



Quantitative Phytochemical Screening And Antimicrobial Properties Of Methanol Extracts Of Nuts And Husk Of *Pinanga Dicksonii* (Roxb.) Blume

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Abstract: *Pinanga dicksonii* (Roxb.) Blume (Arecaceae) is an endemic palm of the Western Ghats of Karnataka and Tamil Nadu. In this study, methanol extracts of *P. dicksonii* nut (PDNM) and *P. dicksonii* husk (PDHM) were evaluated for their antimicrobial, Antimicrobial activity was determined using the well diffusion method, antioxidant activity was assessed using DPPH, Reducing power, and total phenolic and flavonoid contents were quantitatively tested. Methanol extracts of PDNM and PDHM were studied using an *in vitro* wound healing assay. A maximum concentration of 1000 µg/ml of flavonoids was used in both PDNM and PDHM (2458.5 mg and 267 mg RE/g, respectively). Phenolic content was highest in PDNM (1,15,071 mg GAE/g extract) and PDHM (17,190 mg GAE/g) at 1000 µg/ml concentration. The results showed that both extracts had the highest inhibition of fungi against *Candida albicans* (6, 8 and 6, 11, 12 mm) at 25, 50, and 100 µg/ml concentrations. As shown above, the nuts and husk of *P. dicksonii* have excellent antimicrobial properties.

Keywords: Antimicrobial activity, Methanol extract, Nuts and Husk, Quantitative analysis.

I. INTRODUCTION

Clinically, plant-based antimicrobial products are already in use, including essential oils (e.g., tea tree, clove), resins, and purified compounds such as berberine and thymol, which have demonstrated efficacy in preventing and treating infections of the gastrointestinal, respiratory, skin, oral, and urinary systems. Research is ongoing to better standardize extraction methods, understand pharmacokinetics and pharmacodynamics, and optimize formulations to improve the antimicrobial efficacy and safety of these natural products.

Additionally, plant-derived antimicrobials are gaining interest in applications beyond pharmaceuticals, such as in antimicrobial textile finishing for health care products, where natural extracts like neem, aloe vera, *Barleria longiflora* and turmeric confer antimicrobial properties while being environmentally friendly alternatives to synthetic agents, which often pose toxicity and environmental concerns (Kalpana *et al.*, 2016).

Pinanga dicksonii (Roxb.) Blume, belonging to the family Arecaceae, is a species of palm known for its ecological and potential ethnobotanical significance. This plant is native to tropical regions and is characterized by its distinctive morphological features typical of the *Pinanga* genus. Members of this genus have been studied for various biological activities and traditional uses, which highlight their importance in local medicinal and cultural practices. Investigating *Pinanga dicksonii* further can provide insights into its

phytochemical composition and possible applications, including antimicrobial properties, contributing to the broader search for novel bioactive compounds within the Arecaceae family.

Therapeutic plants have yielded almost 13,000 secondary metabolites. In plants, secondary metabolites perform specific tasks or act as defence chemicals. According to reports, the presence of flavonoids, steroids, terpenoids, phenolic acids, and fatty acid derivatives primarily distinguishes members of the Arecaceae family (Mohammed, Fouad, 2022). The aim of the study is to comprehensively investigate antimicrobial activities and provide actionable recommendations of *Pinanga dicksonii* (Roxb.) for effective further research endeavors.

MATERIALS AND METHODS

Dickson's palm or *Pinanga dicksonii* (Roxb.) Flower (Arecaceae) is a thin palm that is 4-6 m high and 6-8 cm in diameter. The complex leaves range in length from 1 to 1.3 m. With toothed edges, two to three leaflets that are 30 to 50 cm long and 2-3 cm wide are joined. Three blooms per segment are unisexual; the lateral flowers are male, whereas the central blossom is female. Males were ovoid lanceolate, 0.2–0.3 cm long, with keel-shaped sepals, petaloid petals, and six stamens. The petals and sepals of the female flowers were rounded and measured 0.1 cm. 6. Ovarian unicellular staminodes. Ovule 1. Fruit: oval, 0.6-1 x 0.2-0.3 cm. Single, elliptical seeds were used.

Phytochemical Quantitative analysis

Estimation of Total Flavanoid Content

Take 0.3 ml of serially diluted test samples (*Pinanga dicksonii* nut methanol (PDNM) and *Pinanga dicksonii* husk ethanol (PDHM)) (0.3 mg/ml in methanol) in separate test tubes. Add 3.4 ml of 30 % methanol. Then, 0.150 ml of 0.5 M sodium nitrite solution was added. Then, 0.150 ml of 0.3 M aluminum chloride solution was added. Incubate for 5 minutes. Then, 1 ml of 1 M sodium hydroxide solution was added and mixed well. An equal volume of methanol was used as a blank sample. The absorbance was measured at 506 nm using a spectrophotometer (Genesys, 10-S, USA) or a multiplate reader.

Estimation of Total Phenolic content (Mahdavi et al., 2010 and Wern et al., 2016)

Different concentrations of 0.4 ml of samples (PDNM and PDHM), standard (Gallic acid), and blank samples were prepared separately. Then, 3.6 ml of distilled water was added to both samples. To the samples, 0.4 ml of Folin-Ciocalteu reagent was added. Then, 4 ml of 7 % sodium carbonate was added to the samples. Both samples were made up to a volume of 10 ml with distilled water, followed by thorough mixing and incubation for 90 min at room temperature. The absorbance was measured at 765 nm using a spectrophotometer (Genesys, 10-S, USA). A calibration curve was plotted using a gallic acid standard. The results were expressed as gallic acid equivalents (mg GAE / 100 ml).

Antimicrobial activity

Preparation of agar media

Beef extract (1 g), yeast extract (2 g), peptone (5 g), Sodium Chloride (5 g), and dextrose (20 g) were dissolved in 900 ml of distilled water. The pH was adjusted to 7.2, and the volume was made up to 1000 ml. Finally, 15 g of agar was added to the media and autoclaved at 121°C for 20 min.

Preparation of inoculum:

Escherichia coli (MTCC 42), *Klebsiella pneumoniae* (MTCC 7407), *Staphylococcus aureus* (MTCC 3160) were used as bacteria and *Candida albicans* (MTCC 183), *Microsporum canis* MTCC (2820) used as fungi was procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh. Loopful of 48 hrs. Old cultures from the slants were transferred to sterile saline and mixed well to prepare a homogeneous inoculum.

Well diffusion method:

The media were cooled to approximately 45-55°C, and approximately 20 ml of each was poured into sterile petriplates. One milliliter of the inoculum was immediately added to the plate and swirled for uniform distribution. Wells were bored using sterile borers. The PDNM and PDHM samples and antibiotics were dispensed into the wells. The plates were incubated overnight at 37°C and observed after 48 h.

Data Analysis

All experiments were performed in triplicate. The experimental data are expressed as the mean and standard deviation. Statistical calculations and analyses were performed by comparing the data between and within groups using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION**Quantitative phytochemical analysis****Determination of Total Flavanoid Content**

Flavonoids and phenolics are the most abundant phytochemicals present in the plants. According to Zhang *et al.* (2022), flavonoids and phenolics possess anti-inflammatory, antibacterial, and antioxidant properties. Flavonoids also influence the body's biochemical reactions and inhibit biomolecules that cause allergies (Madhu *et al.*, 2016). These phytochemicals may be responsible for the observed antioxidant and antimicrobial activities. More than 4,000 flavonoids have been identified, many of which are found in fruits, vegetables, and beverages (tea, coffee, beer, wine, and fruit drinks). The flavonoid content in the methanol extracts of the PDNM and PDHM samples was analyzed (Table 1). The maximum concentration of flavonoids in the PDNM and PDHM extracts was 1000 µg/ml, with flavonoid content (2458.5 mg RE/g and 267 mg RE/g). The PDHM extract exhibited a higher flavonoid content (1892 mg RE/g) at a concentration of 800 µg/ml (Fig. 1). At a concentration of 800 µg/ml, the PDHM extract had the lowest flavonoid content compared to the PDNM extract (210 mg RE/g extract) (Table 1, Fig. 1).

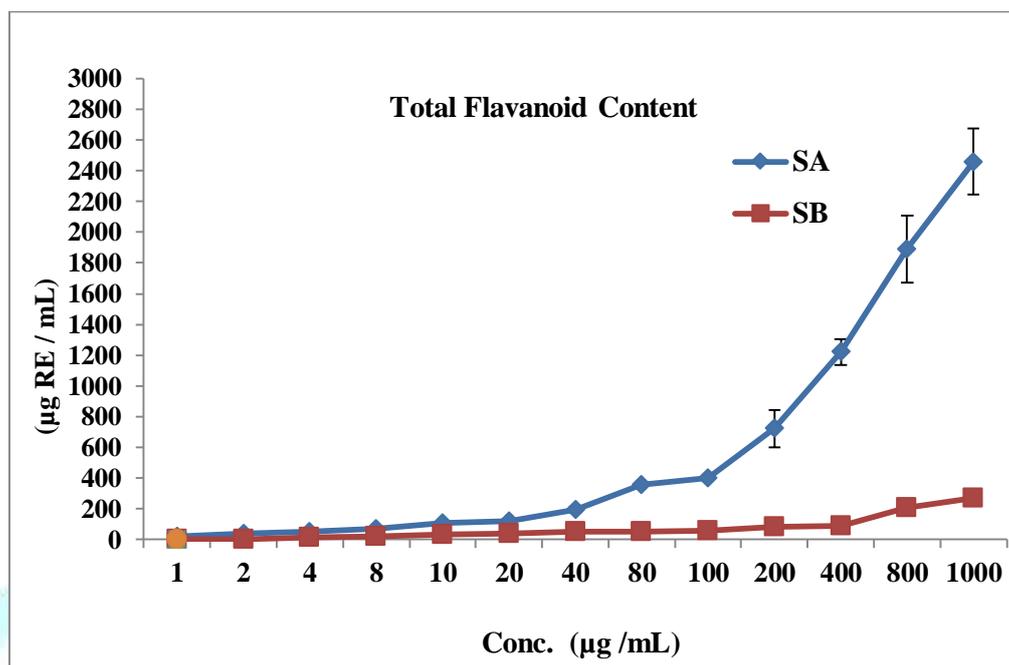
Table 1: Total flavanoid contents of PDNM and PDHM samples

Conc. (µg / mL)	Total Flavanoid content	
	µg CTE / mL	
Rutin	PDNM	PDHM
1	21.5	1.1
2	40	4
4	48.5	13.5
8	70.5	21
10	107.5	30.5
20	118.5	37
40	193.5	48.3
80	354	52.48
100	403.5	55.5
200	723.5	82
400	1220.5	91.5
800	1892	210
1000	2458.5	267

Mean values ± standard deviation were presented (n = 3)

Microgram of Rutin equivalents (CTE)

Fig. 1: Total flavanoid contents of PDNM and PDHM samples



Determination of Total Phenolic content

A summary of the quantitative analysis results for the PDNM and PDHM plant samples is shown in Table 2 and Fig. 2. Table 2 shows the highest phenolic content of the PDNM sample (115.071 mg GAE/g extract) and PDHM sample (17.190 mg GAE/g extract) at a concentration of 1000 $\mu\text{g}/\text{mL}$. Phenol molecules are important chemicals found in plants. They can scavenge because of their hydroxyl groups. They are essential for neutralizing free radicals, adsorbing or breaking down peroxides, and quenching the singlet and triplet forms of oxygen. Phenolic acids, flavonoids, and tannins are examples of phenolic compounds that have a variety of biological properties, including anti-inflammatory, antibacterial, and anticarcinogenic effects.

Table 2: Total phenolic contents of PDNM and PDHM samples

Conc. ($\mu\text{g} / \text{mL}$) Gallic acid	Total Phenolic Content (TPC) (mg GAE/100 mL)	
	PDNM	PDHM
1	4.095	3.714
2	4.738	5.024
4	5.833	9.143
8	7.476	6.452
10	9.881	8.071
20	13.762	7.214
40	18.262	7.690
80	18.738	5.667
100	23.048	12.476
200	35.976	10.595
400	67.143	9.452
800	95.762	13.048
1000	115.071	17.190

Mean values \pm standard deviation were presented (n = 3)

Milligram of Gallic acid equivalents (GAE)

Fig. 2: Total phenolic contents of PDNM and PDHM samples

Antimicrobial activity

Naturally produced chemical compounds have attracted significant interest from academic institutions and the pharmaceutical industry because of their proven effectiveness against a range of bacterial illnesses (Premalatha and Karthi, 2017). The development of resistance of pathogenic bacteria to traditional antimicrobials, which is mostly caused by the extensive abuse and overuse of antibiotics, is partly responsible for this increased attention (Salam *et al.*, 2023). Antibacterial activity was tested by measuring the clear zone developed in a petri dish. Methanol extracts of PDNM and PDHM exhibited dose-dependent antibacterial activity. PDNM exhibited the highest and lowest antibacterial activities against all tested microorganisms at concentrations of 25, 50, and 100 $\mu\text{g}/\text{ml}$ (Table 3). Previous studies using areca nut extracts tested antibacterial activity against two important and common human pathogens: *Staphylococcus aureus*, the cause of various infections such as boils, impetigo, food poisoning, cellulitis, and toxic shock syndrome (Steven *et al.*, 2015), and *E. coli*, which can cause severe food poisoning, septic shock, meningitis, or urinary tract infections (Mobley *et al.*, 2004). The results showed that both extracts formed the largest fungal inhibition zones against *Candida albicans* (6, 8 mm and 6, 11, 12 mm) at concentrations of 25, 50, and 100 $\mu\text{g}/\text{ml}$ (Table 3). The diameter of the bacterial growth inhibition zones of the control samples (Ampicillin, Clotrimazole, and Amphotericin B) was greater than that of the extracts (Plate 1). This is supported by previous studies such as the study by Jam *et al.* (2021), who found that methanolic fruit extract of *A. catechu* showed antibacterial activity against both Gram-positive and Gram-negative bacteria,

with the strongest activity occurring against *E. coli* (MIC 1.56 mg/ml). *E. coli* in PDNM produced significantly maximum zone of inhibition (6.8 mm) than the others at concentrations of 50 and 100µg/mL. According to a study by Chin *et al.* (2013), the ethanol extract of *A. catechu* nut was shown to be effective against *E. coli*, *Klebsiella pneumoniae*, *P. vulgaris*, *P. aeruginosa*, non-typhoidal *Salmonella*, and *Salmonella typhi*, *Salmonella flexneri* and *Vibrio cholerae*. The extract inhibited the growth of all microorganisms, with inhibition zones ranging from 7 to 18 mm. The highest activity was observed against *P. vulgaris* and *Vibrio cholerae*, with a mean zone of inhibition of 16–18 mm. For *S. aureus* and *M. canis*, there was no zone of inhibition in either the PDNM or PDHM extracts (Table 3, Plate 1).

Table 3: *In vitro* antimicrobial activity of PDNM and PDHM samples

S. No.	Organisms	Zone of inhibition (Radius in mm)						
		PDNM sample			PDHM sample			Standard (Ampicillin) (Clotrimazole) (Amphotericin B-) 1mg/600µl
		25 µg/ml	50 µg/ml	100 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	
1	<i>Escherichia coli</i>	0	6	8	0	0	0	14
2	<i>Klebsiella pneumoniae</i>	0	0	6	0	0	0	9
3	<i>Staphylococcus aureus</i>	0	0	0	0	0	0	16
4	<i>Candida albicans</i>	0	6	8	6	11	12	9
5	<i>Microsporium canis</i>	0	0	0	0	0	0	8

Data are expressed as mean ± SD (n = 3). ANOVA (p < 0.001).

Antibiotic resistance is a global issue that affects both economic growth and human health. Consequently, the development of new antimicrobial drugs is required to address this issue. Although they have remarkable antibacterial qualities, secondary antibiotic metabolites produced by a range of bacteria, actinomycetes, and fungi also have serious adverse effects in the human body and inevitably cause resistance (Yuan *et al.*, 2021). To lower the risk of infectious diseases in humans caused by pathogenic bacteria, fungi, viruses, and parasites, a great deal of research has been conducted to identify substances with strong antibacterial action (Mohamed *et al.*, 2020). Additionally, efforts have been directed toward creating natural compounds that may be useful. Eighty percent of the world's population uses plants to treat

a range of human ailments, and approximately fifty thousand plant species have been tested for their therapeutic qualities (Egamberdieva *et al.*, 2017). Plants can fend off pests and diseases before they cause significant harm, thanks to a variety of efficient defense mechanisms, particularly secondary metabolite production. Plant extracts remain a significant source of pharmaceutical chemicals, especially antimicrobial medications, for the treatment of infectious diseases.

Antimicrobial activities refer to the ability of substances to inhibit the growth of or destroy microorganisms such as bacteria, fungi, viruses, and parasites. These activities are critical in combating infectious diseases, preventing contamination, and promoting health across medical, agricultural, and industrial fields. Understanding the mechanisms behind antimicrobial effects, including disruption of microbial cell walls, inhibition of protein synthesis, and interference with metabolic pathways, is essential for developing effective agents. The increasing prevalence of antimicrobial resistance underscores the urgent need for novel compounds and strategies to maintain the efficacy of antimicrobial treatments.

The methanol extracts of *Pinanga dicksonii* nut (PDNM) and husk (PDHM) demonstrate significant antimicrobial potential, particularly against *Candida albicans*, with inhibition zones ranging from 6 to 12 mm at concentrations of 25, 50, and 100 µg/ml. Quantitative analysis reveals a notably high flavonoid content in PDNM (2458.5 mg RE/g) and PDHM (267 mg RE/g) at 1000 µg/ml, alongside substantial phenolic content (PDNM: 115,071 mg GAE/g extract; PDHM: 17,190 mg GAE/g). These bioactive compounds likely contribute to the observed antimicrobial activities. The data indicate that both the nut and husk of *P. dicksonii* are valuable sources of natural antimicrobial agents, aligning with broader evidence of plant-derived secondary metabolites offering effective antimicrobial properties.

Conclusion: The current research has found that the methanol extract of *P. dicksonii* (Roxb.) Blume contains high amounts of flavonoids and phenols. Therefore, this plant can be considered a potential natural source of therapeutically valuable metabolites that can be used to treat various diseases. However, further large-scale and comprehensive *in vivo* and clinical studies are needed to evaluate the safety and efficacy of this plant. Further research is needed to realize the full potential of *P. dicksonii* for pharmaceutical applications.

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