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Phytochemical Compositions and Antimicrobial Activities of *Ananas comosus* Peel (M.) and *Cocos nucifera* Kernel (L.) on Selected Food Borne Pathogens

Dabesor A. P., Asowata-Ayodele A. M.*, Umoiette P.

Department of Biological Sciences, Wesley University, Ondo, Nigeria

Email address:
mojiayodele83@yahoo.com (Asowata-Ayodele A. M.)
*Corresponding author

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Abstract: Plants like pineapple and coconut are able to exhibit inhibitory effect against the growth of most food borne pathogens,due to the presence of various secondary metabolites. The aim of this study is to investigate the phytochemical constituents and antimicrobial activities of aqueous and ethanolic extracts of pineapple (*Ananas comosus*) peel and coconut (*Cocos nucifera*) kernel on some selected food borne pathogens. Antimicrobial analysis of aqueous and ethanolic extracts prepared from coconut kernel and pineapple peel were done by using the agar well diffusion method against the selected food borne pathogens. The ethanolic kernel extracts of coconut showed a remarkable inhibition zone against *Bacillus cereus* (18.0±0.13mm) followed by *Klebsiella pneumonia* (15.0±0.18mm) and *Staphylococcus aureus* (12.0±0.4mm). Whereas, the aqueous kernel extracts of coconut showed maximum inhibition zone against *B. cereus* (16.0±0.6mm) followed by *S. aureus* (15.7±0.9mm) and *Escherichia coli* (15.0±0.10mm). In addition the ethanolic peel extract of pineapple showed maximum inhibition zone against *B. cereus* (15.0±0.6mm) followed by *S. aureus* (14.0±0.22mm) and *E. coli* (12.3.0±0.12mm), whereas its aqueous peel extract resulted in maximum inhibition zone against *B. cereus,* (14.0±0.5mm), *S. aureus* (14.0±0.11mm) and *K. pneumonia* (14.0±0.10mm). Streptomycin, the reference antibiotic, had inhibitory zones on the tested organisms ranged between 10.0 and 24.0 mm. The minimum inhibition zone of the tested extracts against the tested organisms ranged from 5.0 mm to 14.0mm. The phytochemical analysis showed presence of oxalate, alkaloids, phytate, tannins and glycoside. The antimicrobial activities of the extracts of both *C. nucifera* kernel and *A. comosus* peel have capable of inhibitory effect against the target organisms.

Keywords: Phytochemical Constituents, Antimicrobial Activities, *Ananas comosus*, *Cocos nucifera*, Food Borne Pathogens

1. Introduction

Coconut (*Cocos nucifera* L.) is an important member of the family Aracaceae and only accepted species of the genus *Cocos*. The fruit tree is grown all around the world for decorations as well as for its culinary, non-culinary and many other uses, that is why it is often called the ”tree of life”[3]. About 12 different crops of nuts exist under the name coconut palm [5]. Popular medicinal uses of coconut husk fiber against arthritis and diarrhea have been reported [3]. Previous studies as showed that aqueous *C. Nucifera* husk fiber extracts caused important biological activities such as antimicrobial, antiviral, antinociceptive, anti-inflammatory, antioxidant and antineoplasic properties [1-3].

Pineapple (*Ananas comosus L.*) is a tropical plant and fruit belonging to the family of Bromeliaceae, named after the enzyme bromelain, which is one of its most important health promoting compounds [6]. *A. comosus* is known as the fruit of kings, because, despite the fact that the fruit had become familiar to almost the whole world, only the natives of the tropics and the wealthy Europeans, that can have access to the fruit. It is still a true exotic, because it is a member of the bromeliad family, in which edible fruits are rare. Lawal [6] reported that the solvent extracts of the various parts of *A. comosus* exhibit antibacterial, antiviral, antifungal, antiparasitic and anti-inflammatory properties.

There has been a worldwide increased in the demand for novel antimicrobial agents source from nature for preservation of food [4]. These antimicrobial agents with potential benefits over synthetic antimicrobials is said to inhibit the growth of other microorganisms [8]. The exploration of novel antimicrobial agents from natural resources such as plant or plant products and others has been used mainly for treating diseases, food safety and food preservation purpose [4]. Due to the presence of various secondary metabolites, plant like pineapple and coconut are able to exhibit inhibitory effect against the growth of most pathogens. Food borne pathogens include *Escherichia coli, Salmonella* species*, Shigella* species*, Klebsiella pneumonia, Vibrio* species*, Clostridium botulinum, Enterococcus* species and more. Also there is a rapid increase in food borne illnesses caused by the presence of food borne pathogens in food either due to food contamination, food spoilage or mishandling of food. But use of natural antimicrobial agents may prevent or extend the time duration required for spoilage of food [4]. It has been shown from the aforementioned literatures that the antimicrobial activities of solvent extracts of the various parts of *A. comosus* and *C. nucifera* fruit on several microbial species, but there are little or no knowledge of the antimicrobial activities of aqueous and ethanolic extracts of *C. nucifera* kernel and *A. Comosus* peel on selected food borne pathogens. This study, is therefore, aimed to screen the phytochemical composition and antimicrobial activity of both aqueous and ethanolic extracts of *A. comosus* peel and *C. nucifera* kernel on selected food borne pathogens.

2. Materials and Methods

2.1. Collection and Preparation of Plant Materials

The pineapple (*Ananas comosus)* and Coconut (*Cocos nucifera*) fruits used in this study were purchased from the local market in Ondo town, Ondo state, Nigeria.

Extracts were prepared as described by Makanjuola *et al* [7] with slight modifications. The peel of the pineapple and the pulp of the coconut fruits were air dried at room temperature for 16 days and grinded using an electric blender and sieved to a fine powder. The solvents used for the extraction were 98% ethanol and cold distilled water. Exactly 20g each of the dried powder of any of pineapple peel and coconut pulp were soaked with 100 and 200ml of (98%) ethanol and cold distilled water, respectively. Each solution was allowed to stand for 72 hours, after which they were sieved with a muslin cloth and filtered using No. 1Whatman filter paper. The filtrates were collected in a beaker and concentrated in a vacuum at a temperature below 40°C using a rotary evaporator (Heidolph, VE-11). The resulting crude extracts obtained were exposed to UV rays for 24hrs to check for sterility on nutrient agar plates and the resulting dry weights of the dried extracts were measured and reported.

$$\text{Extract recovery (\%)} = (\text{Dry weight of recovered extract} / \text{Initial dry weight of plant part}) \times 100\% \quad (1)$$

2.2. Microbial Culture Collection

The microbial cultures used in this study were obtained from the Microbiological unit of the Department of Biological Science,Wesley University Ondo, Nigeria. The cultures collected were *Escherichia coli*, *Bacillus cereus, Staphylococcus aureus* and *Klebsiella pneumonia.* The cultures were maintained on sterile nutrient agar slants in a refrigerator at 5°C.

2.3. Phytochemical Analysis of the Plant Extract

The extracts were screened for phytochemicals such as alkaloids, tannins, oxalate, phytate and glycosides in accordance with Trease and Evans [9].

2.4. Assay for Antibacterial Activity

The antimicrobial activities of aqueous and ethanolic extract of the kernel of *C. nucifera* and peel of *A. comosus* were determined by the modified agar well diffusion method as described by Esimore *et al.,* [13]. Different extract concentrations of 50, 25, 12.5, 6.25 and 3.125mg/ml were used for the bioassay and standard antibacterial agent while Streptomycin (10µg) was used as positive control. After 24 hour of incubation, inhibition zone diameters formed in the medium were measured in a millimetre (mm) to determine the antibacterial effectiveness of the extracts.

3. Results

Quantitative screening of some extracted phytochemical show that the extracts of coconut kernel and pineapple peel contained alkaloids, oxalate, tannins, phytate and glycosides. Their aqueous and ethanolic extract contained tremendous amount of alkaloid, oxalate and tannins with values (10.31mg, 6.29mg and 2.2%), (11.1mg, 5.46mg and 3.16%) and (12.24mg, 5.31mg and 2.11%) for the extracts of ethanolic coconut kernel, aqueous pineapple peel and ethanolic pineapple peel, respectively. Meanwhile, the aqueous coconut kernel extract with recorded values of 0.093mg, 0.027mg and 0.067% for alkaloid, oxalate and tannins, respectively. On the other hand, phytate and glycoside presented in minute quantity in the extract of the fruits of both plants (Table 1).

Table 1. *Quantitative analysis of Phytochemicals of C. nucifera kernel and A. comusus peel extracts.*

Extract	Alkaloid (mg/g)±SD	Oxalate (mg/g)±SD	Tannins (%))±SD	Phytate (mg/g)±SD	Glycosides (mg/g)±SD
ACK	0.093±0.05	0.027±0.006	0.067±0.003	0.030±0.01	0.13±0.002
ECK	10.31±0.04	6.29±0.02	2.2±0.04	0.60±0.01	0.53±0.6
APP	11.1±0.03	5.46±0.06	3.16±0.014	0.63±0.06	1.25±0.0
EPP	12.24±0.02	5.31±0.02	2.11±0.03	0.52±0.01	1.16±0.01

Values are means ±standard deviation for three samples. Legend: SD = Standard deviation; ACK= Aqueous coconut kernel; ECK= Ethanolic coconut kernel; APP= Aqueous pineapple peel; EPP= Ethanolic pineapple peel.

The results of the antimicrobial activity obtained from aqueous and ethanolic extract of *A. comosus* peel and *C. nucifera* kernel on the tested microorganisms in addition to Streptomycin are represented in Tables 2 and 3. The inhibition zone against various selected food pathogens was measured in mm. Inhibition zones were seen against all the test organisms, confirming the antimicrobial activity of aqueous and ethanolic extract of *A. comosus* peel and *C. nucifera* kernel.

In Table 2, aqueous peel extract of pineapple showed inhibition against all the test microorganisms with maximum inhibition zone for *B. cereus,* (14.0±0.5mm), *S. aureus* (14.0±0.11mm) and *K. pneumonia* (14.0±0.10mm). Meanwhile, the minimum inhibition zone was occurred for *E. coli* (12.0±0.10mm). For ethanolic peel extract the maximum inhibition zone was occurred for *B. cereus* (15.0±0.6mm) followed by *S. aureus* (14.0±0.22mm) and *E. Coli* (12.3.0±0.12mm), and the minimum inhibition zone (12.0±0.20mm) was recorded for *K. pneumonia*.

Table 2. *Antimicrobial activity of aqueous and ethanolic extracts of A. comosus peel on the tested microorganisms.*

Microorganisms	Diameter of inhibition zone (mm)		
	Aqueous extract (g)±SD	Ethanolic extract (g)±SD	Standard antibiotic (Streptomycin 10µg) (g)±SD
B. cereus	14.0±0.5	15.0±0.6	24.0±0.6
E. coli	12.0±0.10	12.3±0.12	16.0±0.2
K. pneumonia	14.0±0.10	12.0±0.20	10.0±0.5
S. aureus	14.0±0.11	14.0±0.22	12.0±0.2

Values are means ± standard deviation for three samples. Legend: SD = Standard deviation

Table 3 shows the antimicrobial activity of aqueous and ethanolic kernel extracts of *C. nucifera* (coconut). Aqueous kernel extract of coconut showed inhibitory effect against all the test microorganisms with maximum inhibition zone for *B. cereus* (16.0±0.6mm) followed by *S. aureus* (15.7±0.9mm) and *E. coli* (15.0±0.10mm). On the other hand, the minimum inhibition zone was recorded for *K. pneumonia* (14.0±0.14mm). Ethanolic kernel extract of coconut resulted in a remarkable inhibition zone against *B. cereus* (18.0±0.13mm) followed by *K. pneumonia* (15.0±0.18mm) and *S. aureus* (12.0±0.4mm) and the minimum inhibition zone was recorded for *E. coli* (5.0±0.11mm).

Table 3. *Antimicrobial activity of aqueous and ethanolic extracts of C. nucifera kernel on the tested miroorganisms.*

Microorganisms	Diameter of inhibition zone (mm)		
	Aqueous extract (g)±SD	Ethanolic extract (g)±SD	Standard antibiotic (Streptomycin 10µg) (g)±SD
B. cereus	16.0±0.6	18.0±0.13	24.0±0.6
E. coli	15.0±0.10	5.0±0.10	16.0±0.2
K. pneumonia	14.0±0.14	15.0±0.18	10.0±0.5
S. aureus	15.7±0.9	12.0±0.4	12.0±0.2

Values are means ±standard deviation for three samples. Legend: SD = Standard deviation

Table 4 shows the percentage yield of extracts obtained. The ethanolic extract of pineapple peel and coconut kernel as well as aqueous extract of coconut kernel had the highest yield of 20%. The least recovery was obtained for aqueous extract of pineapple peel with 17.5%.

Table 4. *Percentage recovery of aqueous and ethanolic extracts of both A. comosus (pineapple) peel and C. nucifera (coconut) kernel.*

Extract	Amount extracted (g)±SD	Percentage recovery (%)±SD
EPP	40.0±0.4	20.0±0.20
APP	35.0±1.3	17.5±0.65
ECK	40.0±1.5	20.0±0.75
ACK	40.0±0.9	20.0±0.45

Values are means ±standard deviation for three samples. Legend: ECK-Ethanolic coconut kernel, ACK- Aqueous coconut kernel, APP-Aqueous pineapple peel, EPP-Ethanolic pineapple peel.

4. Discussion

The present study suggested that, coconut kernel and pineapple peel extract have great potential as antimicrobial effect against the selected food pathogens and they could be used as alternative preservatives in preventing or extending the time duration required for spoilage of food.

The different percentage recovery and phytochemical analysis observed in studied fruit plant extracts may be resulted from various solvents used in extraction as reported by Makanjuola *et al*. [7] who found that different solvents have different extraction capacities and different spectrum of solubility for the phytoconstituents, respectively. The highest percentage of recoveryobserved in ethanolic extract of both *A. comosus* peel and *C. nucifera* kernel may be due to the polar bonds present in ethanol which is more active in extracting plant metabolites. This observation is in agreement with Makanjuola *et al*. [7] who stated that polar solvents have been shown to be more effective in extracting organic and inorganic materials from plants. The obtained results of phytochemical analysis indicated the presence of alkaloid, oxalate, tannins, phytate and glycosides. Phytochemicals are secondary metabolites produced by plants that fight against microorganisms in their environment [10]. There are variations in the phytochemical constituents; this may be due to its solubility in the solvents used for extraction. Ngele *et al.,* [11] showed that phytochemical constituents of the extracts are known to be biologically active and therefore aid

the antimicrobial activities.

In the present study, *A. comosus* peel extracts and *C. nucifera* kernel extracts showed antimicrobial activity against the tested food borne microorganisms. The aqueous extract showed a remarkable inhibition against *S. aureus* (14.0±0.11mm), *B. cereus* (14.0±0.5mm) and *K. pneumonia* (14.0±0.10mm) compared to *E. coli* (12±0.10mm). While, the aqueous extract of *C. nucifera* kernel showed a remarkable inhibition against *B. cereus* (16.0±0.6mm) and *S. aureus* (15.7±0.9mm) compared to *K. pneumonia* (14.0±0.14mm) and *E. coli* (15.0±0.10mm). Ngele *et al.,* [11] reported that Gram negative bacteria are more resistant to antimicrobial agents due to the presence of an outer-membrane permeability barrier, which limits access of the antimicrobial agents to their targets bacterial cell. Ethanolic extracts of both *C. nucifera* kernel and *A. comosus* peel demonstrated the highest antimicrobial activity against *B. cereus* with values (18.0±0.13mm) and (15.0±0.6mm), respectively. While, the ethanolic extract of *C. nucifera* kernel showed the least inhibition against *E. coli* (5.0±0.10mm). Notably, the inhibition zone of the aqueous and ethanolic extracts of *A. comosus* peel and *C. nucifera* kernel against *S. aureus* and *K. pneumonia* are higher than the control, which corroborates the potentials of plant extracts for antibacterial activity. The antimicrobial activity against gram negative (*E. coli* and *K. pneumonia*) and gram positive (*B. cereus* and *S. aureus*) bacteria used in this study is an indication of the broad spectrum activity of the plant extracts.

The variation in the antimicrobial activity of the various extracts (Tables 2 and 3) showed that different extracts may have varying antimicrobial agents with different modes of action and bacteria susceptibility or that not all phytochemicals that are responsible for antibacterial activity are soluble in a single solvent [12].

5. Conclusion

The results obtained in this study showed that aqueous and ethanolic extracts of both *C. nucifera* kernel and *A. comosus* peel have varying degrees of antimicrobial activity against *E. coli*, *S. aureus, K. pneumonia* and *B. cereus*. This suggests that extracts of both plants can be of beneficial effect in developing a preservant that can be used in preserving food against food borne pathogens. The results also revealed the presence of medicinally important constituents in peel and kernel of both plants, respectively. Many evidences gathered in earlier studies have confirmed the identified phytochemicals to be bioactive. Therefore, pineapple peel and coconut kernel fruit could be used as a good source of antibacterial agent against food borne pathogens.

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