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Potentials of Date Palm (*Phoenix dactylifera* L.) and West Indian Wood Nettle (*Laportea aestuans* L.) as Feed Supplements: Insight into their Phytochemicals

Running title: Phytochemicals of Date Seeds and Nettle Leaves

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ABSTRACT

Nutritional supplementation is essential for maximizing livestock health and resilience to stress. In addition to supporting the livestock's immune system, stress management, and general performance, these supplements correct nutritional shortages. Phoenix dactylifera and Laportea aestuans are common fruit and a folk medicine that have been reported for its anticancer, anti-inflammatory and reproductive dysfunction in animals. The phytochemical compositions of Phoenix dactylifera seeds and Laportea aestuans leaves were determined using spectrophotometric method. Flavonoid contents ranged from 98.56 – 126.16 mg QE/g, phenolic acid contents ranged from 133.36 – 274.25 mg TAE/g, alkaloid contents ranged from 7.88 – 11.75 mg/g, and carotenoid content ranged from 30.17 – 240.11 µg/g. Phytochemical compositions of both plant samples are within the acceptable limit in animal nutrition. Recommendation: The inclusion of these plants in animal diets, especially as natural feed additives will have a beneficial impact on animal health, productivity, and the quality of animal-derived products.

Keywords: *Phytochemicals, Phoenix dactylifera, Laportea aestuans, Supplements, Animal nutrition*

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INTRODUCTION

Livestock nutrition presents a significant problem in Nigeria due to factors like high feed costs, scarcity of feed ingredients, and challenges in accessing nutritious feed sources. Nutritional supplementation is essential for maximizing livestock health and resilience to stress. In addition to supporting the livestock's immune system, stress management, and general performance, these supplements correct nutritional shortages. Because livestock are sometimes bred in controlled surroundings with little access to a variety of natural food sources, the significance of these supplements is particularly pertinent in aquaculture. Livestock that receive the right vitamins in their meals may develop more quickly, be healthier, and be more resilient to physiological and environmental stressors. There is a growing emphasis on sustainability in livestock nutrition, with increased use of plants and agricultural by-products (such as fruit residues) as alternative feed ingredients. These plants and by-products are rich in bioactive compounds and phytochemicals, offering both nutritional and health benefits while reducing waste and feed costs (Pugliese *et al.,* 2024).

Date fruits are the products of date palm tree (*Phoenix dactylifera* L.), belonging to the family Arecaceae. Date fruit has been reported to have significance in folk remedies for the treatment of various diseases like diabetes, obesity, cancer and heart diseases (Parvin *et al.,* 2015). Date palm fruit's significance as a nutrient source has drawn the interest of numerous researchers worldwide (Sheikh *et al.,* 2016; Al-Alawi *et al.,* 2017; Al-Shwyeh, 2019; Al-Okbi, 2022).

Laportea aestuans (West Indian Wood Nettle) is an annual herb belonging to family Urticaceae. *Laportea aestuans* has been identified as a rich source of bioactive compounds such as alkaloids, flavonoids, and tannins, which possess antimicrobial and antioxidant properties (Al-Mamary, 2015). The aerial part of *Laportea aestuans* (Urticaceae) commonly called Nettle or West Indian wood nettle is applied on wounds and serves as pain killer (Oloyede and Ayanbadejo, 2014) demonstrated antioxidant, antimicrobial and the anti-inflammatory (Adebajo *et al*., 1991; Oloyede and Ayanbadejo, 2014).

There is a strong focus on optimizing feed efficiency, especially in low- and middle-income countries, with plant by–products to make quality feed more accessible and affordable for smallholder farmers (Balehegn *et al.,* 2020). These plant and by-product are rich in dietary fiber, polyphenols, and other bioactive compounds. These components can promote animal health, act as functional feed additives, and enhance the nutritional value of livestock diets (Chuang *et al.,* 2021; Shah *et al.,* 2025). The growing interest in natural feed additives highlights the need for continued investigation into the most effective sources and formulations of flavonoids for different animal species (Faehnrich *et al*., 2015; Yang *et al*., 2022). This study was aimed at assessing the phytochemical compositions of *Phoenix dactylifera* seeds and *Laportea aestuans* leaves as a possible feed supplement in the diets of livestock.

MATERIALS AND METHODS

Materials collection

Phoenix dactylifera seeds and *Laportea aestuans* leaves were obtained around Nasarawa town and close to dumpsite, respectively. *Laportea aestuans* was identified at Herbarium Unit of Department of Botany, Ahmadu Bello University, Zaria.

Sample preparation

P. dactylifera seeds were prepared to remove dirt and bad seeds, the seeds were weighed and dried. The sun dried seeds were milled into fine flour with a feed mill and were air dried in a room. The flour was packaged in an air tight container until when it was used. *L. aestuans* leaves were picked to avoid dirt and dust. The leaves were air dried in a room and milled into fine flour with mortar and pestle. The flour was packaged in an air tight container until when it was used.

Determination of Phytochemicals

Preparation of rich polyphenol extracts of both samples

The rich phenolic extract was prepared according to the method of Bouhlali *et al*. (2017). Briefly, 30 g of sample powder was extracted with 150 ml of methanol–water (4:1, v/v), at 35°C for 12 h using an orbital shaker-incubator. The mixture was then filtered and the filtrate was concentrated under reduced pressure at 40°C until total evaporation of solvent using a rotary evaporator. The results of methanolic crude extract were kept at –20°C in dark glass bottles until use.

Determination of total phenolic compounds

The total phenolic contents in the samples were determined according to the method described by the International Organization for Standardization (ISO 14502-1). Briefly, 100 µL of the extract was added to 500 µL of a 1/10 dilution of Folin–Ciocalteu reagent in water, and then 400 µL of sodium carbonate solution (7.5% w/v) was added. The mixture was left for 60 min at room temperature and the absorbance was measured at 765 nm. The calibration curve was prepared using Gallic acid. The total phenolic compounds were expressed as Gallic acid equivalent in mg TAE/g dry weight (DW) the samples.

Determination of flavonoid content

The total flavonoid content of the samples was determined

by the method of Kim *et al*. (2003). One ml of the sample extract was mixed with 4 ml of distilled water. Then 0.3 ml of sodium nitrite solution (5%) was added, followed by 0.3 ml aluminum chloride solution (10%). Test tubes were incubated for 5 min at ambient temperature, then 2 ml of sodium hydroxide (1 M) was added to the mixture and then the final volume was made up to 10 ml with distilled water. The mixture was thoroughly vortexed and the absorbance was determined at 510 nm. Measurements were calibrated to a standard curve of prepared Rutin solution and the results were expressed in mg QE/g.

Determination of alkaloid content

The extract (5 mg/mL) was dissolved in dimethyl sulphoxide (DMSO); 1 mL of 2N HCl was added and filtered. The resultant mixture was transferred to a separating funnel; 5 mL each of bromocresol green solution and phosphate buffer was added. The mixture was shaken with 1, 2, 3 and 4 mL chloroform by vigorous shaking, collected in a 10-mL volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg /g of extract (Fazel *et al*., 2010).

Determination of carotenoid content

Carotenoid pigments were extracted by following general procedures (Mı´nguez-Mosquera and Hornero-Me´ndez, 1993; Rodriguez-Amaya, 2001). A homogenous representative sample (5–30 g) was ground with a mortar and pestle with enough cold acetone (4°C) for 15 min to soften the cell wall, which was subsequently filtered through filter paper. The residue was returned to the mortar and macerated again with fresh acetone. The extraction was repeated until exhaustion of colour was achieved (usually three times were enough). Finally, the mortar and filter were washed with a small amount of acetone which was collected with the rest of the extract. A concentration of 0.1% BHT was in acetone, which helped to protect the analytes from eventual oxidation. All operations were carried out under dimmed light to prevent isomerization and photodegradation of carotenoids. The acetone solution was partitioned, using diethyl ether, by adding 10% NaCl. The organic (upper) phase was washed with water, filtered over anhydrous sodium sulphate and evaporated under vacuum (T < 30 C). For saponification, 50 ml of petroleum ether containing 0.1% BHT were added to dissolve the residue, followed by an equal volume of 10% potassium hydroxide in methanol. The mixture was stirred overnight in the dark and under N2. The mixture was partitioned with water, and the upper phase washed with distilled water (four to five times) up to neutral pH of washings, then dried over anhydrous sodium sulphate. The organic phase was concentrated under vacuum (T < 30 C) and re-dissolved in 1 ml of HPLC grade acetone, and stored at 20 C prior to analysis. The analyses were carried out in duplicate.

Data analysis

Student's T-test was used to determine if there was significant difference ($P \leq 0.05$) between the phytochemicals of *Phoenix dactylifera* seeds and *Laportea aestuans* leaves using SPSS version 25.

RESULTS

The result for the phytochemical compositions of *P. dactylifera* seeds and *L. aestuans* leaves is presented in (Table 1). Flavonoid contents ranged from 98.56 – 126.16 mg QE/g, phenolic acid contents ranged from 133.36 – 274.25 mg TAE/g, alkaloid contents ranged from 7.88 – 11.75 mg/g, and carotenoid content ranged from 30.17 – 240.11 µg/g. Both samples have low alkaloid contents. Flavonoids, phenolic acid and alkaloid contents of *P. dactylifera* seeds are all significantly ($P<0.05$) higher than that of *L. aestuans* leaves. Carotenoids content of *L. aestuans* leaves is significantly ($P<0.05$) higher than that of *P. dactylifera* seeds.

DISCUSSION

Phytochemical compositions gotten from both *P. dactylifera* seeds and *L. aestuans* leaves are within the acceptable limit in animal nutrition (Righi *et al*., 2021; Rapisarda *et al*., 2023; Akter *et al*., 2024), except for carotenoids in *L. aestuans* leaves.

Flavonoids act as powerful antimicrobials, helping to control pathogenic microorganisms in the digestive tract. Their antioxidant activity protects animal tissues from oxidative stress, which is linked to improved overall health and immunity (Kalantar, 2018; Yang *et al*., 2022). Flavonoids and phenolic acids also enhance rumen fermentation in ruminant animals by increasing the production of volatile fatty acids while reducing ammonia and methane concentrations (Kalantar, 2018). Flavonoids have been suggested as an alternative to antibiotic growth promoters and has no associated risk of antibiotic residues (Kalantar, 2018). Dietary flavonoids, phenolic acids and carotenoids can improve the quality of animal products, such as increasing the antioxidant content in milk and meat, which can enhance shelf life and nutritional value for human consumers (Faehnrich *et al*., 2016; Kalantar, 2018; Vlaicu *et al*., 2023). As phytogenic pigments, flavonoids can influence feed intake and preferences in livestock, potentially improving feed efficiency and palatability (Faehnrich *et al*., 2016).

Phenolic acids contribute significantly to the antioxidant capacity of animal diets. This antioxidant activity helps

Table 1: Phytochemicals of *Phoenix dactylifera* seeds and *Laportea aestuans* leaves.

Parameters	*P. dactylifera*	*L. aestuans*	P value	Acceptable Limit
Flavonoids (mg QE/g)	126.16 ± 4.25	98.56 ± 2.13	0.028*	150 – 600 mg/kg (19, 21)
Phenolic acid (mg TAE/g)	274.25 ± 5.54	133.36 ± 1.45	0.002*	“
Alkaloids (mg/g)	11.75 ± 0.25	7.88 ± 0.38	0.013*	“
Carotenoids (µg/g)	30.17 ± 1.21	240.11 ± 4.11	0.000*	100 – 140 mg/kg (31)

P values with superscript (*) across rows vary significantly (P≥0.05)

protect animals from oxidative stress, which can improve overall health and productivity (Sinkovič *et al*., 2023; Šimić *et al*., 2025). Due to their beneficial effects, phenolic acids and related compounds are increasingly used as natural feed additives in animal nutrition to replace antibiotics and synthetic additives, aligning with consumer demand for more natural and health-promoting animal products (Kalantar, 2018).

Alkaloids are secondary metabolites found in many plants, including legumes and fodder species, which animals consume (Alarcón and Navarro, 2012; Kasprowicz-Potocka *et al*., 2022). They can influence animal health and nutrition in various ways. They have been reported to demonstrate pharmacological properties beneficial in animal nutrition. Dietary supplementation with berberine (an alkaloid) has been shown to improve growth performance, enhance oxidative and inflammatory markers, and mitigate metabolic dysfunctions in both monogastric and ruminant animals (Ghavipanje *et al*., 2023). Their presence in animal feed can influence the nutritional and health status of animals, which in turn affects the quality of animal-derived food products for human consumption (Cuchillo-Hilario *et al*., 2024).

Carotenoids play a significant role in animal nutrition due to their multiple beneficial effects on animal health, product quality, and overall nutrition. Carotenoids are phytogenic pigments responsible for coloring effects in plants, they serve as visual attractants to herbivores which influence feed intake and preferences in livestock, potentially improving feed efficiency (Faehnrich *et al*., 2016). Carotenoids act as antioxidants, protecting animals against stressors such as UV radiation, reactive oxygen species, and free radicals, which contributes to improved immune function and overall health (Faehnrich *et al*., 2016; de Carvalho and Caramujo, 2017). Carotenoids are also important for vision and act as precursors to molecules that regulate gene transcription and immune responses, thus supporting vital physiological functions in animals (de Carvalho and Caramujo, 2017). In fish and crustacean nutrition, carotenoids are routinely added to diets to promote development, survival, and health. For example, carotenoids improve pigmentation in farmed salmon and trout, which is important for market acceptance and consumer preference (de Carvalho and Caramujo, 2017).

The inclusion of plant materials with these phytochemicals in animal diets, especially as natural feed additives, offers a range of benefits that impact animal health, productivity, and the quality of animal-derived products. However, plants materials with phytochemicals like carotenoids should be added in smaller quantities to avoid exceeding the allowable limit.

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