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Antimicrobial activities of modified and unmodified pectin obtained from peels of *Irvingia gabonensis, Cola milleni*, and *Theobroma cacao* on specific pathogenic organisms

Mariam Taiwo, Oloye-Akorede [1,*], Labunmi Lajide [1], Jamiu Mosebolatan, Jabar [2], Obafemi lamiji Arogundade [3], Solomon Temitope, Oyegoke [4], Izzah Opeyemi, Quadri [5] and Henry Obinwa, Emeka [1]

[1] *Department of Chemistry, University of Medical Sciences, Ondo-City, Ondo-State, Nigeria.*
[2] *Department of Chemistry, Federal University of Technology, Akure, Ondo-State, Nigeria.*
[3] *Department of Chemical Sciences, Crescent University, Abeokuta, Ogun State, Nigeria.*
[4] *Department of Quality Assurance Kroger, Dallas, Texas, United States.*
[5] *Department of Chemistry, University of Ilorin, Kwara State, Nigeria.*

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Abstract

The antibacterial and antifungal activities of ethanolic extracts of pectin derived from the peels of *Theobroma cacao*, *Cola milleni*, and *Irvingia gabonensis* were investigated against phytopathogenic bacteria and fungi. The agar diffusion method was employed to test the extracts against *Staphylococus aureus*, *Pseudomonas aeroginosa*, *Chromobacterium violaceum*, *Streptococcus feacalis, Xanthomonas axonopodis, Erwinia carotovora, Sclerotium rulfsii, Pythophthora palmivora, and Pyricularia oryzae*. Significant differences ($p<0.05$) were observed in all control groups. The extracts inoculated with Streptomycin exhibited the highest activity against all bacterial strains. Conversely, the unmodified extracts of pectin samples from cola, cocoa, and wild mango displayed the least inhibitory activity against the tested bacterial strains, while the modified extracts with Chloro Acetic Acid (CAA), Para Amino Benzoic Acid (PABA), and Para Nitro Benzoic Acid (PNBA) showed varying inhibitory effects. The control sample inoculated with Mancozeb demonstrated the highest zone of inhibition against all fungal strains in the three pectin samples. However, the modified pectin samples exhibited increased inhibitory effects compared to the unmodified pectin extract, although the results were closer to the control. The higher values of modified pectin can be attributed to the presence of modifying agents such as long-chain hydrocarbons, acetic acid, chloro compounds, carboxyl, benzene rings, amino groups, and nitro groups. The presence of secondary metabolites in the pectin samples may also contribute to the higher values observed in both modified and unmodified samples. Overall, the results indicate the fungicidal and bactericidal potential of the extracts against the tested organisms, suggesting their potential use as broad-spectrum antimicrobial agents.

Keywords: Antimicrobial; Pectin; Potato Dextrose Agar (P.D.A) Mancozeb; PABA; CAA and PNBA

1. Introduction

The geometric increase in industrial and population growth has contributed to continuous environmental pollution, with a significant concentration of heavy metals and emerging contaminants posing a threat to various areas of the environment [1]. Antimicrobial substances are widely used in human medicine, food, agriculture, livestock, and household products. The presence of antimicrobial and antifungal compounds in higher plants has been recognized as important for disease resistance, as these compounds exhibit selective toxicity and can effectively control certain plant diseases [8]. However, antimicrobial resistance (AMR) poses a significant challenge to preventing and treating infections caused by bacteria, viruses, and fungi [8]. Pectic polysaccharides, found in higher plants' primary cell walls

* Corresponding author: Oloye-Akorede MT

and middle lamella, play crucial roles in various physiological processes [3]. They act as agents binding cellulose fibrils in cell walls and can be covalently linked to other polymers [2]. Intracellular pectin serves as a channels for nutrient and water transport [15]. Pectin derived from apples, citrus fruits, sugar beet, and sunflower heads are rich sources of pectin and are widely used in the pectin industry [5]. Commercially extracted pectins are commonly used as food additives for their gelling and stabilizing properties in products such as jams, jellies, marmalades, milk, and confectionery items [14]. Pectins also exhibit various biological and physiological functions in human nutrition and health [20]. They can prevent the attachment of pathogens to the intestinal mucosa and are fermented by probiotic bacteria into short-chain fatty acids, which inhibit harmful processes in the colon [18], [2]. Additionally, certain pectins have immune-regulatory effects on the gastric mucosal immune system, stimulate lymphocyte and macrophage proliferation [19], and possess general anti-complementary activity [4].

Chemical modification of polysaccharides is the most important route to alter and enhance the properties of the naturally occurring biopolymers and using these renewable resources in the context of sustainable development will open up new qualities/features for more applications in the industries [13].

Cola *millenii,* found in southwestern Nigeria's deciduous, closed, and transitional forests, belongs to the *Sterculiaceae* family. Its branches bear edible fruits and are known by various names such as monkey kola in English, Obi-Edun in Yoruba (Nigeria), and mbautung-ita in Ibiobio Nigeria [17], [9]. The leaves, flowers, fruits, and bark of the plant have been traditionally used to treat dysentery, diarrhea, vomiting, and cough [9].

Irvingia gabonensis, a tree native to West Africa, produces fruit similar to a mango and is commonly used as food. Irvingia gabonensis seeds have medicinal properties and are employed for various purposes. The tree is also known as the African Mango, Ogbono, Bush Mango, and Dika Nut. Research suggests that daily intake of *Irvingia gabonensis* for 1-3 months can lower blood sugar levels and total cholesterol [7].

Theobroma cacao, also known as the cocoa tree, is a small evergreen tree in the *Malvaceae* family, growing up to 6-12 meters tall [16]. Cocoa beans, derived from their seeds, are used to produce chocolate liquor, cocoa solids, cocoa butter, and chocolate [16]. The tree's leaves are alternate, and unlobed, ranging from 10 to 50 cm in length and 5 to 10 cm in width. Flowers grow in clusters directly on the trunk and older branches, a phenomenon known as cauliflory. The fruit is ovoid, measuring 15-30 cm long and 8-10 cm wide, ripening to yellow or orange, and weighing approximately 500 g when mature. The pod contains 20 to 60 seeds, commonly referred to as "beans," embedded in a white pulp. Each seed contains a substantial amount of fat, known as cocoa butter (40-50%) [16].

The objective of this research is to evaluate the antimicrobial activities of ethanolic extracts of unmodified and modified pectin derived from the peels of *Theobroma cacao*, *Cola milleni,* and *Irvingia gabonensis* against various pathogens.

2. Material and methods

All chemicals used for the extraction were of analytical grade. Fresh samples of *C. milleni, T. cacao,* and *I. gabonensis* fruits were obtained from Idasen farm in Owo, Ondo State, Nigeria. The samples were authenticated at the Department of Biology, Federal University of Technology, Akure, Nigeria.

2.1. Methodology

2.1.1. Samples Preparation

To ensure cleanliness and prevent contamination, the freshly collected fruit samples were thoroughly washed with water. The peels of *Irvingia gabonensis*, pods of *Cola millenii*, and seeds of *Theobroma cacao* were carefully separated from the fruits using a sterile knife. Following the separation, the samples were air-dried for three days under controlled conditions of 30°C temperature and 45% relative humidity. To prepare them for further processing, the dried samples were ground individually using a Marlex electrical blender. After grinding, the resulting ground samples were stored in airtight containers to maintain their integrity until the extraction process [5].

2.1.2. Extraction of Pectin

The extraction of pectin from the samples was conducted using an acidic extraction method, following the modified procedures described by [5]. In 1000 mL beakers, 50 g of each sample (peels of *Irvingia gabonensis,* pods of *Cola millenii,* and seeds of *Theobroma cacao*) were placed. To each sample, 500 mL of acidified water (0.4% HNO_3) was added and stirred. The beakers were then placed in a water bath and heated at 100°C for 1 hour. After heating, the sample mixtures were filtered using a cheesecloth. Ethanol (98%) was added to the filtrate immediately before cooling, forming a pectin

precipitate. The precipitated pectins were separated by filtration and washed three times with ethanol. The isolated pectins were then dissolved in ethanol (98%), re-precipitated to ensure complete removal of impurities, and filtered. The filtered pectins were further washed with distilled water and oven-dried at 50°C. To ensure a constant weight, the pectins were dried in an oven for approximately 12 hours. Once dried, the pectin samples were cooled in a desiccator, broken up, ground, and sieved to obtain a fine powder. The dried pectin samples were stored in airtight containers at room temperature for analysis.

2.1.3. Chemical Modification of Pectin Samples

The pectin samples were subjected to chemical modification using a method based on Prafulla and Shanmugasundaram with slight modifications [13]. Ten grams (10 g) of each pectin sample were added to a flask containing 20 mL of a freshly prepared 50% v/v solution of the modifying chemicals in ethanol. The mixture was stirred for 60 minutes at a temperature of 50°C using a magnetic stirrer in a closed condition. Afterward, the modified product was recovered by filtration and washed repeatedly with distilled water, followed by two washes with 96% ethanol. The product was then dried in a hot air oven at 50°C. The dried product samples were powdered and passed through a sieve with a number 24 mesh size.

2.1.4. Determination of the antibacterial activity of the modified and unmodified samples

The antibacterial activity of both the modified and unmodified pectin samples was determined using the agar well diffusion method, based on [6] with modifications by [11]. Nutrient agar plates were prepared by dispensing 20 mL of nutrient agar into sterile universal bottles, which were then inoculated with 0.2 mL of bacterial culture. The mixture was gently mixed and poured into sterile Petri dishes. Using a sterile number 3-cup borer (6 mm diameter), three to five uniform wells were created in the agar plates. Each well was filled with 0.5 mL of the pectin sample and allowed to diffuse for 45 minutes. The plates were incubated at 37°C for 24 hours, and the zones of inhibition were measured using Digital Vernier Calipers. The experiment was performed in triplicates.

2.1.5. Determination of the antifungal activity of the modified and unmodified pectin samples

For the determination of antifungal activity, the poisoned food technique was employed. The fungi were grown aerobically at 27°C on potato dextrose agar (PDA) plates. Five milliliters (5 mL) of each pectin sample was mixed separately with 20 mL of PDA before being poured into plates and allowed to solidify at room temperature (27°C). A disc cut from a 7-day-old culture of the test fungi was inoculated in the center of the PDA plate containing the pectin sample. Separate control experiments with distilled water and a reference antifungal fungicide (mancozeb) were set up. Both plates were incubated aseptically at 27°C for 72-96 hours. The mycelial growth of the isolates was measured using digital Vernier Calipers, and the percentage of mycelial growth inhibition was calculated using the appropriate formula [6], [11].

$$\frac{dc-dt}{dt} x\ 100 \quad \text{..... (1)}$$

where

- dc = diameter of fungi colony in negative control sets
- dt = average diameter of fungi colony in the experimental plate

3. Results and discussion

3.1. Antibacterial Activities of Modified and Unmodified Pectin in Ethanol

Tables 1, 2, and 3 show the antibacterial activities of the tested pectin extracts on a panel of six bacteria. The Para Amino Benzoic acid-modified pectin samples obtained from cola and wild mango exhibited the highest level of inhibition against *Staphylococcus aureus*, whereas the Para Amino Benzoic acid-modified pectin sample from cocoa demonstrated the most potent inhibitory effect on *Staphylococcus aureus*. Treatment of the wild mango pectin sample with Para Amino Benzoic Acid resulted in the highest inhibitory activity against *Pseudomonas aeruginosa*, while the Para Amino Benzoic Acid modified pectin samples from cola and cocoa exhibited the greatest antibacterial activity against *Pseudomonas aeruginosa.*

Table 1 Antibacterial Activities (%) of *Cola milleni* Pod Pectin

Bacteria	Unmodified	Modifications			Control
		CAA	PABA	PNBA	
A	$12.50^{b}\pm2.31$	$15.20^{b}\pm3.41$	$19.10^{a}\pm1.23$	$18.30^{a}\pm4.35$	$22.60^{c}\pm3.22$
B	$13.70^{b}\pm3.42$	$16.20^{a}\pm2.13$	$18.20^{a}\pm4.32$	$19.00^{a}\pm4.44$	$25.50^{a}\pm3.12$
C	$16.70^{a}\pm2.34$	$16.90^{a}\pm4.12$	$17.50^{b}\pm3.24$	$18.30^{a}\pm2.34$	$23.00^{b}\pm4.34$
D	$12.10^{b}\pm1.23$	$14.00^{b}\pm1.23$	$15.30^{c}\pm4.32$	$16.40^{b}\pm3.42$	$25.80^{a}\pm2.34$
E	$12.40^{b}\pm1.42$	$13.50^{c}\pm2.13$	$15.50^{c}\pm4.44$	$15.00^{b}\pm3.33$	$24.20^{b}\pm3.12$
F	$11.40^{c}\pm1.43$	$12.90^{c}\pm3.42$	$14.00^{d}\pm2.45$	$13.10^{c}\pm2.33$	$22.80^{c}\pm2.22$

Values are Mean ± Standard deviation of triplicate readings

Table 2 Antibacterial Activities (%) of Cocoa Pod Pectin

Bacteria	Unmodified	Modification			Control
		CAA	PABA	PNBA	
A	$6.29^{a}\pm1.22$	$10.30^{b}\pm1.00$	$15.30^{c}\pm2.31$	$15.50^{c}\pm2.31$	$22.60^{d}\pm0.00$
B	$8.33^{a}\pm2.31$	$10.20^{b}\pm1.11$	$11.30^{b}\pm0.98$	$12.10^{b}\pm1.25$	$25.50^{c}\pm1.55$
C	$3.74^{a}\pm0.23$	$09.00^{b}\pm1.23$	$10.10^{b}\pm3.11$	$10.20^{b}\pm2.22$	$23.00^{c}\pm5.02$
D	$3.28^{a}\pm0.00$	$07.20^{b}\pm1.21$	$10.30^{c}\pm2.31$	$10.00^{c}\pm0.99$	$25.80^{d}\pm2.23$
E	$10.10^{a}\pm1.23$	$12.50^{b}\pm1.21$	$13.50^{b}\pm1.22$	$14.20^{b}\pm1.23$	$24.20^{c}\pm1.21$
F	$04.06^{a}\pm1.11$	$08.60^{b}\pm0.34$	$10.20^{c}\pm1.22$	$11.50^{c}\pm1.11$	$22.80^{d}\pm2.31$

Values are Mean ± Standard deviation of triplicate readings

Table 3 Antibacterial Activities (%) of Wild mango peel Pectin

Bacteria	Unmodified	Modifications			Control
		CAA	PABA	PNBA	
A	$14.30^{a}\pm1.23$	$15.00^{a}\pm2.55$	$17.50^{b}\pm2.32$	$17.20^{b}\pm3.41$	$20.60^{c}\pm1.21$
B	$17.40^{a}\pm1.22$	$18.30^{a}\pm1.67$	$20.30^{b}\pm1.23$	$20.40^{b}\pm2.09$	$25.50^{b}\pm3.49$
C	$19.00^{a}\pm2.31$	$20.20^{a}\pm1.89$	$22.40^{b}\pm0.34$	$22.90^{b}\pm2.09$	$23.00^{b}\pm3.19$
D	$19.10^{a}\pm2.33$	$21.00^{a}\pm2.78$	$21.40^{a}\pm2.31$	$20.90^{a}\pm1.73$	$25.80^{b}\pm4.09$
E	$13.50^{a}\pm2.41$	$15.50^{a}\pm1.66$	$18.30^{a}\pm2.31$	$19.50^{a}\pm1/82$	$24.20^{b}\pm4.05$
F	$16.50^{a}\pm2.33$	18.90b±0.23	$18.50^{b}\pm1.23$	$18.50^{b}\pm1.90$	$22.50^{c}\pm4.77$

Values are Mean + Standard deviation of triplicate readings A – *Staphylococus aureus, B – Pseudomonas aeroginosa, C – Chromobacterium violaceum D – Streptococcus feacalis, E – Xanthomonas axonopodis, F – Erwinia carotovora Control: Streptomycin*

The Para Amino Benzoic Acid modification of pectin samples from cola and cocoa showed the highest inhibitory activity against the *Chromobacterium violaceum* bacterial strain, whereas the Para Amino Benzoic Acid modification in wild mango displayed the most pronounced inhibitory activity. The Para Amino Benzoic Acid modified pectin sample from cola exhibited the highest inhibitory activity against *Streptococcus faecalis*, while the Para Amino Benzoic Acid modification in cocoa and wild mango resulted in the highest inhibition against *Streptococcus faecalis*. *In Xanthomonas axonopodis* tested strain, the Para Amino Benzoic Acid modification of pectin samples from cola and wild mango displayed the highest inhibitory activity, whereas the Para Amino Benzoic Acid modification in cocoa showed the highest inhibitory activity. Among the tested strains, the Para Amino Benzoic Acid modification of pectin samples from

cocoa and wild mango showed the highest inhibition against *Erwinia carotovora*, whereas the Para Amino Benzoic Acid modification of pectin samples from cola exhibited the highest antibacterial activity.

3.2. Antifungal Activities of Modified and Unmodified Pectin in Ethanol

Table 4 Antifungal Minimum Inhibitory Concentration (%) of *Cola milleni* Pod Pectin

Fungal	Unmodified	Modification			Control
		CAA	PABA	PNBA	
SR	$67.00^{a}\pm2.31$	$69.50^{a}\pm5.46$	$70.20^{a}\pm0.97$	$71.50^{a}\pm0.09$	$76.80^{b}\pm6.43$
PP	$61.30^{a}\pm2.47$	$66.80^{b}\pm3.45$	$66.50^{b}\pm2.34$	$66.50^{b}\pm2.67$	$67.40^{b}\pm5.44$
PO	$67.00^{a}\pm8.91$	$70.50^{a}\pm0.34$	$71.50^{a}\pm7.09$	$72.30^{b}\pm8.75$	$74.80^{b}\pm2.34$

Values are Mean ± Standard deviation of triplicate readings

Table 5 Antifungal Minimum Inhibitory Concentration (%) of Cocoa Pod Pectin

Fungal	Unmodified	Modification			Control
		CAA	PABA	PNBA	
SR	$59.30^{a}\pm2.34$	$65.20^{b}\pm7.81$	$64.10^{b}\pm6.78$	$69.70^{c}\pm4.59$	$76.80^{d}\pm9.04$
PP	$58.50^{a}\pm7.89$	$55.00^{a}\pm1.34$	$60.80^{a}\pm4.45$	$61.50^{a}\pm2.22$	$67.40^{b}\pm4.56$
PO	$55.50^{a}\pm2.31$	$61.30^{b}\pm4.53$	$65.50^{c}\pm2.32$	$70.30^{c}\pm3.32$	$74.80^{d}\pm7.89$

Values are Mean ± Standard deviation of triplicate readings

Table 6 Antifungal Minimum Inhibitory Concentration (%) of Wild Mango Peel Pectin

Fungal	Unmodified	Modification			Control
		CAA	PABA	PNBA	
SR	$59.10^{a}\pm3.45$	$62.50^{a}\pm2.31$	$65.80^{b}\pm6.89$	$66.70^{b}\pm4.72$	$76.80^{c}\pm9.22$
PP	$38.90^{a}\pm6.72$	$52.30^{b}\pm2.47$	$55.90^{b}\pm4.56$	$57.30^{b}\pm8.43$	$67.40^{c}\pm4.29$
PO	$58.20^{a}\pm6.78$	$64.80^{b}\pm2.31$	$60.30^{b}\pm7.92$	$64.80^{b}\pm8.78$	$74.80^{c}\pm5.72$

Values are Mean ± Standard deviation of triplicate readings SR – *Sclerotium rulfsii*, PP – Pythophthorapalmivora, PO – *Pyriculariaoryzae,* Control – Mancozeb

The results from Tables 4, 5, and 6 indicate the fungicidal potential of the extracts. The Para Amino Benzoic Acid modification of pectin extracts obtained from *cola milleni*, cocoa, and wild mango exhibited the highest inhibition against *Sclerohum rulfsii* [12]. Treatment of the pectin extract from *cola milleni*, modified with chloroacetic acid, resulted in the highest antifungal activity against *Phytophthora palmivora*. Additionally, the Para Amino Benzoic Acid modification of pectin extracts from cocoa pods and wild mango peels showed the highest inhibition against *Phytophthora palmivora*.

Both Chloro Acetic Acid and Para Amino Benzoic Acid modifications of the pectin extract from wild mango peels exhibited the same level of inhibition against *Pyricularia oryzae,* which was the highest observed. Furthermore, the highest inhibition against *Pyricularia oryzae* was observed in the Para Amino Benzoic Acid modification of pectin extracts from cocoa and cola. The antifungal activity against *Pyricularia oryzae and Phytophthora palmivora could* be attributed to the presence of reagents like ethanol, long-chain hydrocarbons, carboxyl esters, and other metabolites such as *simiarenol* (pentacyclic triterpene), one diterpene, and two phytosteroids [8], [12] .

4. Conclusion

The Streptomycin-inoculated extracts exhibited the highest activity against all tested bacterial strains. Conversely, the unmodified pectin extracts from cola, cocoa, and wild mango displayed the lowest inhibitory activity against the

bacterial strains. However, the modified extracts treated with Chloro Acetic Acid (CAA), Para Amino Benzoic Acid (PABA), and Para Nitro Benzoic Acid (PNBA) showed varying levels of inhibitory effects.

Regarding the Mancozeb-inoculated extracts, the control sample demonstrated the highest zone of inhibition against all tested fungal strains in the three pectin samples. However, the modified pectin samples exhibited greater inhibition compared to the unmodified pectin extracts, although the results were closer to the control. The increased inhibitory effects observed in the modified pectin samples can be attributed to the presence of compounds such as long-chain hydrocarbons, acetic acid, chloro compounds, carboxyl groups, benzene rings, amino groups, and nitro groups. Additionally, the presence of phytochemicals inherent in the three pectin samples may contribute to the higher values observed in both modified and unmodified samples. These results suggest the potential fungicidal and bactericidal properties of the extracts against the tested organisms, indicating their potential use as broad-spectrum antimicrobial agents.

Compliance with ethical standards

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Disclosure of conflict of interest

No competing interests were declared.

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Authors' contributions

Oloye-Akorede, M. T., Lajide L., and Jabar, J. M. designed and conducted the research. Obinwa, H. E. and Quadri, I. O. helped with sampling research materials. Arogundade, O.L. provided the necessary reagents for the research, and Oyegoke, S.T. did the data analysis. All authors proofread and approved the manuscript.

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