93^{b} \pm 0.03$	$7.08^{b} \pm 0.14$
400	$4.42^{c} \pm 0.14$	$1.79^{c} \pm 0.04$	$8.77^{c} \pm 0.03$	$4.08^{c} \pm 0.14$
200	$3.50^{d} \pm 0.00$	$0.67^{d} \pm 0.02$	$5.66^{d} \pm 0.03$	$2.83^{d} \pm 0.14$
100	$2.33^{e} \pm 0.14$	-	$2.66^{e} \pm 0.03$	$1.50^{e} \pm 0.00$
50	-	-	$1.77^{f} \pm 0.03$	-
25	-	-	$1.00^{g} \pm 0.03$	-
10	-	-	-	-
Distilled water	-	-	-	-

$^{a-g}$values are means of three replicates, and those with different letters within the same column are the difference is statistically significant; "–": Resistance.

The antibacterial ability of cashew nut testa extract may come from bioactive compounds such as tannins, catechins, saponins, and coumarins (Oliveira et al., 2015). According to Farha et al. (2020), tannins are natural compounds that have the potential to replace antibiotics due to their good antibacterial ability. Tannins inhibit bacterial growth through the following mechanisms: tannins form complexes with iron from the environment so that bacteria cannot use iron; inactivate enzymes involved in cell wall synthesis, inhibiting bacterial cell wall synthesis; bind directly to the peptidoglycan layer and destroy the integrity of the cell wall; form hydrogen bonds with membrane proteins, leading to changes in the permeability of the cell membrane, causing the membrane to denature and be destroyed (Dong et al., 2018; Salehi et al., 2019).

4. Conclusions

This study's findings revealed the presence of various phytochemical compounds in the cashew nut testa extract, including saponins, coumarins, triterpenoids, tannins, flavonoids, and alkaloids, as determined through phytochemical screening. The antimicrobial results demonstrated that the CNT extracts had significant antimicrobial efficacy against four bacterial strains known for causing food poisoning: *Bacillus cereus*, *Staphylococcus aureus*, *Shigella* spp., and *Salmonella typhimurium*. Notably, the extract showed inhibitory action against *Staphylococcus aureus*, with an inhibition zone diameter of 1.00 mm at a concentration of 25 mg/mL and the largest diameter of 12.93 mm at 800 mg/mL. The Minimum Inhibitory Concentration (MIC) values were determined to be 200 mg/mL for *Salmonella typhimurium*, 100 mg/mL for both *B. cereus* and *Shigella* spp., and 25 mg/mL for *Staphylococcus aureus*. However, the antibacterial activity of the extract was only evident at a high concentration of 800 mg/mL. Therefore, the antibacterial results of this study may not apply to food preservation in practical settings. Nevertheless, our findings are crucial as a foundation for further research on cashew nut testa and other similar materials.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This research was financed by Project B2022 - NLS- 02 (Vietnam's Ministry of Education and Training). We would like to thank Anh Dang Thi Ngoc, Mi Nguyen Diem and Hue Do Thi Kim for their support in preparing cashew nut testa extracts and antibacterial test samples.

References

Abbas, M., Saeed, F., Anjum, F. M., Afzaal, M., Tufail, T., Bashir, M. S., Ishtiaq, A., Hussain, S., & Suleria, H. A. R. (2017). Natural polyphenols: An overview. *International Journal of Food Properties* 20(8), 1689-1699. https://doi.org/10.1080/10942912.2016.1220393.

Ajila, C. M., Brar, S. K., Verma, M., Tyagi, R. D., Godbout, S., & Valéro, J. R. (2011). Extraction and analysis of polyphenols: recent trends. *Critical Reviews in Biotechnology* 31(3), 227-249. https://doi.org/10.3109/07388551.2010.513677.

Balachandran, C., Duraipandiyan, V., Balakrishna, K., Sundaram, R. L., Vijayakumar, A., Ignacimuthu, S., & Al-Dhabi, N. A. (2013). Synthesis and medicinal properties of plant-derived

vilangin. *Environmental Chemistry Letters* 11, 303-308. https://doi.org/10.1007/s10311-013-0408-4.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis* 6(2), 71-79. https://doi.org/10.1016/j.jpha.2015.11.005.

Dao, T. M. T. (2024). *Optimizing the process of extracting polyphenol from cashew nut testa using a mixture of cellulase and pectinase enzymes* (Unpublished master's thesis). Nong Lam University, Ho Chi Minh City , Vietnam

D'Archivio, M., Filesi, C., Benedetto, R. D., Gargiulo, R., Giovannini, C., & Masella, R. (2007). Polyphenols, dietary sources and bioavailability. *Annali-Istituto Superiore di Sanita* 43(4), 348.

Das, P., Sreelatha, T., & Ganesh, A. (2004). Bio oil from pyrolysis of cashew nut shell-characterisation and related properties. *Biomass and Bioenergy* 27(3), 265-275. https://doi.org/10.1016/j.biombioe.2003.12.001.

Dong, G., Liu, H., Yu, X., Zhang, X., Lu, H., Zhou, T., & Cao, J. (2018). Antimicrobial and anti-biofilm activity of tannic acid against Staphylococcus aureus. *Natural Product Research* 32(18), 2225-2228. https://doi.org/10.1080/14786419.2017.1366485.

Farha, A. K., Yang, Q. Q., Kim, G., Li, H. B., Zhu, F., Liu, H. Y., Gan, R. Y., & Corke, H. (2020). Tannins as an alternative to antibiotics. *Food Bioscience* 38, 100751. https://doi.org/10.1016/j.fbio.2020.100751.

Godghate, A., & Sawant, R. (2013). Qualitative phytochemical analysis of chloroform extract of leaves of *Adhatoda vasica* Nees. *Rasayan Journal of Chemistry* 6(2), 107-110.

Gul, R., Jan, S. U., Faridullah, S., Sherani, S., & Jahan, N. (2017). Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan. *The Scientific World Journal* 2017(1), 5873648. https://doi.org/10.1155/2017/5873648.

Gutiérrez-del-Río, I., López-Ibáñez, S., Magadán-Corpas, P., Fernández-Calleja, L., Pérez-Valero, Á., Tuñón-Granda, M., Miguélez, E. M., Villar, C. J., & Lombó, F. (2021). Terpenoids and polyphenols as natural antioxidant agents in food preservation. *Antioxidants* 10(8), 1264. https://doi.org/10.3390/antiox10081264.

Harris, S. J., Cormican, M., & Cummins, E. (2012). Antimicrobial residues and antimicrobial-resistant bacteria: impact on the microbial environment and risk to human health-A review. *Human and Ecological Risk Assessment: An International Journal* 18(4), 767-809. https://doi.org/10.1080/10807039.2012.688702.

Iqbal, E., Salim, K. A., & Lim, L. B. (2015). Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts *of Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. *Journal of King Saud University-Science* 27(3), 224-232. https://doi.org/10.1016/j.jksus.2015.02.003.

Kamath, V., & Rajini, P. S. (2007). The efficacy of cashew nut (*Anacardium occidentale* L.) skin extract as a free radical scavenger. *Food Chemistry* 103(2), 428-433.

Lubi, M. C., & Thachil, E. T. (2000). Cashew nut shell liquid (CNSL)-A versatile monomer for polymer synthesis. *Designed Monomers and Polymers* 3(2), 123-153. https://doi.org/10.1163/156855500300142834.

Markus, J., Wang, D., Kim, Y. J., Ahn, S., Mathiyalagan, R., Wang, C., & Yang, D. C. (2017). Biosynthesis, characterization, and bioactivities evaluation

of silver and gold nanoparticles mediated by the roots of Chinese herbal *Angelica pubescens* Maxim. *Nanoscale Research Letters* 12, 1-12. https://doi.org/10.1186/s11671-017-1833-2.

Mazzetto, S. E., Lomonaco, D., & Mele, G. (2008). Cashew nut oil: Opportunities and challenges in the context of sustainable industrial development. *Quimica Nova* 32(3), 732-741. https://doi.org/10.1590/S0100-40422009000300017.

Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., & Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences* 25(2), 361-366.

Nguyen, M. T., Nguyen, V. T., Le, V. M., Trieu, L. H., Lam, T. D., Bui, L. M., Nhan, L. T. H., & Danh, V. T. (2020). Assessment of preliminary phytochemical screening, polyphenol content, flavonoid content, and antioxidant activity of custard apple leaves (Annona squamosa Linn.). *IOP Conference Series: Materials Science and Engineering* 736(6), 062012. https://doi.org/10.1088/1757-899X/736/6/062012.

Nguyen, T. T., Tran, T. P. N., Phan, T. H., Tran, T. A. T., & Nguyen, T. T. (2021). Physicochemical properties, antioxidant and antibacterial activities of spray-dried powder and custard apple peel extract (*Annona squamosa L.*). *Journal of Science and Technology-IUH* 49(01), 57-66.

Oliveira, N. F., Leal, R. S., & Dantas, T. N. C. (2015). The importance of the cashew nut (*Anacardium occidentale* L.) coat: A review. *American International Journal of Contemporary Scientific Research* 2(8), 09-41.

Paramashivappa, R., Kumar, P. P., Vithayathil, P. J., & Rao, A. S. (2001). Novel method for isolation of major phenolic constituents from cashew (*Anacardium occidentale* L.) nut shell liquid. *Journal of Agricultural and Food Chemistry* 49(5), 2548-2551. https://doi.org/10.1021/jf001222j.

Salehi, B., Gültekin-Özgüven, M., Kırkın, C., Özçelik, B., Morais-Braga, M. F. B., Carneiro J. N. P., Bezerra, C. F., Silva, T. G., Coutinho, H. D. M., Amina, B., Armstrong, L., Selamoglu, Z., Sevindik, M., Yousaf, Z., Sharifi-Rad, J., Muddathir, A. M., Devkota, H. P., Martorell, M., Jugran, A. K., Martins, N., & Cho, W. C. (2019). Anacardium plants: chemical, nutritional composition and biotechnological applications. *Biomolecules* 9(9), 465. https://doi.org/10.3390/biom9090465.

Singh, V., & Kumar, R. (2017). Study of phytochemical analysis and antioxidant activity of *Allium sativum* of Bundelkhand region. *International Journal of Life-Sciences Scientific Research* 3(6), 1451-1458. http://dx.doi.org/10.21276/ijlssr.2017.3.6.4.

Sruthi, P., & Naidu, M. M. (2023). Cashew nut (*Anacardium occidentale* L.) testa as a potential source of bioactive compounds: A review on its functional properties and valorization. *Food Chemistry Advances* 3, 100390. https://doi.org/10.1016/j.focha.2023.100390.