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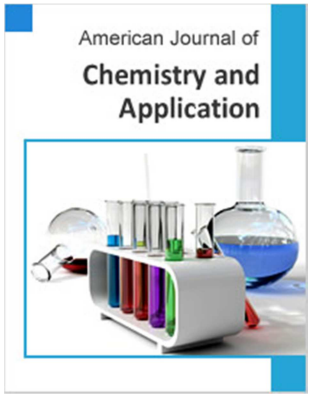

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Phytochemical Profile, Antimicrobial and Antioxidant Activities of *Ptychosperma macarthurii* and *Archontophoenix tukeri* Seeds Extracts

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Abstract
Ptychosperma macarthurii and *Archontophoenix tuckeri* are unexploited and underutilized palms primarily cultivated as ornamentals. The seeds collected from mature fruiting palms were evaluated for phytochemical constituents and biological activity using standard protocols. *A. tuckeri* seeds contain high amount of alkaloids, terpenes, flavonoids, tannins, deoxy-sugars, cardiac glycosides and total polyphenols; saponins, tannins and deoxy sugars were dominant in *P. elegans* seeds. *A. tuckeri* extract exhibited significant antibacterial activity (MIC, 75 µg/mL) against *Staphylococcus aureus*, *Escherichia coli* and *S. dysenteria*; DPPH radical scavenging (89.59%, 2.0 mg/mL) and iron chelating activity (70.40%, 0.5 mg/mL). The well-endowed phytochemical profile of *A. tuckeri* seeds is worth exploiting for potential bioactive molecules via bioassay guided isolation.

1. Introduction

Ptychosperma macarthurii (H. Wendl. Ex H. J. Veitch) H. Wendl. Ex Hook. f. (Arecaceae) is a slender cluster palm endemic to Australia and New Guinea; commonly cultivated as ornamental tree in both temperate and tropical regions. The fruits are globose, about 1.3 cm long, bright red when mature and single seeded; seed grooved and pointed at both ends [1] (Figure 1). Few studies on *P. macarthurii* revealed the proximate, fatty acids and total carotenoids content of pulp and kernel [2]; Lewis and Zona [3] also reported the absence of cyanogenesis in the leaf and shoot meristem.

Archontophoenix tuckeri, the Rocky River palm or Cape York palm (Arecaceae) is native to Northern Australia. *A. tuckeri* is a large, single-trunked palm reaching 22 meters in height and 26 cm in diameter with an expanded base. The red fruits (17-25 mm long) produced by the mature palm at maturity possess both thin and thick mesocarp fibres in two different layers bound together [4] (Figure 2). The nutritional and toxicity potentials of *A. tuckeri* seeds [5], biological activity of fruit pericarp of *A. tuckeri* [6], and composition of flours made from *Archontophoenix alexandrae* residue obtained after heart-of-palm production [7]. Nature provides in most plants a complex array of

molecules with therapeutic significance which could be tailored to solve man's problem of drug resistance and development of new drug leads. A vast number of potential drug plants are untapped, unexploited and underutilized. The mature ripe fruits of *P. macarthurii* and *A. tukeri* are regarded as waste in the environment of cultivation. This study is aimed at evaluating the antimicrobial and antioxidant potentials, as well as the class of secondary metabolites endowed in *P. macarthurii* and *A. tuckeri* seeds.

Figure 1. *P. macarthurii seeds.*

Figure 2. *A. tuckeri seeds.*

2. Materials and Methods

2.1. Plants Materials and Extraction

The mature ripe fruits of *P. macarthurii* 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; seeds were cracked to obtain the kernels. The kernels were shade-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°C.

2.2. Phytochemical Screening

Standard methods for phytochemical screening of alkaloids, flavonoids, saponins, tannins, carbohydrates, and terpenes were employed. Alkaloids determination was done using Mayer's and Dragendoff's reagents [8]. The persistent frothing, sodium bicarbonate and carbonate tests, as described by Trease and Evans [9] were used for saponins. The methods described by Trease and Evans [9] and Harborne [10], were used for the determination of flavonoids, phenols, cardiac glycosides, carbohydrates, terpenes, tannins and phlobatannins.

2.3. Determination of Total Phenolics and Flavonoids

The amount of total phenols and flavonoids in the palm extracts was determined with the Folin-Ciocalteu's reagent using the method of Meda et al. [11]. Total phenol and flavonoid values were expressed in terms of gallic acid equivalent (GAE) (mg/g of extract).

2.4. Determination of Tannins

The tannins content in each extract was analysed using the method described by Kalpana *et al*. [12]. Tannin content was expressed as mg tannic acid equivalent per g of extract.

2.5. 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°C. 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.

2.6. 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.

2.7. Evaluation of Antimicrobial Activity

Antimicrobial activity was tested using a modified discs diffusion assay method [13]. The results were recorded by measuring the zones of growth inhibition. The minimum inhibitory concentration (MIC) of the extracts were determined using tube dilution method [14]. All data on antimicrobial activity were average of triplicate readings.

2.8. DPPH Radical Assay

The free radical scavenging activity of the palm extracts was determined using the modified method of Blois [15]. 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 was 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:

$$\% \text{ scavenging activity} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

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 an 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 [16].

$$\% \text{ Iron chelating activity} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sampl}}{\text{Absorbance of Control}} \times 100$$

3. Results and Discussion

The phytochemical profile of *P. macarthurii* and *A. tuckeri* is presented in Table 1. The evaluation revealed the high content of saponins, tannins, and deoxy-sugars in *P. macarthurii* while alkaloids, terpenes, flavonoids, tannins, phlobatannins, deoxy-sugars and phenols were dominant in *A. tuckeri* kernels. Total phenols were also high in *A. tuckeri* (184 mg GAE/g) compared with *P. macarthurii* (Table 2). In contrast, *A. tuckeri* fruit pericarp is less endowed with secondary metabolites [6] compared with the seed; the fruit pericarp was shown to contain high amount of flavonoids, phenols and deoxy-sugars.

P. macarthurii and *A. tuckeri* seed extracts exhibited significant antibacterial and antioxidant activities (Table 3). The extracts demonstrated varying degrees of microbial inhibition (6-30 mm) against the test organisms; improved antimicrobial activity was concentration dependent. *P. macarthurii* displayed potent antibacterial activity against *P. aeruginosa* (30 mm zone of inhibition) and *A. tuckeri* against *E. coli* (15 mm zone of inhibition) compared with the standard drug, streptomycin (15mm and 10mm zones of inhibition respectively). M. I. C. values indicated a stronger antibacterial potency of *A. tuckeri* on *E. coli* and *S. dysenteria* (75 µg/mL) and *P. macarthurii* activity on *P. aeruginosa* (75 µg/mL) in the assay. The broad spectrum antimicrobial activity of the seed extracts could be attributed to their distinct phytochemical profile (Table 1), however a notable enhanced antimicrobial activity was exhibited by *A. tuckeri* extract. Similarly, research has shown that the presence of a high concentration of phenolic compounds with astringent properties in *Livistona chinensis* palm fruit resulted in *S. aureus* DNA, enzyme and protein denaturing [17]. Essien *et al*. [6] showed that *A. tuckeri* fruit pericarp extract demonstrated profound antibacterial activity on *B. subtilis* and *S. aureus* (MIC, 50 µg/mL) compared with the inhibitory effect on gram negative bacteria (75-100 g/mL).

Table 1. *Phytochemical profiles of P. macarthurii and A. tuckeri.*

Metabolite	*P. macarthurii*	*A. tuckeri*
Alkaloids	++	+++
Terpenes	+	+++
Flavonoids	-	+++
Saponins	+++	++
Tannins	+++	+++
Phlobatannins	++	+++
Deoxy-sugars	+++	+++
Cardiac glycosides	-	+++
Reducing sugars	-	-
Anthraquinones	+	++
Phenols	++	+++

+++ = High; ++ = Moderate; + = Trace; - = Not detected

Table 2. *Total Phenolic content of P. macarthurii and A. tukeri nuts.*

Phenolic	*P. macarthurii*	*A. tuckeri*
Total Phenols (mg GAE/g)	89.0	184.0
Total flavonoids (mg GAE/g)	5.5	37.5
Tannins (mg/g)	42.0	68.0

Table 3. *Antimicrobial activity of P. macarthurii and A. tukeri extracts.*

Organism	Zone of inhibition (µg/mL)										M. I. C. (µg/mL)	
	P. macarthurii				*A. tuckeri*				Std. drug			
	100	200	300	400	100	200	300	400	STP	NYS	P. E	A. T
B. subtilis	6.0	8.0	9.0	10.0	7.0	8.0	8.0	9.0	12.0	NT	100	100
S. aureus	7.0	9.0	12.0	15.0	7.0	8.0	9.0	10.0	20.0	NT	75	75
P. aeruginosa	10.0	15.0	25.0	30.0	6.0	7.0	7.0	8.0	15.0	NT	75	100
S. typhii	6.0	7.0	7.0	8.0	7.0	8.0	9.0	10.0	10.0	NT	100	100
P. mirabilis	6.0	7.0	8.0	8.0	6.0	7.0	10.0	12.0	12.0	NT	100	100
E. coli	7.0	8.0	9.0	10.0	7.0	8.0	10.0	15.0	10.0	NT	100	75
S. dysenteria	-	6.0	7.0	8.0	7.0	8.0	9.0	10.0	11.0	NT	150	75
C. albicans	6.0	7.0	7.0	8.0	7.0	8.0	12.0	14.0	NT	24.0	100	100

STP: Streptomycin; NYS: Nystatin; Std.: standard; NT: Not tested; -: No activity; P. E: *P. macarthurii*; A. T: *A. tuckeri*

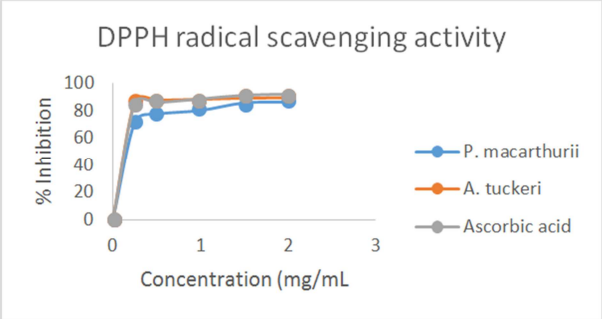

Figure 3. *DPPH radical scavenging activity of palm seeds' extracts.*

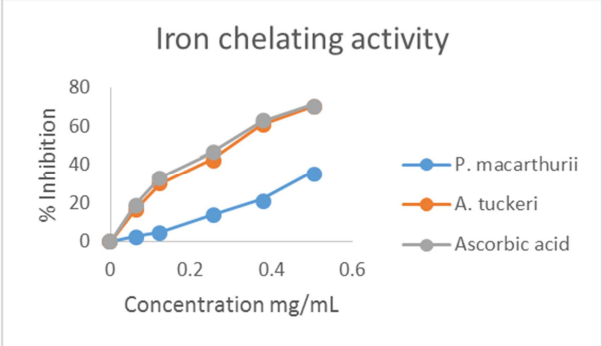

Figure 4. *Iron chelating activity of Palm seeds' extracts.*

The antioxidant models (DPPH radical scavenging and iron chelating activities) of *P. macarthurii* and *A. tuckeri* extracts are depicted in Figure 3 and Figure 4 respectively. In both antioxidant assays, the extracts displayed radical scavenging and chelating effects in a concentration dependent fashion. In Figure 3, *A. tuckeri* showed a slightly higher scavenging activity than *P. macarthurii*, but comparable with ascorbic acid (2.0 mg/mL, 86.56%, 89.59% and 92.32% for *P. macarthurii*, *A. tuckeri* and ascorbic acid respectively). This ascribes the potential of antioxidant compounds in the extracts to reduce the stable, purple-coloured DPPH to the yellow coloured DPPH-H. Similarly, high radical scavenging by palm kernel extracts of *L. chinensis*, *Areca catechu*, and *Saribus rotundifolius* have been reported [18]. In contrast, Essien *et al*. [6] recorded a lower radical scavenging activity for (57.36%, 2.0 mg/mL) for *A. tuckeri* fruit pericarp extract. The iron chelating activity of *A. tuckeri* extract (Figure 4) was stronger than *P. macarthurii*, though comparable with ascorbic acid (0.5 mg/mL, 36.23%, 70.4% and 71.26% for *P. macarthurii*, *A. tuckeri*, and ascorbic acid respectively). Interestingly, there is a correlation between the total phenolic content of the extracts (Table 2) and the observed antioxidant activities. *A. tuckeri* fruit pericarp extract is reported to demonstrate a lower iron chelating activity (0.5 mg/mL, 62.31%) [6] compared with the seed extract in this study.

4. Conclusions

The seeds extract of *A. tuckeri* has a higher profile of phytochemicals: alkaloids, terpenes, flavonoids, tannins, and the phenolics compared to *P. macarthurii* extract. *A. tuckeri* seeds extract exhibited a stronger anti-microbial activity against the test organisms as well as a stronger anti-oxidant activity than the *P. macarthurii* seeds extract. The bioactive potentials of these neglected and wasted palms' nuts could serve as a good source of new anti-microbial and anti-oxidative agents and are worth investing further for their pharmacological relevance and possible applications in food or drug preparations.

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