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Antimicrobial Activities of Pineapple Peel (*Ananas comosus*) Extract on Selected Microbes

RESEARCH ARTICLE

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ABSTRACT

Pineapples (*Ananas comosus)* have exceptional juiciness and a vibrant tropical flavor that balances the tastes of sweet and tart. It has a wide variety of health benefits. The aim of the study is to evaluate the antibacterial activity of pineapple peel extract on against selected bacterial pathogens. The antimicrobial activities of the peel extract were determined using agar well diffusion method and broth dilution technique which include Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The antimicrobial activity was tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*. They produced different sized zones of inhibition against the growth of organisms of various concentrations 12.5mg/ml, 25mg/ml, 50mg/ml and 100mg/ml respectively. The zone of inhibition exhibited by methanolic extracted against the tested organisms ranges from 3.3mm to 15 mm. *Salmonella typhi* showed significant difference ($p \leq 0.05$) than *Staphylococcus aureus* and *Pseudomonas aeruginosa* that has no significant difference. The chloramphenol standard antibiotic show highest grand inhibitory between 22.3mm to 26.00mm with significant difference ($p \leq 0.05$). The Minimum Inhibitory Concentration (MIC) values were 25mg/ml, 50mg/ml and 12.5mg/ml for *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* respectively. While the Minimum Bactericidal Concentration (MBC) was at 100mg/ml and 12.5mg/ml for *Staphylococcus aureus* and *Salmonella typhi* respectively while *Pseudomonas aeruginosa* did not show bactericidal effect. The antibacterial activity of the peel extract showed a greater inhibition at 25mg/ml, 50mg/ml and 12.5mg/ml for *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* respectively this is dependent on the dosage. The data obtained shows that the pineapple peel contains potential antimicrobial component that may be of great use for the development of pharmaceutical industries as a therapy against various diseases and drinking the juice or its peel and eating can help hydrate the body and restore the immune system.

Keywords: Antimicrobial activities, Pineapplepeel Extract, Microbes

INTRODUCTION

The increase in antibiotic resistant bacteria is larger due to the generalized use of antibiotics in medicine, in animal care, in agriculture. Due to the lack of antibiotics to attack the resistant bacteria, researchers and scientist have been initiated in the search of new antimicrobial agents in order to counter the resistant bacteria [1].

There is a great demand of fruit juices in treatment of various illnesses [2]. Extraction of bioactive molecules from medicinal plant facilitates pharmacological studies which lead to a synthesis of a more potent drug with a reduced toxicity [3-4]. Plant based extract can be extracted from any parts of plants like barks, leaves, fruits, seeds and fruit rinds etc [5].

Pineapple is a favourite of the lovers of fruits in its fresh form, sweetness as well as in its preserve like jams and jellies. It is the 3rd most import fruit crop in the tropical region of the world after banana and citrus fruit in terms of production [6].

Pineapple (*Ananas comosus*), a tropical plant with enable multiple fruit consisting coalesced berried named for resemblance to the pine cone, is the most economically important plant in the *Bromeliaceae* family [7]. The pineapple is a rich source of manganese, which helps build and maintain bone strength and it also has plenty of vitamin C. It also contains bromelain, an enzyme extract, studies has shown that the enzyme found in pineapples can reduce swelling, bruising, healing time and pain associated with injury and surgical intervention [8].

The word pineapple in English was first recorded to describe the reproductive organs of conifer trees (now termed pine cones). When the European explorers encountered this tropical fruit in the Americas during the 1400s, they called them "Pineapples".

In 1496, along with tame parrots, tomatoes, tobacco and pumpkins, Christopher Columbus brought back a load of pineapples from the New World. Fortunately, at least one of them didn't rot and was given to the Spanish King Ferdinand II of Argon. Peter Martyr, tutor to the Spanish prices, said king tasted the pineapple and declared that "its flavour excels all other fruits". By the middle of the seventeenth century, depictions of the pineapple often showed it with crown around the top, enhancing its symbolism with royalty and the realm of God. Eventually Charles II of England gained access to pineapples via England colonies in the West Indies. Feeling very proud of his accomplishment in international trade, the kind adopted the pineapple as his primary status symbol, nevertheless, pineapple couldn't be grown in northern clime – it was simple too cold there.

In the scientific binomial *Ananas comosus*, *ananas*, the original name of the fruit, comes from the tupi word nanas, meaning "excellent fruit" [9].

The Netherlands constructed the first green house in 1658, beginning what could be called the pineapple wars in Europe. Unfortunately, growing pineapples in green house was an expensive, labour intensive endeavour. In the world, China is the main producers of pineapple supplying 52% of the total output. Other important producers include India, Nigeria, Kenya, Indonesia, Mexico and Costa Rico. Production of pineapple has grown since 1960 with a wide range. Even though Nigeria is recognized as one of the main producers of the pineapple in the world, she is the number 1 producer in Africa, followed by South Africa. Currently Nigeria is presently the 8th largest producers of the fruit in the world with a production capacity of 1,052,000 metric tons. Almost all consumed locally; the export market is yet to be exploited.

Bromelain is a non toxic substance notable for its effect in the reduction of inflammation [10,11,], but scope of its benefits is increasing. It is also used for hay fever, treating of bowel condition that include swelling and ulcers, removing dead and damage tissue after a burn, improving the absorption of antibiotics, preventing cancer, shortening labour and helping the body get rid of fat. Bromelain is a mixture of Thiopepdidases such Asananain and Comosain, Phosphatases, Glucosidases, Peroxidises, Cellulose, Glycoprotein's, Proteinase inhibitors such as cystatin [12]. Owing to the fact that pineapples (*Ananas comosus)* have exceptional juiciness and a vibrant tropical flavor that balances the tastes of sweet and tart as well as its wide variety of health benefits, this study is aimed at evaluating antibacterial activity of pineapple peel extract on selected bacterial pathogens.

EXPERIMENTAL PROCEDURES

Study area

The study was carried out in the microbiology, Department of Biological sciences, Benue State University, Makurdi. Makurdi lies between the latitude 07° 15-07° 45N, longitude 08° 15-08° 40E. The town lies in the guinea savannah vegetative belt and the bank of second largest river in Nigeria. The river divides the town into two North and South bank and the town lower an area of 16km². The river constitute the main source of the water supply for the inhabitants of the town, the climate condition of Makurdi is influence by two air masses. The warm, most south westerly air mass is a rain bearing wind brings about rainfall from the month of May to October; the dry North-easterly air mass blows over the region from November to April. Hereby, bringing about seasonal drought, the main annual rainfall in Makurdi is 1,290mm. Temperature in Makurdi is however generally high throughout the year, with February and March as the hottest month. Makurdi has a monthly temperature between 27°C to 38°C, The town like most other cities in the lower Benue valley is drain by the Benue river and its tributatories, other minor river that drain in Makurdi town in turn empty their water in the river Benue including river Idye, Genebe, Urudu, Kpege and Kereke due to the general low relief of Makurdi sizeable portion of the area is water logged and flogged during heavy rain and storm.

Bacteria strains

The test bacteria of the American Type Culture Collection (ATCC) were obtained on Friday by 12pm from the Bacteria section, Department of Microbiology & Biotechnology (MB & BT), National Institute for Pharmaceutical Research and Development (NIPRD), Idu Layout, Abuja. This include the strains of gram positive bacteria; *Staphylococcus aureus* ATCC 25923, and gram negative bacteria; *Pseudomonas aeruginosa* ATCC 29953 and *Salmonella typhi* ATCC 13311 . The stock culture was then stored at 4°C.

Sample collection

Ripened pineapples were bought from Wurukum market, Makurdi, Benue state. Pineapple was washed and then peeled off. They were then washed twice once with tap water and then with distilled water to remove dirt and soil particles. Peels were allowed to dry at room temperature at room temperature for a period of 24hrs, then it was modified and dried at 40°c in an oven for 24hrs.The dried pieces were grounded with ordinary grinder to powder [13]

Preparation of extract

The peel samples were prepared following [14] method of extraction. 40g grounded powder of pulp was dissolved in 400ml of methanol and allowed to soak for 72 hrs with shaking of the extracts in the intermediated time. After 3 days the extract was filtered by using standard filter paper what man number One (1). The collected extract was further separated by rotary evaporator at 40°C reduced temperature. Finally the crude extract was placed in a water bath to evaporate any remaining methanol by succination. It was then placed in a desiccator containing CaCl. The dried extracts were put in the refrigerator for further use. The dry weight of the extracts were obtained by allowing the solvent to evaporate and also used to determine concentration in mg/ml.

Antimicrobial activity

In preparation of media, Mueller Hinton Agar (MHA) powder was collected and prepared according to the manufacturer`s instruction. 13.68g MHA was weighed on analytical weighing balance and dissolved in 360ml of distilled water, shook and heated for 10 minutes and autoclaved at121°C for 15mins (15 PSI). After sterilization 20ml of the agar was dispensed into the Petri dish and allowed to gel.

Preparation of sample

0.5g of the sample was weighed on analytical weighing balance and dissolved in 5ml of methanol. The concentration of the sample gave 0.5g or 100mg as stock. Further dilution 1: 2 dilution of the stock solution to 50mg, 25mg and 12.5mg in sterile tubes.

Test Organism preparation

A wire loop of a 24hrs culture of each test organisms (*Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus*) was transferred onto 5ml of sterile broth of nutrient broth and sub cultured and incubated between 3hrs and less than 18hrs and then compared with 0.5 McFarland Standard to give the population of test organisms as 10^6colony forming unit (cfu/ml).

Preparation of McFarland standard

McFarland standards are used to standardize the approximate number of bacteria in a liquid suspension by comparing the turbidity of the test suspension with that of the McFarland standard. A McFarland standard is a chemical solution of barium chloride and sulfuric acid; the reaction between these two chemicals results in the production of a fine precipitate, barium sulfate. When shaken well, the turbidity of McFarld and standard is visually comparable to bacterial suspension of known concentration [16].

Procedures

1g of Barium chloride ($Bacl_2$) was weighed and dissolved in 99ml of distilled water to get 1% $Bacl_2$. 1ml of conc. Sulfuric acid (H_2SO_4) was dissolved in 99ml of distilled water to get 1% H_2SO_4 9.95ml of 1% H_2SO_4 was dissolved in 0.05ml (50 micro litres) of 1% $BaCl_2$; the solution was dissolved with a stirring rod to become turbid.

Agar dilution technique

Sterile nutrient agar plate were prepared and allowed to solidify. Standardized organism 0.1ml (approximately 5 drops) of the inoculum was introduced into the plates and sterile cotton swab was used to spread the inocula evenly on the surface of the agar. The plates were left on the bench for 1hour so that the inocular will diffuse into agar. A sterile cork borer of 5mm diameters was used to make 4 ditches on the plates. Varying concentrations of extracts; 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml was made and 0.5ml of the extract was dropped in each of the appropriately labelled plate and positive control of chloramphenicol and negative control of methanol of each organisms was done on a different plate labelled. The plates were triplicate and left on the bench for 20 minutes for the extract to diffuse into the agar and later incubated at 37°C for 24 – 48 hours. After incubation the zone of clearance around each well was measured using a metric ruler by taking measurement from the edge of the plate to the point where the growth of the organism started. The diameter of the zone of inhibition which represents antibacterial activity was measured.

Determination of the minimum inhibitory concentration (MIC) of the extract

The broth dilution method was used to determine MIC. Six test tubes was used, the 1st containing 5ml of nutrient broth the rest containing 2ml each of nutrient broth representing the Varying concentration of the of the extract to be used ranging from 3.125mg/ml to 100mg.ml. 0.5g of the extract is weighed into the 1st test tube and serial dilution is done to the last test tube. 0.1ml of standardized test organism of bacterial cells is introduced into the tubes. The tubes were incubated for 24hrs at 37°C. Controls were equally set up by using solvent and test organisms without the extract[17].

Determination of the minimum bactericidal concentration (MBC)

A sample of test tubes used in MIC determination which did not show any visible growth after the period of incubation when compared with the positive control is streak on Nutrient agar

plates. The lowest concentration of the extract indicating a bacterial effect after 24hour of incubating at 37°C was regard as the Minimum Bactericidal Concentration [17].

Phytochemical analysis

Alkaloids and sterols were determined by the method described by [18]. Saponin was determined by the method described by AOAC (2000). Flavonoid was determined by the method described by [18]. Ethanol extract of the sample was be used for the test. The dried sample were soaked in the solvent overnight and filtered before heading to one quarter volume of flask [17].

Test for alkaloids

The extract (1.0g) was shaken with 5.0ml of 2% HCL on a steam bath and filtered. To 1ml of filtrate. Hagers reagent (Iodine in potassium iodide solution) was added. The formation of orange red precipate confirms that it's presence.

Test for saponin

(Frosting test) 1g of the extract was diluted in 1ml of water and shaken vigorously. Persistence foam indicates the presence of saponin.

Test for tannins

(Ferric chloride test) 1g of the extract was added ot 2ml of 1%HCL. Deposition of red precipitate shows the presence of tannin.

Test for steroid

The extract 1g was dissolved in 2ml of chlorofoam in a test tube and the 1ml of concentrated sulfuric acid was added. Formations of reddish brown color at the interphase indicate the presence of steroid.

Test for phenol

(Ferric chloride test) the extract 1g was added with 1ml of 10% ferric chloride. The formation of a greenish brown indicates the presence of phenols.

Test for flavonoid

The extract 1g was diluted in 1ml of diluted NaOH. Formation of yellow precipitate indicates the presence of flavonoid.

Test for steroids

0.30 g of the extract and add 2ml of acetic anhydride and the 2ml of concentrated of sulphuric acid. A blue or green colour change indicates the presence of steroids.

Test for glycosides

1g of the extracts and add 3ml of chlorofoam and shake vigorously and 3ml of 10% ammonia solution. The formation of a pink colour indicates the presense of glycosides.

RESULTS AND DISCUSSION

The result of this work as shown in this chapter is as of regards to the phytochemical analysis, the antibacterial screening of the fruit peel extracts on the test organisms, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). Table 1 shows the alkaloids, flavonoids and saponin had the highest abundance as compared to tannin, phenol, glycoside, terpenoids and steroids are presense at different interference.The presence of this phytochemicals confirmed the medical properties of the pineapple peel.

There was an increase in the zone of inhibition after a period of 48hrs (two days) ranging from 3.3mm to 15 mm. *Staphylococcus aureus* and *Pseudomonas aeruginosa* has a wide zone of inhibition at 50mg/ml concentration while *Salmonella typhi* has at 100mg/ml concentration. This

is observed in Table 2 and 3. The comparative mean was done against the various concentrations with the positive control. The chloramphenicol has the greatest zone of inhibition over methanol extract of peel. The result of the MIC clarity or growth (turbidity) was observed as compared to the positive control using broth dilution method showed a significant MIC among the organisms with *Staphylococcus aureus* at 25mg/ml, *Pseudomonas aeruginosa* at 50mg/ml and *Salmonella typhi* at 12.5mg/ml while the MBC result was taken after culturing for 24hrs from the MIC tubes to observe the cidal of *Staphylococcus aureus* at 100mg/ml and *Salmonella typhi* at 12.5mg/ml as shown in Table 5, 6 and 7.

Table 1: The qualitative test on phytochemical properties of the pineapple peel:
Akaloids, flavonoids and saponin have the highest abundance of the phytochemical properties

Phytochemical components	Relative abundance Methanol extract
Alkoloids	+++
Steroids	+
Glycoside	**+**
Flavonoids	**+++**
Tannins	**++**
Phenolic	**++**
Saponin	**+++**
Terpenoids	**++**

Keys: - Absent
+ Present in trace amount
++ Moderately present
+++ Present in Abundance

Table 2: Mean inhibitory effect of methanolic extract on test organisms

Here there is a zone of inhibition on the various test organisms as the within 48hrs. *Staphylococcus aureus* at 25mg/ml and 50mg/ml, *Pseudomonas aeruginosa* at12.5mg/ml and 50mg/ml while *Salmonella typhi* at 50mg/ml and 100mg/ml.

	Zone of inhibition (mm)					
Concentration of peel extract(mg/ml)	***Staphylococcus aureus***		***Pseudomonas aeruginosa***		***Salmonella typhi***	
	Day 1	**Day 2**	**Day 1**	**Day 2**	**Day 1**	**Day 2**
12.5	5.00	3.67	8.70	3.00	2.33	1.67
25.0	8.30	6.67	6.30	2.00	2.67	0.67
50.0	8.30	6.67	10.30	2.67	8.67	4.00
100.0	5.30	4.33	3.00	0.67	9.33	5.33
LSD(P≤0.05)	6.52NS	5.52NS	7.17NS	2.43NS	5.90	3.65NS

Foot note: means tagged with alphabets are not significant.

NS = No significant difference.

Table 3: Overall mean inhibitory effect of methanolic extract on test organisms

Inhibitory effect of *Staphylococcus aureus* is at 25mg/ml and 50mg/ml, while *Pseudomonas aeruginosa* at 12.5mg/ml and 50mg/ml and *Salmonella typhi* at 50mg/ml and 100mg/ml.

	Zone of inhibition (mm)		
Concentration of peel extract (mg/ml)	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Salmonella typhi***
12.5	8.70	11.70	4.00
25.0	15.00	8.30	3.30
50.0	15.00	13.00	12.70
100.0	9.70	3.70	14.70
LSD(P≤0.05)	11.59NS	9.13NS	8.68

Foot note: means tagged with alphabets are not significant.

NS = No significant difference.

Table 4: Comparative mean inhibitory effect of methanolic extract and chloramphenicol on the three test organisms

Here Chloramphenicol show a inhibitory effect on the test organisms as compared to the peel extracts on the organisms

Concentration of peel extract (mg/ml)	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Salmonella typhi*
12.5	8.70	11.70	4.00
25.0	15.00	8.30	3.30
50.0	15.00	13.00	12.70
100.0	9.70	3.70	14.70
Chloramphenicol	22.30	22.30	26.00
LSD(P≤0.05)	10.23NS	8.15	7.55

Foot note: means tagged with alphabets are not significant.

NS = No significant difference.

Table 5: The Minimum Inhibitory Concentrations

		ORGANISMS		
Concentration	**Dilution factor**	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Salmonella typhi***
100mg/ml	10^1	Clear	Clear	Clear
50mg/ml	10^2	Clear	Clear	Clear
25mg/ml	10^3	Clear	Turbid	Clear
12.5mg/ml	10^4	Turbid	Turbid	Clear
6.125mg/ml	10^5	Turbid	Turbid	Turbid
3.125mg/ml	10^6	Turbid	Turbid	Turbid
Positive control	-	Turbid	Turbid	Turbid
Negative control	-	Nil	Nil	Nil

Table 6: The minimum inhibitory concentration (MIC) of test organisms

Test organism	Minimum Inhibitory Concentration
Staphylococcus aureus	25mg/ml
Pseudomonas aeroginosa	50mg/ml
Salmonella typhi	12.5mg/ml

Table 7: The minimum bactericidal concentration (MBC) of test organisms

Test organisms	Minimum bactericidal concentration
Staphylococcus aureus	100mg/ml
Pseudomonas aeruginosa	----
Salmonella typhi	12.5mg/ml

Phytochemical also known as phytonutrients are naturally occurring secondary metabolites found in plant life which act as antioxidant, anti-inflammatory and antimicrobial agents. They play a vital role in detoxification of damaging and deleterious chemicals from the body [19]. From the result it shows that alkoloids, saponin and flavonoids are the abundance as compared to tannins. Phenolic and terpenoids which are moderately abundant, Steroids and glycoside were also present. The presence of these phytochemical shows the antioxidant, anti-inflammatory and antimicrobial activity of the pineapple peel extract. This result with regards to the phytochemical properties using methanolic extraction is in agreement with [20] which showed presence of the phytonutrients using methanolic as a solvent for extraction. It's been reported that the peel itself contain a lot nutritional value that strengthens bones, the fertility of man, reduce blood pressure, supply high magnesium and calcium to the body. Flavonoid plays a crucial role in the antimicrobial activity of pineapple peel. The methanol used as a solvent for extraction of the peel showed an inhibitory effect toward the three test organisms; *Staphylococcus aureus* (Gram positive), *Pseudomonas aeruginosa* and *Salmonella typhi* (both gram negative). All the organisms were observed within two days incubation period (24 - 48hrs), it showed the organism was still inhibited after 24hrs leading to a wider zone of inhibition. Using agar well diffusion technique it showed that at 25mg/ml and 50mg/ml *Staphylococcus aureus* gave a zone of inhibition of 15mm which was its highest with no significant difference, while *Pseudomonas aeruginosa* gave it highest zone of inhibition of 13mm at 50mg/ml, *Salmonella typhi* gave its highest zone of 14.7mm at 100mg/ml this findings agrees with [20] that evaluated the antimicrobial, antioxidant of the flavonoids isolated from the peels of the Ananas comosus using six various solvents; ethanol, methanol, n-hexane, ethyl acetate, chloroform and acetone to see its inhibitory effect on *Escherichia coli, Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillius amyloliquefaciens*. it was observed that *Escherichia coli* was inhibited by extracts made in ethanol, methanol and n-hexane, giving a maximum inhibition length of 11mm in methanolic extract as compared to extracts prepared in ethanol and n-hexane giving a inhibition of length of 10mm and 4mm respectively. *Bacillus amyloliquefaciens* was inhibited to grow by extracts of ethanol, methanol and n-hexane as compared by *Staphylococcus aureus* and *Pseudomonas aeruginosa* which was inhibited by only ethanolic and methanolic extracts. *Staphylococcus aureus* gave more inhibitory length in methanol extract (6mm) than ethanol extract (5mm). When compared with the standard antibiotics used in inhibiting the bacteria, the positive control (Chloramphenicol) gave a higher zone of 22.3mm, 22.3mm and 26mm for *Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi* respectively. There was a significant difference of ($p \leq 0.05$) between the peel extract and the positive control of *Pseudomonas aeruginosa* and *Salmonella typhi*. This also agrees with [13] gave a zone of inhibition of 9mm and 6mm respectively of the organisms.

The MIC and MBC of the samples against the test organisms were also determined using the broth dilution method. The MIC varied between 3.125mg.ml to 100mg/ml. From the analysis carried out for the MIC of the organisms were at 25mg/ml, 50mg/ml and 12.5mg.ml for *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* respectively. While the MBC is at 100mg/ml and 12.5mg/ml for both *Staphylococcus aureus* and *Salmonella typhi* respectively but no MBC for *Pseudomonas aeruginosa*. Studies showed that extract was effective against *Escherichia coli* with a zone of inhibition of 26mm at concentration 1000mg/ml. But with *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus* it was found to be 20mm, 22mm and 23mm respectively all inhibited at a concentration of 1000mg/ml this is due to the increase in the mg/ml showing a greater zone of inhibition as the dosage increases. Natural substances have demonstrated antibacterial action mainly because most plants used in

alternative medicines are composed of flavonoids which act on bacterial cells disrupting the cytoplasmic membrane and inhibiting the enzymatic activity. In this present study the extract used has significant antimicrobial action on the entire bacterial agent shows that the higher the concentration the higher the inhibition.

CONCLUSION

The data obtained showed that the fruit extract of *Ananas comosus* possess various beneficial properties. The extracts have the ability to inhibit the bacterial strains used during the research work, therefore the antimicrobial component and therapeutic properties serve as a potential for the development of antibiotics in the pharmaceutical industries. The peel itself has a huge nutritive value that can be modified to body supplements. If extract is screened and further purified it would yield a higher zone of inhibition than most standard antibiotics. The results of the study show that it can be used for the development new drugs and alleviation of some illness.

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