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In vitro Cytotoxic, Antioxidant and Antimicrobial Activities of *Adoninia merrilli* and *Archontopheonix tukeri* Fruit Pericarps

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Abstract

Adonidia merrilli (Becc.) Becc. and *Archontophoenix tuckeri* are palms primarily cultivated for ornamental purposes due to their exotic appearance. A number of ornamental palms have been used in traditional medicine and scientifically explored to ascertain their medicinal value. Methanol extracts of *A. merrilii* and *A. tuckeri* pericarps were screened for phytochemicals, and tested for cytotoxicity against various human carcinoma cell lines, antioxidant and antimicrobial activity using standard protocols. The phytochemical screening revealed the presence of phenolics, flavonoids, deoxy sugars, cardiac glycosides and saponins. Total phenolics, flavonoids and tannin content were 52.05, 20.68, 2.58 mg/g and 40.58, 32.73, 3.09 mg/g for *A. merrilli* and *A. tuckeri* respectively. *A. merrilli* and *A. tuckeri* extracts demonstrated notable anti-proliferative activity against cervical (HeLa), lung (H460), breast (MCF-7) and prostate (PC-3) cancer cells at 30 µg/mL; *A. merilli* (52.90-66.51% kill; IC_{50}: 26.53-29.70), *A. tuckeri* (55.62-62.21% kill; IC_{50}: 18.73-.25.20). Minimum inhibitory concentrations (25-100 µg/mL) of the extracts against *Bacillus subtilis*, *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Salmonella typhi, Shigella dysenteriae* and *Candida albicans* were recorded. *A. merrilli* and *A. tuckeri* pericarp extracts contain potential bioactive compounds for phytomedicine and deserve further scientific investigations as the related ornamental palms, *Livistona chinensis* and *Areca catechu*. The chemical and biological studies of *A. tukeri* palm is reported for the first time.

1 Introduction

Adonidia merrilli (syn. *Normanbya merrilli* (Becc.), or *Veitchia merrilli* (Becc.) H. E. Moore (Arecaceae) is an ornamental palm commonly known as *Adonidia* palm, Manila palm or Christmas palm. Manila palm is native to the Philippines and Sabah[1]. *A. merrilli* is an erect, solitary, unarmed, monoecious palm, 5-10 m high. The fruit has a thin epicarp and dry yellowish, thin fleshy mesocarp, and thin, fragile endocarp. *A. merrilli* fruit is used as a masticatory when ripe, but is an inferior substitute for betel-nut[2]. The fruit and seed extracts have been shown to exhibit antimicrobial properties[3] and cytotoxicity in Huh-7 and Hep G2 human liver cancer cells[4] respectively.

Archontophoenix tukeri is indigenous to Northern Australia, where it is commonly found in rainforest or along streams. *A. tuckeri* is a large, single-trunked palm reaching 22 meters in height, trunk to 26 cm in diameter and expanded at the base. The mature palm produces red fruits having both thin and thick mesocarp fibres in two different layers held together[1].

Several ornamental palms have been found valuable for their aesthetics and therapeutic potentials; for example, the palms, *Livistona chinensis and Areca catechu. L. chinensis* seeds and fruits have shown promising and profound antitumour, cell protective and antibacterial activities[5-8]. Research has shown that phytochemicals, such as phenolics, are one of such bioactive molecules involve in the inhibition of atherosclerosis and cancer. These bioactivities of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase, and scavenge free radicals[9,10]. The paucity of scientific investigation

on the therapeutic relevance of *A. merrilli* and *A. tuckeri* palms necessitates the evaluation of their cytotoxic, antioxidant and antimicrobial activities.

2 Materials and methods

2.1 Plants materials and extraction

The mature ripe fruits of *A. merrilli and A. tuckeri* were collected in the month of April – July, 2015 within Uyo metropolis, Akwa Ibom State, Nigeria. Plant samples were identified and authenticated by a taxonomist, M. E. Bassey, Department of Botany and Ecological Studies, University of Uyo, where voucher specimens were deposited. The fruits pericarp was peeled to expose the seeds. The fruit pericarps were sun-dried, pulverized and macerated in methanol (95%). The maceration process was repeated twice (for exhaustive extraction) to obtain a good yield of extracts. The extracts were concentrated and stored in a refrigerator at 4 ºC.

2.2 Phytochemical screening

Standard methods for phytochemical screening of alkaloids, flavonoids, saponins, tannins, carbohydrates, sterols and triterpenoids were employed. Alkaloids determination was done using Mayer's and Dragendoff's reagents[11]. The persistent frothing, sodium bicarbonate and carbonate tests, as described by Trease and Evans[12] were used for saponins. The methods described by Trease and Evans[12] and Harborne[13], were used for the determination of flavonoids, phenols, cardiac glycosides, carbohydrates, sterols and triterpenes, tannins and phlobatannins.

2.3 Determination of total phenolics

The amount of total phenols in the palm extracts was determined with the Folin-Ciocalteu's reagent using the method of Meda *et al.*[14]. 2.5 ml of 10% Folin Ciocalteu's reagent was added to 0.5 ml of each concentration (2, 1.5, 1.0, 0.5 and 0.25 mg/ml) of the extract and then 2 ml of 2% w/v of Na_2CO_3 was introduced, incubated at room temperature (28 ºC) for 30 minutes. The absorbance was measured at 760 nm using a uv/vis spectrophotometer (Unisio, Shanghai-China). Total phenol values were expressed in terms of gallic acid equivalent (GAE) (mg/g of extract).

2.4 Determination of flavonoids

The method of Meda *et al.*[14] was used to determine the total flavonoid content. 2.5 ml of aluminium trichloride ($AlCl_3$) in methanol was mixed with different concentrations (2, 1.5, 1.0, 0.5 and 0.25 mg/ml) of the palm extracts. Absorption readings at 415 nm using uv/vis spectrophotometer (Unisco, Shanghai - China) were taken after 30 minutes. The total flavonoid content was determined using a standard curve prepared with gallic acid and expressed as GAE mg/g of extract.

2.5 Determination of tannins

The tannin content in each extracts was analysed using the method described by Kalpana *et al.*[15]. 3 ml of extract or standard solution of tannic acid (0.0625-0.5 mg/ml) were mixed with 1 ml of Folin-Ciocalteau reagent and 1 ml of 3.5% Na_2CO_3 solution was added. The volume was made to 5 ml with distilled water, and absorbance read at 725 nm after 30 minutes of incubation. Tannin content was expressed as mg tannic acid equivalent per g of extract.

2.6 Cell culture

HeLa (Cervical Cancer) cells were cultured in Minimum Essential Medium Eagle (MEME), supplemented with 5% of fetal bovine serum (FBS), 100 IU/ml of penicillin and 100 µg/ml of streptomycin in 75 cm^2 flasks, and kept in 5% CO_2 incubator at 37°C. Exponentially growing cells were harvested, counted with haemocytometer and diluted with DMEM[16].

H460 (Lung cancer) cells were cultured in RPMI medium, supplemented with 10% of fetal bovine serum (FBS), 100 IU/ml of penicillin and 100 µg/ml of streptomycin in 75 cm^2 flasks, and kept in 5% CO_2 incubator at 37 ^{o}C. Exponentially growing cells were harvested, counted with haemocytometer and diluted with RPMI[17].

MCF-7 Breast cancer (ATCC No. HTB-2)[18] and PC-3 prostate cancer cells (ATCC No. CRL-1435)[16] were cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 5% of fetal bovine serum (FBS), 100 IU/ml of penicillin and 100 µg/ml of streptomycin in 75 cm^2 flasks, and kept in 5% CO_2 incubator at 37 ^{o}C. Exponentially growing cells were harvested, counted with haemocytometer and diluted with DMEM.

2.7 Cytotoxicity Screening

Cytotoxic activity of compounds was evaluated in 96-well flat-bottomed micro plates by using the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay[16]. HeLa cells were plated into 96-well cell culture plates at 6 x 10^4 cells per well, H460 cells (4 x 10^4), MCF-7 (8 x 10^3) and PC-3 cells at 1 × 10^5 cells per well. The volume in each well was 100 µL for all cell types. After overnight incubation, supernatant fluid was removed by suction and 200 µL of fresh medium containing extract/ compound was added in triplicate, giving a final concentration of 30 µg/mL or 30 µM. Standard drug used in the MTT assay was doxorubicin. After the addition of the sample, plates were incubated for 48 h at 37 °C; 200 µL MTT (0.5 mg/ml) was added to each well and incubated further for 4 hrs. Formazan crystals, formed by reduction of MTT were dissolved in 100 µL DMSO and absorbance was taken at 570 nm using micro-plate reader (Spectra Max plus, Molecular Devices, CA, USA). The % inhibitions were processed by using Soft- Max Pro software (Molecular Device, USA). If extracts/compounds showed 50% or more percent inhibition, they were further processed for IC_{50} calculation. The cytotoxicity was recorded as concentration

causing 50% growth inhibition (IC_{50}) for all cell lines. The percent inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = 100 - \frac{\text{OTC} - \text{ONC}}{\text{OPC} - \text{ONC}} \text{ X } 100$$

Where, OTC: mean of O.D of test compound; ONC: mean of O.D of negative control; OPC: mean of O.D of positive control

2.8 DPPH radical assay

The free radical scavenging activity of the palm extracts was determined using the modified method of Blois[19]. 2.5 ml of different concentrations (0.25-2.0 mg/ml) of the extracts and standard drug (ascorbic acid) was separately measured into test tubes, and then 2.5 ml of 0.1 mM DPPH in methanol were added. The mixtures were incubated in a dark chamber for 30 minutes after which the absorbance was measured (in triplicates) at 517 nm against a DPPH control (containing reagents except test samples). Percentage scavenging activity was calculated using the expression:

% Scavenging actiivty = $[(A_C – A_S)/A_C]$ x 100

Where A_C is the absorbance of the control and A_S is the absorbance in the presence of the extracts or standards

2.9 Iron chelating activity

The reaction mixture containing 1 ml of *o*-phenanthroline (0.025 M), 2 ml of ferric chloride (0.05 M) and 2 ml of extract at various concentrations (0.0625-0.50 mg/ml) was incubated for 10 minutes at ambient temperature. The absorbance at 510 nm was recorded. Ascorbic acid was added instead of extract and absorbance obtained was taken as equivalent to 100% reduction of all ferric ions. The readings were taken in triplicate[20] and the percent iron chelating activity calculated thus:

$$\% \text{ Iron chelating activity} = \frac{\text{AC} - AS}{AC} X \text{ } 100$$

Where AC is the absorbance of the control and AS is the absorbance in the presence of the extracts or standards.

2.10 Collection of bacterial and fungus isolates

Clinical bacterial and fungus isolates were collected from St. Lukes Hospital, Anua, Uyo and University of Uyo Teaching Hospital, Uyo, Akwa Ibom State, Nigeria. These isolate were transported on slants to Microbiology Laboratory, University of Uyo, Nigeria. The test organisms were sub-cultured into nutrient broth and incubated for 48 hrs at 37 ºC. The microbes were sub-cultured on a nutrient agar slant for the isolation of pure culture. Isolates were identified using standard cultural, microscopic and standard biochemical methods such as motility test, gram staining, oxidase test, oxidation fermentation test, indole test, catalase test, gelatin liquefaction test, citrate utilization, esculin hydrolysis, urease activity, decarboxylase reactions and hydrogen sulphide production tests. The Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and fungus (*Candida albicans*) were serially diluted to factor three using 10 fold dilution. Gram negative isolates (*Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Salmonella typhi* and *Shigella dysenteriae*) were serially diluted to factor five using 10 fold dilution. The isolates were sub-cultured into their selective media based on their exhibited morphological characteristics. They were preserved in a refrigerator at 4 °C and later used for this work.

2.11 Preparation of antimicrobial discs

A 5 mm diameter plunger was used to punch a Whatman no.1 absorbent filter paper to obtain 5 mm diameter paper discs. The discs were properly labeled and then sterilized by autoclaving for 15 min at 121°C. The disc were impregnated with the plant extracts (100- 400 µg/ml), dried and stored off in sterile bottles.

2.12 Evaluation of antimicrobial activity

Antimicrobial activity was tested using a modified discs diffusion assay method[21,22]. A loop of culture from the nutrient agar (NA) slant stock was cultured in Mueller Hinton medium overnight and spread with a sterile swab into Petri-plates. Each microbial swab was spread on separate plates. Sterile disc (5 mm in diameter) impregnated with the plant extracts were placed on the cultured plates. Control experiment was carried out using commercial antibiotics, antifungal and solvent (stock). The solvent loaded disc without extracts served as control in the study. Streptomycin was used for bacterial isolates and Nystatin for fungal isolates; plates were incubated for 24 hrs and 48 hrs respectively. The results were recorded by measuring the zones of growth inhibition. Clear inhibition zones around the discs indicated the presence of antimicrobial activity. All data on antimicrobial activity were average of triplicate readings.

2.13 Determination of minimum inhibitory concentration

The minimum inhibitory concentrations of the extracts were determined using tube dilution method[23]. The initial concentration of each extracts was diluted using double fold dilution and standard volume of each diluted isolate (0.1 mL) was aseptically inoculated into different concentrations of the extract. Control experiment was carried out without the crude extracts. All tubes were incubated at 37 °C for 24 hrs. Minimum inhibitory concentrations (MIC) were determined as the lowest concentration without turbidity.

3 Results and Discussions

The phytochemical composition of *A. merrilli* and *A. tuckeri* are presented in Table 1. The result indicated the presence of phenolics, flavonoids, deoxy sugars and cardiac glycosides in the high amount; and saponins in varied amount in both samples, whereas alkaloids, anthraquinones and reducing sugars were not detected. Lewis and Zona[24] reported that the leaf and root tissues of *A. merrilli* and *Archontophoenix*

purpurea leaf tested negative for cyanogenesis; indicating that separate plant organs within the same plant may differ in their cyanogenic potential and could be difficult to extrapolate from the results the cyanogenic activity of the flowers and fruits.

Table 1: Phytochemical composition of *A. merrilli* and *A. tukeri* palms

Test	*A. merrilli*	*A. tuckeri*
Saponins	++	+
Tannins	+	+
Cardiac glycosides	++	+
Terpenes	+	+
Alkaloids	-	-
Flavonoids	+++	+++
Anthraquinones	-	-
Reducing sugars	-	-
Phenols	+++	+++
Phlobatannins	-	-
Deoxy sugars	+++	+++

+++: High, ++: Moderate, +: Trace, -: Not detected

Table 2 indicates the concentration of antioxidant components (mg/g) in the palm pericarp extracts. Total phenolics in *A. merrilli* were higher than *A. tuckeri*, while the flavonoid and tannin contents in *A. merrilli* were lower compared with *A. tuckeri.* The phytochemical content of *A. merrilli* and *A. tuckeri* has not been previously reported.

Table 2: Polyphenols content of *A. merrilli* and *A. tuckeri* palms

Phytochemical (mg/g)	*A. merrilli*	*A. tuckeri*
Total phenol	52.05 ± 0.23	40. 58 ± 0.50
Total flavonoids	20.68 ± 0.55	32.73 ± 0.15
Tannins	2.58 ± 0.11	3.09 ±0.01

However, the fruit of ornamental palm, *A. catechu* has been shown to contain isorhamnetin, quercetin, liquiritigenin, 5,7,4'-trihydroxy-3',5'-dimethoxyflavanone, (+)-catechin, resveratrol, ferulic acid, vanillic acid, 5,8-epidioxiergosta-6,22-dien-3beta-ol, stigmasta-4-en-3-one, beta-sitosterol, cycloartenol, and de-O-methyllasiodiplodin[24]. *A. catechu* seed is also reputed to contain high amount of polyphenols (114.14 mg/g and 155.80 mg TAE/g)[25,26].

The results from the cytotoxicity screening of *A. merrilli* and *A. tuckeri* fruit pericarps are presented in Table 3. *A. merrilli* and *A. tuckeri* methanol extracts at a low concentration of 30 µg/mL demonstrated strong anti-proliferative effect against all cell lines in the assay with 52.90-66.51% kill and IC_{50} value, 26.53-29.70; 55.62-62.21% kill and IC_{50} value 18.73-.25.20 respectively. *A. tuckeri* extract exhibited higher percent inhibition against cervical, breast and prostate cancer cells compared with *A. merrilli*, except for lung cancer. The cytotoxicity of *A. merrilli* fruit pericarp is potent at 30 µg/mL compared with the reported induction of cytotoxicity in hepatocellular carcinoma cells by ethyl acetate seed extract of *A. merrilli* against Huh-7 and Hep G2 (IC_{50}: 48.0 & 75.6 µg/mL)[4]. The observed induction of cytotoxicity by *A. merrilli* and *A. tuckeri* extracts may be attributed to high phenolic content, especially flavonoids (Table 1 and 2). The efficacy of polyphenols as anticancer agent are influenced by varied factors such as cancer type, working dose and chemical structure[28]. These anticancer effects may be related, at least partly, to their antioxidant activities. Phenolics isolated from *L. chinensis* palm fruits are reputed to demonstrate potent antioxidant, anti-proliferative and apoptosis against HepG2 human liver cancer, HL-60 human myeloid leukemia, K562 human myeloid leukemia, CNE-1 human nasopharyngeal and HeLa carcinoma cell lines[8,29,30]. Similarly, ethanol extracts of *L. chinensis* fruits and *A. catechu* seeds showed anti-proliferative activity against *HL-60* (Human promyelocytic leukemia cells) and MCF-7 cells, respectively[5,31].

The results of the antioxidant activity models are displayed in Fig 1 & 2. In the DPPH radical assay, the palm extracts (0.25-2.0 mg/ml) significantly scavenged the DPPH radical in a concentration dependent manner (Fig. 1). The result also showed that at 2.0 mg/ml dose, *A. merrilli* and *A. tuckeri* extracts inhibited DPPH radical by 64.89% and 57.36% respectively compared to the standard, ascorbic acid (97.26%). This is an indication of the antioxidant potential in the extracts to reduce the stable, purple-coloured radical DPPH to the yellow coloured DPPH-H form.

Similarly, high antioxidant activity was observed for iron chelating activity (Fig. 2) in a concentration dependent pattern. The percentage inhibitions at 2.0 mg/ml for *A. merrilli, A. tuckeri* and ascorbic acid were 66.21, 62.31 and 73.14% respectively. In both assays, *A. merrilli* extract displayed stronger antioxidant potential compared with *A. tuckeri* extracts. The correlation coefficient values for TPC and DPPH assay; TPC and metal chelating assay were significant. The higher the total phenolics content (Table 2), the higher the antioxidant activity exhibited by the extracts. The ornamental palm fruit pericarp of *A. catechu* and *Dypsis lutescens* have also been reported to possess strong antioxidant activity using DPPH and reducing power assays[31,32].

A. merrilli and A. tuckeri extracts showed significant antimicrobial activity (Table 4 & 5). The results revealed that

both extracts showed varying degree of microbial inhibition (6.0-13.5 mm) against the test microbial strains.

Table 3: Cytotoxicity activity of *A. merrilli* and *A. tuckeri* palms

	Percent Inhibition			
Sample	**HeLa [a]**	**H-460 [a]**	**MCF-7 [a]**	**PC-3 [a]**
A. merrilli	56.27	66.51	52.90	55.89
A. tuckeri	57.21	62.21	55.62	56.91
Doxorubicin [b]	95.90	95.90	89.19	81.97
	IC_{50} Value ± S.D			
A. merrilli	27.21 ± 0.005	29.02± 0.004	29.70 ± 0.01	26.53 ± 0.05
A. tuckeri	20.94 ± 0.8	18.73 ± 0.05	25.20 ± 0.05	24.80 ± 0.004
Doxorubicin	0.20 ± 0.03	0.21 ± 0.01	0.92 ± 0.1	0.25 ± 0.008

[a] % kill at 30 μg/mL; [b] % kill at 30 μM

Table 4: Zones of inhibition of *A. merrilli* and *A. tuckeri* extracts in millimetres (mm)

Isolate	*A. merrilli* (μg/mL)				*A. tuckeri* (μg/mL)				Standard	
	100	200	300	400	100	200	300	400	STP	NYS
B. subtilis	7.0	7.5	12.0	15.0	7.0	8.0	10.0	13.5	12.0	NT
S. aureus	6.0	7.0	8.5	14.0	8.0	9.0	10.5	12.0	20.0	NT
P. aeruginosa	6.0	6.0	8.0	8.5	8.0	8.5	9.0	10.0	15.0	NT
S. typhii	6.0	6.0	7.5	8.5	7.0	7.5	8.5	10.0	10.0	NT
P. mirabilis	6.0	6.5	9.0	11.0	7.0	7.0	7.0	7.5	12.0	NT
E. coli	7.0	7.5	8.5	10.0	6.0	6.0	6.5	7.0	10.0	NT
S. dysenteria	7.0	7.0	8.0	8.5	6.0	6.0	7.0	7.5	11.0	NT
C. albicans	7.0	7.0	10.5	12.0	6.0	6.0	6.5	9.0	NT	24.0

STP: Streptomycin; NYS: Nystatin; NT: Not tested

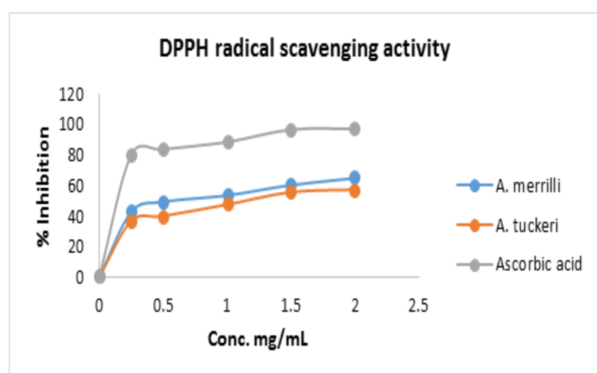

Fig 1: DPPH radical scavenging activity

The zones of inhibition varied with the extract and the organism tested. It was observed that the zones of inhibition increased with increase in concentration, as improved antimicrobial activity was concentration dependent. The antibacterial activity was highly comparable to the standard drug, streptomycin, except against *P. mirabilis, E. coli, S. dysenteria* and *C. albicans*. Minimum inhibitory concentration (M.I.C.) (the lowest concentration of an extract that inhibits completely the growth of micro-organism in 24 hours) ranged from 25-100 μg/mL on tested bacteria and fungus (Table 5).

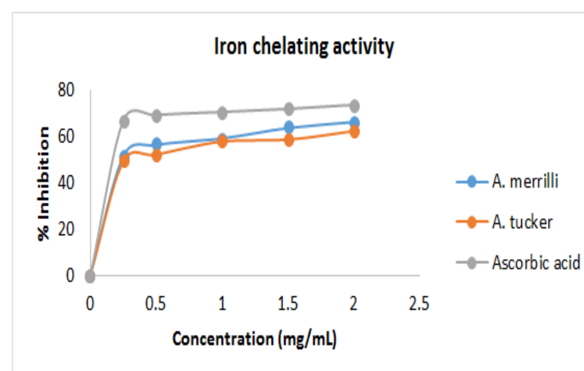

Fig 2: Iron chelating activity

Table 5: Minimum inhibitory concentrations of palm extracts

Organism	*A. merrilli* (µg/mL)				*A. tuckeri* (µg/mL)			
	25	50	75	100	25	50	75	100
B. subtilis	-	✓	✓	✓	-	✓	✓	✓
S. aureus	-	-	-	✓	-	✓	✓	✓
P. aeruginosa	-	-	-	✓	-	-	✓	✓
S. typhii	-	-	-	✓	-	-	✓	✓
P. mirabilis	-	-	-	✓	-	-	-	✓
E. coli	-	-	✓	✓	-	-	-	✓
S. dysenteria	-	-	✓	✓	-	-	-	✓
C. albicans	-	-	-	✓	-	-	-	✓

Antimicrobial agents with low activity against an organism have a high MIC while a highly active antimicrobial agent gives a relatively low MIC.

B. subtilis was more susceptible to the inhibitory effects of the extracts than *S. aureus*; *A. tuckeri* extracts showed better activity on *S. aureus* (MIC, 50 µg/mL) compared with *A. merrilli* (100 µg/mL). *P. mirabilis* and *C. albicans* were the most resistant strains to the antimicrobial effects of both extracts. *A. merrilli* extracts exhibited stronger action against *E. coli* and *S. dysenteria* (MIC, 75 µg/mL) while *A. tuckeri* inhibitory effect was dominant against *P. aeruginosa* and *S. typhii* (MIC, 75 µg/mL) in the assay. This result is consistent with the report by Tangjai and Rutatip[3], which showed that ethanol fruit extracts of *A. merrilli* demonstrated notable antimicrobial activity against *S. aureus, B. cereus* and *Micrococcus sp.* (15.625 ppm) and lower effects against *E. coli, P. aeruginosa, Saccharomyces cerevisiae* and *C. krusei* using agar well diffusion method. Evidence has shown that Gram-negative bacteria are less susceptible to chemical agent than Gram-positive bacteria due to variation in their cell wall structures[34]. This is obvious from the sensitivity of the Gram-positive bacteria to the tested extracts, though with differences in the degree of inhibition. The observed antimicrobial property may be attributed to the antimicrobial compounds inherent in each extract, especially the high amount of phenolic compounds. Kaur and Singh[6] reported that the presence of high concentration of phenolic compounds with astringent properties in *L. chinensis* fruit resulted in *S. aureus* DNA, enzyme and protein denaturing. Tannins have also been shown to have astringent properties, hasten the healing of wounds and inflamed mucous membrane[35].

4 Conclusions

The fruit pericarp extracts of *A. merrilli* and *A. tuckeri* demonstrated potent anti-proliferative activity against HeLa, H 460, MCF-7 and PC-3 cells. The *in-vitro* antioxidant activity of *A. merrilli* extract was more profound compared with *A. tuckeri*. The extracts exhibited notable antibacterial activity against *B. subtilis* and *S. aureus* compared with the gram-negative bacteria strains (*P. aeruginosa, E. coli, P. mirabilis, S. typhi* and *S. dysenteriae*) and *C. albicans*.

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6 Conflicts of Interest

The authors declare no conflict of interest.

7 Author Contributions

EEE and BSA conceived and designed the experiments; EEE and AUS performed the experiments; EEE and BSA wrote the manuscript; MIC supervised the experiments.

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