Nigerian Research Journal of Chemical Sciences **(ISSN: 2682-6054)**
Volume 12, Issue 1, 2024

Evaluation of Antioxidant Capacity, Phytochemical and Antimicrobial Activities of the Methanol Extract of the Shell, Pulp, and Seed of *Balanites aegyptiaca*

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Accepted: May 29, 2024. Published Online: June 11, 2024

ABSTRACT

Numerous ethnobotanical investigations have revealed that *Balanites aegyptiaca* (desert date) possesses therapeutic characteristics, such as anthelminthic, purgative, leukoderma, and emetic effects. This study aims to evaluate the phytochemical, antioxidant and antimicrobial activities of the methanol extract of *Balanites aegyptiaca* seed, pulp, and shell. Samples were collected and extracted in methanol for 72 hours by cold maceration. The extracts were concentrated by using a rotary evaporator and analysed for their phytochemical constituents using standard methods. The Agar well diffusion method was used to measure the antibacterial activity of extracts against *Salmonella typhi, Escherichia coli,* and *Staphylococcus aureus* while chloramphenicol was used as a control. The antioxidant activities of the extract were evaluated based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The phytochemical screening demonstrated the presence of saponins, flavonoids, alkaloids, tannins, and phenol in the three samples. The DPPH antioxidant evaluation showed that pulp and shell had their highest activities at 0.5 mg/mL (76.72% and 72.33% respectively) while seed samples had their highest antioxidant activity at 0.25 mg/mL (76.53%). These compared favourably to the values from ascorbic acid used as a control. The least activities recorded for all samples were at 0.0625 mg/mL with the shell having the lowest activity (19.66%). *E. coli* was resistant to the extracts except that of seed. *S. aureus* was resistant to seed extract while *K. pnuemonae* was susceptible to the three extracts. Methanolic extracts of *Balanites aegyptiaca* parts may be selectively applied in industries due to their antioxidant, antibacterial activities, and phytochemical constituents.

Keywords: *Balanites aegyptiaca*, methanol extract, shell, pulp, seed, antimicrobial, antioxidant

INTRODUCTION

B*alanites aegyptiaca* (L.) Del. belongs to the Balanitaceea family—eleven intra-specific taxa and nine species make up the complete genus Balanites [1]. Balanites are used for various purposes and have a broad range of benefits, including food, fodder, shade, oil, and traditional medicine [2, 3]. But the fruits of the tree matter most [4] because of their edibility and use as beverage drinks. The fruit is a long, slender drupe that is 2.5 to 7 cm long and 1.5 to 4 cm in diameter. When ripe, the green, tomentose young fruits turn golden and glabrous. Its four layers are the flesh pulp (mesocarp), the woody shell (endocarp), the seed (kernel), and the outer skin (epicarp). Each of these can be applied to pharmaceutical and industrial uses [5]. Its pulp is edible and tastes bitter-sweet. The seed is pyrene, or stone, which is light brown, fibrous, and hard. It is 1.5 to 3 cm long. About 50–60% of the fruit is composed of seed.

Several ethnobotanical investigations have revealed that various parts of the plant possess therapeutic characteristics, including anthelmintic, purgative, leukoderma, and emetic effects [6, 7]. It also functions as an effective antidiabetic and antioxidant [8, 9], as well as an anticancer, antiviral, and antibacterial [10, 11].

Numerous phytochemical components have been documented to be present in both the fruit pulp and the seed of desert date (DD) [12]. The pharmacological properties of date seeds, such as their anti-inflammatory, antioxidant, antidiabetic, antibacterial, and antiviral properties, have been the subject of numerous studies [13]. However, the fruit's shell has received less attention. Thus, in addition to the pulp and seed, this study attempted to see the nutritional, pharmacological impact that the shell of DD fruit can have.

This study is aimed at comparing the phytochemical components, antibacterial activities, and antioxidant properties of the seed, the shell, and the pulp of this fruit. This will provide nutritional insight into the shell and the seed in comparison with the pulp, the former often thrown off as a wasteful part of the fruit.

Plate 1a: Fresh fruit of desert date

Plate 1b: Dry fruit of desert date

MATERIALS AND METHOD

Sample collection and treatment

Desert date fruits were collected from the medicinal plants garden at the Sheda Science and Technology Complex in Abuja, Nigeria. It was washed and dried. The shells were removed and blended to powder, and the fleshy pulp was scrapped from the seed. The seed was crushed and blended.

Extraction of treated samples

Approximately 50 g each of the samples were weighed and macerated in 250 mL methanol for 72 hours. The extracts were concentrated by using a rotary evaporator.

Antimicrobial studies

To make the stock solution, 100 mg of extracts were weighed into sterile tubes. About 2 mL of 10% dimethyl sulfoxide (DMSO) was used to dissolve the extracts and mixed thoroughly using a vortex machine. From the stock solution, different concentrations were made through serial dilution to obtain concentrations of 10 mg/mL, 5 mg/mL, and 2.5 mg/mL. Three microorganisms were obtained from the Biotechnology Advanced Research Center at the Sheda Science and Technology Complex: *Salmonella typhi, Escherichia coli,* and *Staphylococcus aureus.* The Agar well diffusion method was used to measure the antibacterial activity. On tryptic soy agar, all the bacteria were cultivated for a full day. After inoculating three colonies into Muller-Hinton broth and letting them incubate for four hours, the turbidity was measured using 0.5 MacFarland and adjusted as necessary. Each inoculum was applied to the Muller Hinton Agar (MHA) plates using a sterile swab. Using a 6 mm cup borer, four wells were drilled into the media. The wells were sealed with MHA, and the plates were left for fifteen minutes. A 100 µL aliquot of varying extract concentration was introduced into a distinct well and left there for 30 min. The fourth well served as the control and held 100 µL of chloramphenicol at a concentration of 10 mg/mL. The zone of inhibition on the plates was measured in millimeters after 18 to 24 hours of incubation [14].

Qualitative phytochemical screening

The extracts were screened for their phytochemical constituents using standard procedures previously reported by Fadeyi and Akiode [15].

Quantitative phytochemical analysis

Investigations were conducted to determine flavonoids [16], alkaloids [17], phenols [18], tannins [19], and saponins [20] in the samples.

Antioxidant capacity screening using the DPPH method

Following the method of Sungthong and Srichaikul [21], the protocol employed different concentrations of the extracts which were made from the stock solution of the extracts. About 0.5 mL of 0.1mM DPPH solution was introduced as the free radical. A blank solution was prepared. The samples were then incubated in a dark cupboard for 30 minutes. The extracts showed their radical scavenging capacity by bleaching the purple DPPH to orange. Absorbance was read at 517 nm on a UV-visible spectrophotometer (CECIL Aquarius). The extract's free radical scavenging prowess was calculated using the formula given in equation (1) for each extract concentration.

$$\%\text{Inhibition} = \frac{\text{Absorbance (blank)} - \text{Absorbance (sample)}}{\text{Absorbance (blank)}} \times 100 \quad (1)$$

RESULTS AND DISCUSSION

The phytochemical screening indicates the presence of saponins, cardiac glycosides, flavonoids, alkaloids, phenols, tannins, and terpenoids in the pulp, the seed, and the shell of the fruit (Table 1).

Table 1: Phytochemical screening

Phytochemicals	DD pulp	DD seed	DD shell
Saponins	+++	+++	++
Flavonoids	+	+	+
Alkaloids	+	+	+
Phenols	+	+	+
Tannins	+	+	+
Glycosides	+	+	+
Terpenoids	-	+	-
Cardiac glycosides	++	+	++

+++ = present; ++ = present; + = present; - = absent

The phytochemical quantification is presented in Table 2.

Table 2: Quantitative phytochemical analysis (mg/g)

Sample	Alkaloids	Saponins	Tannins	Flavonoids	Phenols
DD pulp	1.256	1.722	0.996	0.874	1.318
DD shell	0.222	1.838	0.532	0.994	1.370
DD seed	1.012	1.642	0.900	1.032	1.204

The tested parts of the fruit are rich in saponins and phenols. The redox characteristics of phenolic compounds found in herbs enable them to function as metal chelators, hydrogen donors, reducing agents, and free radical quenchers in addition to acting as antioxidants [22, 23]. Free radical damage to the body is prevented by flavonoids [24]. They are also used to

cure diarrhea [25], lower fever (antipyretic), relieve pain (analgesic), stop spasms (spasmolytic), and fight cancer. Important anticancer, antibacterial, antiallergic, anti-inflammatory, and antioxidant properties are found in phenolic and flavonoid molecules. They are essential to growth and reproduction. Additionally, they offer defense against dangerous pathogenic microorganisms and predators [26, 27]. It is well known that tannins have immune-stimulating properties. Tannins are crucial for accelerating the healing of wounds. It is also known that tannins function as the main scavengers of free radicals and antioxidants [28].

Table 3 shows the antioxidant measurement result of the tested parts of the fruit of desert date and ascorbic acid which was used as a control while Figures 1 and 2 demonstrate the comparison of the scavenging activities of the tested fruit's parts with the ascorbic acid used as control.

Table 3: Radical scavenging activities

Conc. (mg/mL)	DD seed	DD pulp	DD shell	AA
0.0625	52.48	22.14	19.66	80.89
0.125	72.71	45.23	55.53	79.65
0.25	76.53	63.93	65.84	79.32
0.5	73.66	76.72	72.33	77.43
1.0	66.79	72.32	70.80	78.61

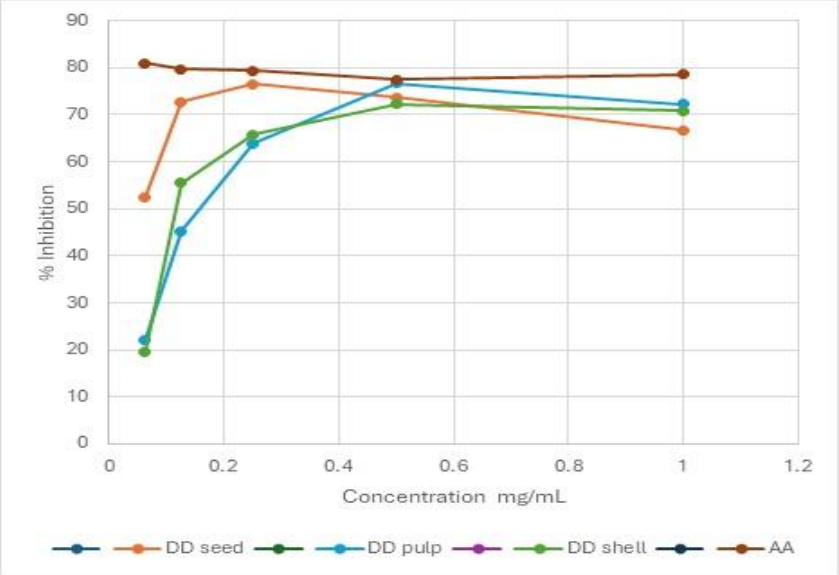

Figure 1: Antioxidant capacities of Seed, pulp, and shell of desert date, with ascorbic acid

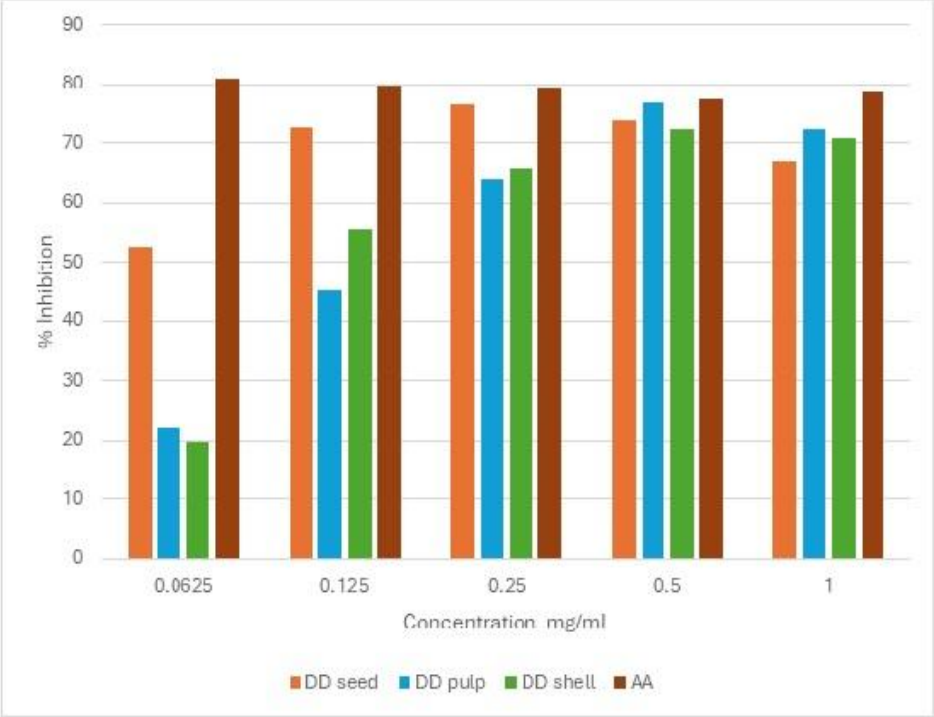

Figure 2: Comparative antioxidant capacities of the pulp, seed, and shell of desert date and ascorbic acid

From the result, both the seed, pulp, and shell exhibited good antioxidant capacity at high concentrations but at low concentrations, only the seed demonstrated good antioxidant power comparable to the standard used. The presence of flavonoids, tannins, polyphenols, phenolic compounds, or flavonoids may be the reason for the antioxidant activity shown in the methanol extracts. Phytochemicals like flavonoids and phenols are known natural antioxidants. Flavonoids may be better antioxidants than Vitamin C [29], hence the good antioxidant capacity of the seeds over the pulp and shell can be easily linked to the fact that the seeds contained the highest quantity of flavonoids compared to the other parts as shown in the quantitative phytochemical screening (Table 2).

The high level of phenols in the shell may also have contributed to the good antioxidant capacity it exhibited. This is consistent with the research that was carried out by Ahmad *et al* [30]. Alkaloids have stimulating, analgesic, and sedative effects. It is employed to treat hypertension. Alkaloids are nitrogen-containing chemical and natural substances that also have sedative and analgesic effects in addition to being physiologically active. They can help lessen the symptoms of depression and stress. Because of their stimulatory properties, which cause excitation linked to cell and nerve problems, alkaloids are typically toxic when consumed in large quantities [31].

The pharmacological activity of saponins, also known as triterpenoid or steroidal glycosides, has been demonstrated to have a range of effects, including antiallergic, antiphlogistic, cytotoxic, antitumor, antiviral, immunomodulatory, antihepatotoxic, molluscicidal, and antifungal properties [32]. Since saponins function as an adjuvant and enhance immune response, they are widely used in animal vaccinations. Many of these are helpful for intracellular protein molecules that are accessible to antibodies during intracellular histochemistry labeling. This indicates that phytochemicals dissolved readily in polar solvents and are more likely to scavenge free radicals such as DPPH. Table 4 displays the result of the antimicrobial study carried out using three test organisms and chloramphenicol as the control drug.

Table 4: Antibacterial activity

BACTERIA	DD SHELL (mg/mL)				DD PULP (mg/mL)				DD SEED (mg/mL)			
	100	50	25	C	100	50	25	C	100	50	25	C
ZONE OF INHIBITION in mm												
S. aureus	16	0	0	40	0	25	15	40	0	0	0	45
E. coli	0	0	0	30	0	0	0	35	20	24	0	35
K. pnuemonae	12	8	0	35	11	10	8	35	26	0	30	35

C= Control (Chloramphenicol)

E. coli was resistant to the shell and the pulp extracts but susceptible to the seed extract. However, both *K. pneumonia* and *S. aureus* were susceptible to the shell and the pulp extracts, especially at a high concentration of 100 mg/mL).

CONCLUSION

The methanolic extracts of the shell, pulp, and seeds of *Balanites aegyptiaca* have shown the presence of various bioactive phytochemicals as well as remarkable antioxidant activity against DPPH and moderate antibacterial activity. This shows that these parts of the fruit can be exploited to manage some diseases and lead to drug discovery and development. The study has revealed that the fruit's shell and seed components, which have been regarded as wastes, can now be put to useful use because of their phytochemical compositions and their biological activities. In addition, when used profitably, it will leave our surroundings cleaner

and advance the field of green chemistry and sustainable environmental practices. However, further studies such as the toxicity of the parts and chemical profiling may need to be conducted to fully exploit their usefulness to man.

Acknowledgment

The authors wish to acknowledge the Management of the Sheda Science and Technology Complex (SHESTCO) for the use of their laboratory at the Chemistry Advanced Research Centre (CARC).

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