International Journal of Life Science and Agriculture Research
ISSN (Print): 2833-2091, ISSN (Online): 2833-2105
Volume 03 Issue 05 May 2024
DOI: https://doi.org/10.55677/ijlsar/V03I5Y2024-12
Impact Factor: 6.774 , Page No : 432-438

Antibacterial Activity of Pineapple (*Ananas comosus*) Fruit Peel Extract against *Escherichia coli*

Erlin Dwi Cahyani[1], Ida Munfarida[2], Amrullah[3]
[1,2,3] Department of Environmental Engineering, Islamic State University of Sunan Ampel Surabaya, Surabaya, Indonesia.

ABSTRACT: *Escherichia coli* is generally considered a harmless bacterium, but numerous strains are well-known for causing diseases globally and frequently exhibit antibiotic resistance. Therefore, it's crucial to discover new natural products to combat these bacteria. Meanwhile, a significant amount of pineapple (*Ananas comosus*) peel ends up as waste. This study aims to examine the antibacterial activity of pineapple (*Ananas comosus*) fruit peel extract against *E. coli*. The preparation of pineapple (*Ananas comosus*) peel extract involves grinding, maceration with 96% ethanol, and evaporation. The phytochemical test was conducted to assess the presence of active compounds. Flavonoids were identified using Shinoda's test, tannins with Ferric Chloride, and saponins by detecting foaming development. Antibiotic analysis was carried out following the Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. Disks soaked in *Ananas comosus* peel extract at concentrations of 25%, 50%, 75%, and 100% were placed on Mueller-Hinton (MH) agar inoculated with an *E. coli* suspension. One-way ANOVA was employed to analyze differences between treatments. The phytochemical test results revealed the presence of flavonoids, tannins, and saponins in pineapple peel extract. The highest inhibition zone, measuring 2.231 mm, was observed with 100% *Ananas comosus* peel extract. Statistical analysis confirmed significant differences between the groups with a significance value of 0.000 ($p<0.05$). This suggests that pineapple peel extract could be useful in removing *E. coli*, rather than being discarded as waste. Further research is needed to gain a better understanding of the antibacterial mechanism.

Published Online:
May 31, 2024

KEYWORDS: *Ananas comosus*, Antibacterial, *Escherichia coli*, Peel Extract

Corresponding Author:
Ida Munfarida

INTRODUCTION

Escherichia coli, a Gram-negative rod-shaped bacterium, is typically harmless and lives in the gastrointestinal tract of all warm-blooded animals. However, certain strains, including enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroaggregative (EAEC), enteroinvasive (EIEC), and enterohemorrhagic (EHEC) *E. coli*, can cause gastroenteritis. These strains are classified based on their pathogenic mechanisms. All types of *E. coli* are transmitted through the fecal-oral route (Pepper *et.al*., 2015). It is well known that ETEC is a major cause of diarrheal illness in resource-poor regions worldwide, significantly contributing to high morbidity and ongoing mortality rates, especially among young children (Fleckenstein & Kuhlmann, 2019). Chervy *et.al* (2020) reported that a specific pathotype of *Escherichia coli* associated with Crohn's Disease abnormally colonizes the intestinal mucosa in patients. A previous researcher discovered that the ETEC pathotype is one of the leading causes of diarrhea in Iran, noting that the most prevalent DEC pathotype identified was enterotoxigenic *E. coli* (ETEC) (Alizade *et.al*, 2019). A study conducted in Aceh, Indonesia, revealed that the ingestion of enteric pathogens, including *E. coli*, plays a role in the pathogenesis of stunting in children (Rinanda *et.al*., 2023).

Both developed and developing countries, including Indonesia, continue to battle against *E. coli* contamination. Indonesia has established standards for the presence of *E. coli* in surface water for various purposes, dividing these standards into four (4) classes: Class One water is designated for drinking water sources and other uses requiring similar quality. Class Two water is suitable for recreational facilities, freshwater fish farming, animal husbandry, crop irrigation, and other uses with comparable quality needs. Class Three water is intended for freshwater fish farming, animal husbandry, crop irrigation, and similar uses and Class Four water is designated for crop irrigation and other uses requiring the same quality. For example, the Government has stated that for Class One criteria, the maximum allowable fecal coliform level is 100 MPN/100 m (Government Regulation No. 22 of 2021). Indonesia has established specific regulation governing the quality standards for drinking water. These standards are outlined in Minister of Health Regulation No. 2 of 2023, which serves as the implementation guideline for Government Regulation No. 66 of 2014 concerning Environmental Health. According to this regulation, drinking water must adhere to stringent criteria, including the absence of *E. coli* and total coliform bacteria, to ensure its safety for consumption.

The most common method for treating diarrhea diseases is the use of antibiotics such as amoxicillin-clavulanate and cephalosporins (Tanır Basaranoğlu *et.al*, 2023). The global recognition of these antibiotics for treating diseases related to fecal coliform is well-established. It is known that certain antibiotics, such as Phenoxymethylpenicillin, Doxycycline, and Clarithromycin, have varying degrees of effectiveness in reducing *E. coli* in the human gut, ranging from minor to significant (Elvers *et.al*, 2020). However, the use of antibiotics changes the composition of the intestinal microbiota, resulting in fluctuations in the abundance of certain bacteria such as *E. coli* and a rise in antibiotic resistance (Zimmermann & Curtis, 2019). Microbial antibiotic resistance continues to cause a significant global challenge to this day. Recently, extensive research has focused on developing new antibiotics and exploring the potential of alternative and complementary medicines to combat antibiotic-resistant and multidrug-resistant (MDR) pathogens. Phytochemicals, botanical extracts, and natural compounds are attracting considerable research attention as agents that modify resistance against multi-drug resistant (MDR) pathogens. For instance, propolis produced by honeybees (*Apis mellifera*) and stingless bees has proven effective in treating MDR pathogens (Kasote *et.al*, 2023). Meanwhile, there is an abundance of plant waste that can be used as antibacterial agents. For example, the ash from *Vigna mungo* plant waste has been found to have antibacterial activity against *Escherichia coli* (Mazumder *et.al*, 2024).

Ananas comosus, commonly known as pineapple, is a perennial tropical fruit plant that belongs to the Bromeliaceae family and is widely recognized worldwide (Wali, 2019). Although the health benefits of pineapple have been well-documented, the peels are often discarded and have not been as extensively studied, especially regarding their antibacterial properties. Agricultural waste products, such as pineapple peels, are commonly found throughout Indonesia. Pineapple production in the country reaches 74,815 tons annually (Nurcholis *et.al*, 2020). Assuming that 30 percent of the pineapple fruit is peel, this results in 22,444 tons of waste from pineapple peels, which can potentially pollute the environment. Using peels as antibacterial agents not only benefits the environment but also offers potential in combating pathogenic bacteria like *E. coli* in medicinal applications. It is essential to explore the potential of natural antibacterial agents derived from *Ananas comosus* plant waste. Therefore, this study focuses on analyzing the antibacterial activity of pineapple (*Ananas comosus*) peel extract against *E. coli*.

MATERIALS AND METHODS

E. coli Cultures Preparation

E. coli culture was obtained from the Biology Laboratory of UIN Sunan Ampel Surabaya. The inoculum was placed in 0.9% NaCl and compared to the 0.5 McFarland Standard. The selected inoculum was then used for testing antibacterial activity.

Plant Material Preparation

Ananas comosus peels were collected from a traditional market in Surabaya, Indonesia. The peels were air-dried for 6 hours at room temperature. A total of 1000g of the dried peels were ground into fine powder using a manual grinder. The fine powder then underwent maceration, during which it was placed in a container and covered with 96% ethanol. This process was carried out over 5 days, starting with 1 day of maceration followed by 4 days of re-maceration, with the solvent being replaced every 24 hours. The final step involved removing the solvent from the extract using a rotary evaporator.

Ananas comosus Peels Extract Bioassay

A phytochemical screening test was performed to identify the presence of flavonoids, tannins, and saponins in the pineapple peel extract. It was identified through color reactions and foam-foaming using specific reagents. Flavonoids were identified using Shinoda's test: to 0.5 ml of the extract from *Ananas comosus* peels, magnesium turnings and drops of hydrochloric acid were added. The presence of flavonoids was indicated by the appearance of a crimson red color after 10 minutes. To analyze tannins, the Ferric chloride test was employed. One milliliter of the extract was combined with two milliliters of a 5% neutral ferric chloride solution. The development of a dark blue/green color indicated the presence of tannins (Kancherla *et.al*, 2019). Meanwhile, to detect saponins, 2 ml of distilled water was mixed with 2 ml of the extract sample and shaken vigorously in a graduated cylinder for 15 minutes. The formation of a foam layer indicated the presence of saponins in the test samples (Ali *et.al*, 2018).

Antibacterial Activity

The antibiotic analysis was conducted based on the Kirby-Bauer Disk Diffusion Susceptibility Test Protocol (Hudzicki, 2016). Disks soaked in the *Ananas comosus* peel extract 25%, 50%, 75% and 100% were placed on Mueller-Hinton (MH) agar inoculated with the *E. coli* suspension. The diameter of the bacterial growth inhibition zone, measured in millimeters, is the area where bacteria do not grow around the disk, indicated by the presence of a clear zone.

Statistical Analysis

We conducted a one-way ANOVA to analyze the differences between the treatments. The One-Way Analysis of Variance (ANOVA) is a statistical method utilized to assess whether there exists a discrepancy in means among two or more distinct groups, where the grouping is based on the outcomes of a single categorical variable. In essence, it broadens the examination of mean disparities beyond two groups to encompass multiple groups (Murray, 2017).

Consider a scenario with $K \geq 2$ groups. The null hypothesis suggests that the means of all groups are equivalent, while the alternative hypothesis proposes that the mean of at least one group differs from that of another group (Murray, 2017).

Null hypothesis: $\mu 1 = \mu 2 = ... = \mu K$

Alternative hypothesis: $\mu i \neq \mu j$ for some $i \neq j$

The statistical analysis was performed using SPSS software version 25.

RESULTS

Ananas comosus Peels Extract Bioassay

The results of the phytochemical test, displayed in Table 1 and Figure 1, indicate that pineapple (*Ananas comosus*) peel extract contains flavonoids, tannins, and saponins. Flavonoids were indicated by the formation of a crimson red color, tannins by a dark green color, and saponins by the presence of bubbles and foam.

Table 1. Phytochemical Test Results of *Ananas comosus* Peel Extract

Phytochemical Criteria	Result	Result Test
Flavonoids	a crimson red color appearance	+
Tannins	a dark green color appearance	+
Saponins	a foam layer appearance	+

Flavonoids

Tannins

Saponins

Figure 1. Phytochemical Test Results of *Ananas comosus* Peel Extract

Antibacterial Activity

The results of the antibacterial test using *Ananas comosus* peel extract are presented in Table 2 and Figure 2. The antibacterial activity of *Ananas comosus* peel extract was indicated by the inhibition zone diameter (in mm) formed in a petri dish containing *E. coli*. The average inhibition zones for *Ananas comosus* peel extract concentrations of 25%, 50%, 75%, and 100% were 0.598 mm, 0.784 mm, 0.711 mm, and 2.231 mm, respectively. The highest inhibition zone, 2.231 mm, was formed by the 100% *Ananas comosus* peel extract, while the lowest inhibition zone, 0.598 mm, was formed by the 25% extract. Meanwhile, the control (96% ethanol) produced an inhibition zone of 0.366 mm.

Table 2. Antibacterial Activity from Different Concentrations of *Ananas comosus* peel extract

Peel Extract Concentration	Antibacterial Activity (Inhibition zone diameter in mm)				Average
	1	2	3	4	
25%	0,375	1,186	0,495	0,335	0,598
50%	0,986	0,755	0,525	0,870	0,784
75%	0,945	1,178	0,210	0,512	0,711
100%	2,756	2,801	1,123	2,245	2,231
Control	0,540	0,320	0,255	0,350	0,366

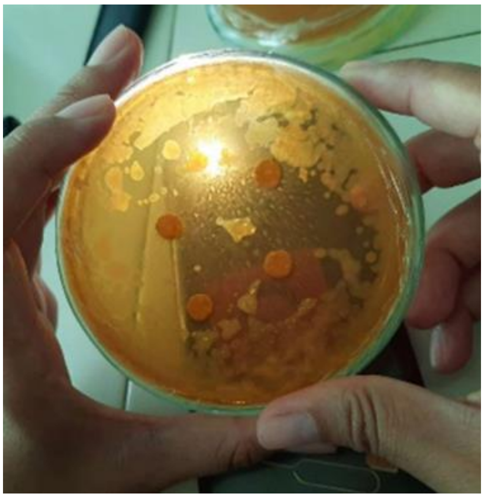

Figure 2. The Inhibition Zone Appearance

Statistical Analysis

The results of the statistical analysis are presented in Table 3. The significance value is 0.000, which is less than p (0.05), indicating that the null hypothesis is rejected. This means there are significant differences between the groups, specifically between the four treatments.

Table 3. One-Way Anova Analysis Result

ANOVA	**Sum of Squares**	**df**	**Mean Aquares**	**F**	**Sig**
Between Groups	6.821	4.000	1.705	10.912	000
Within Groups	2.344	15.00	156.0		
Total	9.165	19.00			

DISCUSSION

***Ananas comosus* Peels Extract Bioassay**

Ananas comosus or Pineapple, a perennial tropical fruit plant, is widely recognized globally and belongs to the Bromeliaceae family (Wali, 2019). While the health benefits of pineapple have been extensively researched, the peels are often discarded and have not been studied as thoroughly, particularly for their antibacterial properties. A previous study demonstrated that out of 11 methanol leaf extracts from various plants, *Punica granatum* and *Syzigium cumini* exhibited significant antibacterial activity against the predominant bacterial isolates *P. aeruginosa, S. aureus, K. pneumoniae*, and *E. coli* (Pallavali *et.al*, 2019). Another researcher discovered that the traditional antibacterial use of species native to Argentina, especially *L. meyenii* extract, proved to be the most effective, showing the lowest minimum inhibitory concentration (MIC) against both Gram-positive and Gram-negative bacteria (Chabán *et.al*, 2019). There is evidence suggesting that their effective components could be potential candidates for combating bacterial infections. Numerous researchers have identified that phytochemicals play a role in antibacterial mechanisms. Among these, polyphenols and terpenes were found to be the most active. The number and position of phenolic hydroxyl groups, double bonds, delocalized electrons, and conjugation with sugars in flavonoids appear to be crucial for their antimicrobial properties. It's essential to highlight that polyphenols exhibit significant chemical diversity and can be categorized into various families according to their chemical structures, such as flavonoids, lignans, tannins, and phenolic acids, among others (Álvarez-Martínez *et.al*, 2021). Additionally, saponins, which are natural nonionic surfactants, also possess a broad spectrum of antibacterial effects. Triterpenoid saponins like Sapindoside A (SA) and Sapindoside B (SB) have demonstrated synergistic antibacterial activity against *M. luteus* (Wei *et.al*, 2021).

The bioassay of *Ananas comosus* peel extract revealed that it contains phytochemicals such as flavonoids, tannins, and saponins, indicating its potential to combat bacterial infections. Recent research on the characteristics of dried pineapple (*Ananas comosus* L.) peel powder revealed that it contains fat, protein, crude fiber, and carbohydrates. Additionally, pineapple peel powder has phenolic content, flavonoid content, and antioxidant capacities. HPLC analysis identified catechin, vanillic acid, and sinapic acid as the top three most notable phenolic compounds, succeeded by ferulic acid, rutin, caffeic acid, quercetin, gallic acid, and coumaric acid. (Mala *et.al*, 2024).

Antibacterial Activity

Microorganisms play a major role in food spoilage, and certain pathogenic bacteria in the environment can pose serious health and safety risks to humans. Currently, there is substantial evidence indicating a rise in bacterial resistance, particularly with an increase in multidrug-resistant strains. To address the emergence of these resistant bacteria or multidrug-resistant strains, there is an urgent need for research and the development of innovative antibacterial medications. Natural sources are known to possess various chemical properties that can be utilized in antibacterial treatments. This study revealed that *Ananas comosus* peel extract has the potential to combat bacteria, specifically *E. coli*. A recent study has revealed that waste peels of *Ananas comosus* have antibacterial properties against microbial organisms such as *S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa*, and *V. cholera*. These properties are attributed to the carbon quantum dots (CQDs) derived from Ananas comosus agro-waste through a simple hydrothermal treatment process (Surendran *et.al.*, 2021). According to Table 2, the highest concentration of *Ananas comosus* peel extract produced the largest inhibition zone. This indicates that higher concentrations of the extract are more effective at combating bacterial resistance. Statistical analysis has conclusively demonstrated significant differences between the treatment groups as shown in Table 3.

It has been found that *Ananas comosus* peel extract contains phytochemicals such as flavonoids, tannins, and saponins, which may play a role in antibacterial mechanisms. According to Álvarez-Martínez *et.al* (2021), the interaction of polyphenols, such as flavonoids, with bacterial plasma membranes can produce a range of effects that enhance their antibacterial properties. Extensive evidence indicates that plant extracts and polyphenols can disrupt the bacterial plasma membrane's structure, leading to pore formation, leakage, changes in electrical charge and polarity, increased permeability, alterations in fluidity, delocalization of membrane proteins, and other actions that contribute to their antibacterial activity. Tannin exhibits antibacterial qualities, as indicated by research findings. Metabolomic studies shed light on how tannin works against bacteria, involving processes such as osmotic regulation, pH control within cells, amino acid synthesis and metabolism, glycolysis, the TCA cycle, and iron metabolism. These findings suggest that tannin's effects are diverse, potentially causing damage to cell membranes, restricting amino acids, disrupting energy metabolism, and depriving the iron on *Staphylococcus aureus* metabolism (Liu *et.al*, 2020). Meanwhile, saponins have been reported to cause severe damage to several bacteria by degrading the cell wall and disrupting the cytoplasmic membrane and membrane proteins, leading to leakage of the cell contents (Dong *et.al*, 2020). The antibacterial mechanism of *Ananas comosus* peel extract may be similar to those of flavonoids, tannins, and saponins. However, further studies are necessary to gain a better understanding of the antibacterial properties of *Ananas comosus* peel extract.

CONCLUSION/RECOMMENDATION

This study has verified the presence of phytochemicals, including flavonoids, tannins, and saponins, in *Ananas comosus* peel extract, suggesting their potential involvement in antibacterial mechanisms against *E. coli*. Furthermore, given that *Ananas comosus* peel extract is typically discarded as waste, the development of this innovative technique for bacterial removal could transform this waste into a valuable medicinal resource. However, further research is necessary to analyze the metabolomics of the active compounds within *Ananas comosus* peel extract, thereby enhancing our understanding of their mechanisms against *E. coli* and other bacteria.

REFERENCES

1. Álvarez-Martínez, F.Z., Barrajón-Catalán, E., Herranz-López, M., and Micol, V. (2021). Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action. Phytomedicine. 90. 1-16. https://doi.org/10.1016/j.phymed.2021.153626.
2. Ali, S., Khan, M.R., Irfanullah, Sajid, M., Zahra, Z. (2018). Phytochemical investigation and antimicrobial appraisal of Parrotiopsis jacquemontiana (Decne) Rehder. BMC Complement Altern Med. 18(1). 1-15. doi: 10.1186/s12906-018-2114-z.
3. Alizade, H., Hosseini Teshnizi, S., Azad, M., Shojae, S., Gouklani, H., Davoodian, P., and Ghanbarpour, R. (2019). An overview of diarrheagenic Escherichia coli in Iran: A systematic review and meta-analysis. Journal of Research in Medical Sciences 24(1). 1-14. DOI: 10.4103/jrms.JRMS_256_18.
4. Chabán, M.F., Karagianni, C., Joray, M.B., Toumpa, D., Sola, C., Crespo, M.I., Palacios, S.M., Athanassopoulos, C.M., and Carpinella, M.C. (2019). Antibacterial effects of extracts obtained from plants of Argentina: Bioguided isolation of compounds from the anti-infectious medicinal plant Lepechinia meyenii. Journal of Ethnopharmacology. 239. 1-9. https://doi.org/10.1016/j.jep.2019.111930.
5. Chervy M, Barnich N., and Denizot J. (2020). Adherent-Invasive E. coli: Update on the Lifestyle of a Troublemaker in Crohn's Disease. International Journal of Molecular Sciences 21(10). 1-34. https://doi.org/10.3390/ijms21103734.

6. Dong, S., Yang, X., Zhao, L., Zhang, F., Hou, Z., and Xue, P. (2020). Antibacterial activity and mechanism of action saponins from Chenopodium quinoa Willd. husks against foodborne pathogenic bacteria. Industrial Crops and Products. 149. 1-14. https://doi.org/10.1016/j.indcrop.2020.112350.
7. Elvers, K.T., Wilson, V.J., Hammond, A., Duncan, L., Huntley, A.L., Hay, A.D., Van der Werf, E.T. (2020). Antibiotic-induced changes in the human gut microbiota for the most commonly prescribed antibiotics in primary care in the UK: a systematic review. BMJ Open 10 (e035677). 1-24. doi: 10.1136/bmjopen-2019-035677.
8. Fleckenstein, J.M., and Kuhlmann, F.M. (2019). Enterotoxigenic Escherichia coli Infections. Current Infectious Disease Reports 21 (9). 1-9. https://doi.org/10.1007/s11908-019-0665-x.
9. Government Regulation No. 22 of 2021. Implementation of Environmental Protection and Management. Indonesia.
10. Hudzicki, J. (2016). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. American Society for Microbiology. New York.
11. Kancherla, N., Dhakshinamoothi, A., Chitra, K., and Komaram, R.B. (2019). Preliminary Analysis of Phytoconstituents and Evaluation of Anthelminthic Property of Cayratia auriculata (In Vitro). Maedica (Bucur). 14(4). 350-356. doi: 10.26574/maedica.2019.14.4.350.
12. Kasote, D.M., Sharbidre, A.A., Kalyani, D.C., Nandre, V.S., Lee, J.H.J., Ahmad, A., and Telke, A.A. (2023). Propolis: A Natural Antibiotic to Combat Multidrug-Resistant Bacteria. In: Wani, M.Y., Ahmad, A. (eds) Non-traditional Approaches to Combat Antimicrobial Drug Resistance. Springer, Singapore. https://doi.org/10.1007/978-981-19-9167-7_12.
13. Liu, M., Feng, M., Yang, K., Cao, Y., Zhang, J., Xu, J., Hernández, S.H., Wei, X,, and Fan, M. (2020). Transcriptomic and metabolomic analyses reveal antibacterial mechanism of astringent persimmon tannin against Methicillin-resistant Staphylococcus aureus isolated from pork. Food Chem. 30 (309). 1-8. doi: 10.1016/j.foodchem.2019.125692.
14. Mala, T., Piayura, S., and Itthivadhanapong, P. (2024). Characterization of dried pineapple (Ananas comosus L.) peel powder and its application as a novel functional food ingredient in cracker products. Future Foods. 9. 1-11. https://doi.org/10.1016/j.fufo.2024.100322.
15. Mazumder, D., Mittal, R., and Nath, S.K. (2024). Green synthesis of silver nanoparticles from waste Vigna mungo plant and evaluation of its antioxidant and antibacterial activity. Biomass Conversion and Biorefinery. 1-12. https://doi.org/10.1007/s13399-024-05375-x.
16. Minister of Health Regulation No. 2 of 2023. Implementation Guideline for Government Regulation No. 66 of 2014 concerning Environmental Health. Indonesia.
17. Murray, J.M. (2017). One-Way Analysis of Variance (ANOVA). University of Wisconsin-La Crosse.
18. Nurcholis, J., Saturu, B., Syaifuddin, and Buhaerah. (2020). Aplikasi pupuk organik cair limbah kulit nenas terhadap pertumbuhan tanaman kacang panjang. Jurnal Agrisistem. 16 (2). 1-8.
19. Pallavali, R.R., Avula, S., Degati, V.L., Penubala, M., Damu, A.G., and Durbaka, V.R.P. (2019). Data of antibacterial activity of plant leaves crude extract on bacterial isolates of wound infections. Data in Brief. 24. 1-6. https://doi.org/10.1016/j.dib.2019.103896.
20. Pepper, I.L., Gerba, C.P., and Gentry, T.J. (2015). Environmental Microbiology, Third Edition. Academic Press. United Stated. 152-153. https://doi.org/10.1016/C2011-0-05029-9.
21. Rinanda, T., Riani, C., Artarini, A., and Sasongko, L. (2023). Correlation between gut microbiota composition, enteric infections and linear growth impairment: a case–control study in childhood stunting in Pidie, Aceh, Indonesia. Gut Pathog 15 (54). 1-14. https://doi.org/10.1186/s13099-023-00581-w.
22. Surendran, P., Lakshmanan, A., Priya, S.S., Geetha, P., Rameshkumar, P., Kannan, K., Hegde, T.A., and Vinitha, G. (2021). Fluorescent carbon quantum dots from Ananas comosus waste peels: A promising material for NLO behaviour, antibacterial, and antioxidant activities. Inorganic Chemistry Communications. 124. 1-9. https://doi.org/10.1016/j.inoche.2020.108397.
23. Tanır Basaranoğlu, S., Karaaslan, A., Salı, E., Çiftçi, E., Aydın, Z.G.G., Kocabaş, B.A., Kaya, C., Bayturan, S.S., Kara, S.S., Çiftdoğan, D.Y., Çay, U., Aktürk, H.G., Çelik, M., Ozdemir, H., Somer, A., Diri, T., Yazar, A.S., Sütçü, M., Tezer, H., Oncel, E.K., Kara, M., Çelebi, S., Parlakay, A.O., Karakaşlılar, S., Arısoy, E.S., Tanır, G., Kara, T.T., Devrim, I., Erat, T., Aykaç, K., Kaba, O., Güven, S., Yeşil, E., Yılmaz, A.T., Durmuş, S.Y., Çağlar, I., Günay, F., Özen, M., Dinleyici, E.C., and Kara, A. (2023). Antibiotic associated diarrhea in outpatient pediatric antibiotic therapy. BMC Pediatr 23 (121). 1-9. https://doi.org/10.1186/s12887-023-03939-w.
24. Wali, N. (2019). Chapter 3.34 - Pineapple (Ananas comosus), Editor(s): Seyed Mohammad Nabavi, Ana Sanches Silva. Nonvitamin and Nonmineral Nutritional Supplements. Academic Press. United States. 367-373. https://doi.org/10.1016/B978-0-12-812491-8.00050-3.

25. Wei, M., Yu, H, Guo, Y., Cheng Y., Xie, Y., and Yao, W. (2021). Antibacterial activity of Sapindus saponins against microorganisms related to food hygiene and the synergistic action mode of Sapindoside A and B against Micrococcus luteus in vitro. Food Control. 130. 1-9. https://doi.org/10.1016/j.foodcont.2021.108337.
26. Zimmermann, Z., and Curtis, N. (2019). The effect of antibiotics on the composition of the intestinal microbiota - a systematic review. Journal of Infection 79 (6). 471-489. https://doi.org/10.1016/j.jinf.2019.10.008.