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Comparative antifeedant activities of the epicarp and mesocarp methanolic extracts of the seeds of *Balanites aegyptiaca* against cowpea bean (*Vigna unguiculata*) storage pest (*Callosobruchus maculatus*)

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Abstract
Farmers in West Africa including northern Nigeria use various parts of plants to preserve cereals and legumes against damage by storage pest. Phytochemical screening of both the epicarp and mesocarp methanolic extracts of the seeds of *Balanites aegyptiaca* have been conducted and results indicated the presence of alkaloids, flavonoids, tannins, saponins, carbohydrates and terpenoids. In the present study the antifeedant efficacies of the methanolic extracts on cowpea bean (*Vigna unguiculata*) against the cowpea weevil storage pest, *Callosobruchus maculatus*, have been investigated**.** The test insects were cultured and the first generation progenies that emerged were used for the study. The beans were dressed with different doses (0.05, 0.10, 0.15, 0.20, and 0.25g) of the extracts and the weevil perforation index (WPI) was observed to have decreased as concentration of extracts increased. Compared to the control which has WPI of 50%, both extracts were found to be effective antifeedants with the mesocarp extract being more effective for the period taken for the study. The ability to protect the seeds from damage has been clearly shown by the percent protectant ability (PPA) whose value at 0.25g dose for the mesocarp extract was 100% while for the epicarp extract it was 81.18%. The mesocarp extract was therefore more effective than the epicarp extract at the highest dose used. The results of this study indicated that *Balanites aegyptiaca* seeds' mesocarp and epicarp extracts possessed antifeedant activity which may be associated with the presence of the secondary metabolites detected by the phytochemical screening.

Keywords: Antifeedant, cowpea beans, *Balanites aegyptiaca*, *Callosobruchus maculatus,* weevil-perforation-index, percent-protection-ability

1. Introduction

The cowpea beans, *Vigna unguiculata* (L.) Walp., is an important legume in West Africa and Nigeria accounts for about 70% of the world's production of cowpea beans [1]. However, cowpea beans are seriously damaged by insect pests of which the cowpea weevil, *Callosobruchus maculatus* is the most important [2]. The cowpea weevil is a major field to storage pest of cowpea with damage infestation starting in the field and progressing rapidly during storage. It causes significant losses in the seeds manifested by perforations and reduction in weight, market value and germinability [3]. The occurrence of this pest therefore constitutes the major storage problem contributing to huge food shortage in the production countries. It has been reported that at least 4% of the total annual production valued at over 30 million US dollars is an annual lost in Nigeria alone to this cowpea weevil [4].

It is important to acquire more improved traditional storage techniques so as to reduce this damage of cowpea beans by *C. maculatus*. Methods of protecting stored cowpeas is conventionally known to depend so much on the use of synthetic chemical insecticides. In Nigeria, fumigants like aluminum phosphide, dusts like Gammalin "A" dust and Lindane dust have been extensively used to control these pests [5]. Control of cowpea pests using chemical insecticides is, however, being discouraged because of health hazards to humans and environmental concerns amongst others [6]. Moreover, there is a distinction between antifeedants and insecticides. Insect antifeedants are compounds that temporarily or permanently reduce or prevent insects from feeding and therefore antifeedants differ from insecticides by their indirect, rather than direct, action. Thus an antifeedant should not kill the insect although death can be caused by starvation due to inhibited feeding.

An alternative approach for the reduction of the attack by the weevil is therefore the use of natural products of plants origin. Farmers in northern Nigeria indigenously use various plants to protect cereals and legumes against pest damage during storage. The low cost and safety of botanical extract is gaining more importance in controlling cowpea pests [7, 8] and it has been concluded that plant-derived extracts are environmentally safe, degradable, and target specific insecticides [9]. Plant powders' for antifeedant activity tests are easy to prepare and apply but extracts of fresh or dry plants materials are usually more effective than these powders [10]. Mostly the extracts are effective against adult beetles, being repellents or toxicants to the beetles. The need to study the effects of some solvent extracts of the *Balanites aegyptiaca* seeds has been realized. The present research, therefore, has evaluated and compared the antifeedant efficacies of the methanolic extracts of the epicarp and mesocarp of the seeds of *Balanites aegyptiaca*.

Balanites aegyptiaca Del. (Zygophyllaceae), popularly known by the English name "desert date", is also called "Kurna" among the Hausa people of West Africa. It is a widely grown tree found throughout Africa and is also found in the Middle East and India. The fruit or the seed of *Balanites aegyptiaca* is made up of four layers, namely the epicarp (the outer skin), the mesocarp (the fleshy pulp), the endocarp (which is the hard woody part of the seed) and the kernel (the inner part of the seed). Each seed, weighing 5-8 g, is composed of 5-9% epicarp, 28-33% mesocarp, 45-54% endocarp and 8-12% kernel [11]. The thick mesocarp is edible and the hard woody endocarp encloses an oil-rich seed kernel which is reported to be rich in saturated fatty acids and is used as cooking oil [12]. The seed kernel has also been reported to contain high amount of protein varying in amount with different sources [13].

All parts of *Balanites aegyptiaca* are been used in a variety of traditional medicines in many parts of the world particularly in Africa and Asia. Extracts from several parts of the plant were shown to exhibit antifeedant, antidiabetic, molluscicide, anthelmintic and contraceptive activities [14-18]. For the treatment of asthma, about 10 gm of seed powder is taken with water in the morning for 10 days [19]. The aqueous extract of the mesocarp was reported to have prominent antidiabetic effect in streptozotocin-induced diabetic mice [20, 21] and the aqueous suspensions of dried fruits are being used as abortifacient by local healers [22]. From this aqueous extract, two new steroidal saponins and two known saponins were isolated and identified. The individual saponins have shown no antidiabetic activity, but when combined these saponins produced a significant antidiabetic activity. Two known flavonol glycosides were also reported by the same study to have been isolated from the ethanolic extract of the epicarps. The plant contains phytochemicals including saponins, flavonoids and alkaloids which are claimed to contribute to its pharmacological activities including anti-inflammatory, analgesic, antioxidant, anti-tumor and antidiabetic [23]. However, saponins have been implicated as the main secondary metabolites behind most of the medicinal uses of *Balanites aegyptiaca*. These compounds are also considered to be the major effective component of many traditional medicines.

2. Materials and Methods

2.1. Experimental Cowpea

The cowpea beans, black-eyed, (*Vigna unguiculata* L. Walp.) used for this experiment were purchased from Wudil market, Kano State, Nigeria and the beans were left in their pods. The beans were only removed from the pods when needed for the insect culture and the antifeedant study.

2.2. Insect Culture

Adult insects (*Callosobruchus maculatus*) were collected from previously infested cowpea beans at Wudil market. Twenty fresh black-eyed peas (*Vigna unguiculata*) removed from bean pods were placed each in two different plastic Petri dishes (150 x 25 mm) and in each twenty insects (5 males and 5 females) were introduced to allow for mating and oviposition. The Petri dishes were covered with fine nylon mosquito netting held in place by means of rubber bands to prevent the escape of the insects but allow passage of air. The culture was maintained on a laboratory bench at room temperature (25-27 °C) with indirect outdoor window lighting and ambient humidity (ranging from 46%-60% RH) for 6 weeks. The parent insects were sieved out after oviposition and the first generation progeny that emerged were removed and used for the antifeedant study.

2.3. Preparation of Plant Material

Seven hundred and fifty grams (750 g) of sun-dried seeds (130 seeds) of *Balanites aegyptiaca* were secured and the epicarp removed manually. The epicarp was grounded to powder and the remains of the seeds were divided into two and packed in two 2L-volumetric flasks.

2.4. Extraction of Plant Material

250 g of the powdered epicarp were put in a maceration bottle and covered with 1000ml of methanol for 72 hr at room temperature. The methanol epicarp extract was filtered and another 500ml of the methanol was again poured onto the residue and extracted for another 48 hr; the extract again was filtered into the previous one to get the epicarp methanolic extract. The remains of the seeds packed in the two 2L-volumetric flasks were covered each with 1200ml of methanol. Three days (72 hr) were allowed before filtering and 1000ml of methanol added to extract more for two more days. The two filtrates were combined to afford the mesocarp methanolic extract. The solvent of the two extracts was removed under reduced pressure using a rotary evaporator.

2.5. Phytochemical Screening

Phytochemical screening using standard chemical tests [24] was carried out on the epicarp and mesocarp methanolic extracts of the seeds of *Balanites aegyptiaca* to investigate the presence of alkaloids, terpenoids, tannins, saponins, carbohydrates, steroids, flavonoids and cardiac glycosides.

2.6. Antifeedant Bioassay

Five different doses (0.05, 0.10, 0.15, 0.20, and 0.25g) of each of the epicarp and mesocarp methanolic extracts were weighed and dissolved in 6ml of acetone. For each dose of the extracts, 20 cowpea beans were placed in a beaker. The beans were then dressed with the prepared acetone solutions. Another 20 beans were dressed with acetone only as a negative control. Five males and five females of newly emerged cowpea weevils were then introduced into each of the eleven beakers of the test samples (five for epicarp and five for mesocarp methanolic extracts) plus that of control. All the beakers were covered with a net that allows the passage of air but prevents the escape of the insect. The eleven beakers were allowed to stay for seven weeks and the numbers of infested and un-infested beans were counted in each beaker including the control. Weevil Perforation Index (WPI), which measures the protection ability of the treatment materials, has been calculated according to one standard method [25].

Weevil Perforation Index value exceeding 50% is regarded as enhancement of infestation by the weevil or negative ability of the plant material tested. To obtain the Percent Protection Ability (PPA), the value of WPI is subtracted from 100.

$$WPI = \frac{\text{\% Number of infested seeds}}{\text{\% Number of infested seeds} + \text{\% Number of infested seeds in control}} \times 100$$

3. Results and Discussion

3.1. Phytochemical Screening

The results of the phytochemical screening, as shown in table 1 below, have indicated the presence of alkaloids, flavonoids, steroids, saponins, carbohydrates, tannins and terpenoids in both the methanolic extracts of the epicarp and mesocarp of the seeds of *Balanites aegyptiaca* except that tannins was not indicated in the epicarp extract and cardiac glycosides were not found in both extracts.

Table 1: Phytochemical Screening of Methanolic Extracts of Epicarp and Mesocarp

Phytochemical	Methanolic epicarp extract	Methanolic mesocarp extract
Alkaloids	+	+
Steroids	+	+
Flavonoids	+	+
Cardiac glycoside	-	-
Terpenoids	+	+
Saponins	+	+
Tannins	-	+
Carbohydrates	+	+

Key: + = detected, - = not detected

3.2. Antifeedant Bioassay

From table 2 below, the results obtained for the antifeedant bioassay of both the methanolic extracts of the epicarp and mesocarp of the seeds of *Balanites aegyptiaca* revealed that the Weevil Perforation Index (WPI) is inversely proportional to the concentration of the extracts. This, on the other hand, indicates that both extracts were effective at high dosage rate and so higher concentration is required for higher antifeedant activity.

Table 2: Effects Methanolic Extracts of Epicarp and Mesocarp of *Balanites aegyptiaca* seeds on Cowpea seed damage

Extract/Control	Concentration (g/20 seeds)	Number of infested	% number of infested	WPI	PPA
Epicarp methanolic extract	0.05	15	75	44.12	55.88
	0.10	12	60	35.29	64.71
	0.15	9	45	26.47	73.53
	0.20	7	35	20.59	79.41
	0.25	3	15	18.82	81.18
Mesocarp methanolic extract	0.05	7	35	20.59	79.41
	0.10	5	25	14.71	85.29
	0.15	2	10	5.88	94.12
	0.20	1	5	2.94	97.06
	0.25	0	0	0.00	100
Control	0.00	19	95	50.00	50.00

Key: WPI = Weevil Perforation Index; PPA = Percent Protectant Ability

As indicated in table 2, in all treatments the antifeedant activity was directly proportional to the concentration of the extract. From the table the Weevil Perforation Index (WPI) for epicarp methanolic extract decreases from 44.12% to 18.82% as the dosage level increases from 0.05g to 0.25g. The same trend was also observed for the mesocarp extract whose WPI decreases from 20.59% to 0.00% as concentration increases from 0.05g to 0.25g, indicating that the mesocarp extract to be much more effective than the epicarp extract at the highest dose. The ability to protect the seeds from damage has been clearly shown by the Percent Protectant Ability (PPA) whose value at 0.25g dose for mesocarp extract was 100% while for epicarp extract it was 81.18%. The two extracts are therefore found to serve as antifeedants against the cowpea weevil storage pest when compared to the control which has the highest WPI of 50% for the five weeks period allowed for the study. The Weevil Perforation Index indicates the ability of an extract or insecticide to protect the cowpea beans from damage and values of this index exceeding 50% is regarded as enhancement of infestation by the weevil or negative ability of the plant material [26].

Secondary metabolites such as phenolic compounds, alkaloids, flavonoids and terpenoids have been identified to exhibit feeding deterrent activity [27, 28]. As has been indicated in table 1, both the epicarp and mesocarp methanolic extracts contained alkaloids, flavonoids, terpenoids and saponins. Flavonoids have been considered as one of the plant's defensive systems against phytophagous insects [29] and together with their related compounds, flavonoids have also been indicated to possess antifeedant activities against the subterranean termite *Coptotermes formosanus* [30]. Structure-activity relationship study of these compounds showed that the pyran ring was responsible for the antifeedant activity whereas the absence of this ring increased feeding-preference activity. Saponins have also been reported to exhibit clear insecticidal properties in several pest insects [31, 32] and it is also contributing in the plant's defense. Some of the reported observed effects of saponins are increased mortality, lowered food intake, weight reduction, retardation in development and decreased reproduction [32]. Terpenoids, among other secondary metabolites, have been reported to be the most successful pesticides [28] and the tricyclic sesquiterpenes isolated from *Senecio palmensis* has been reported to have high antifeedant potency on the Colorado potato beetle, *Leptinotarsa decemlineata* [33]. The alkaloids isolated from the seeds, seed pods and flowers of *Erythrina latissima* were reported to have antifeedant activities against *Spodoptera littoralis* [34]. Some of these secondary metabolites could be responsible for the antifeedant property observed in the methanolic extracts of both epicarp and mesocarp of *Balanites aegyptiaca* seeds. In fact complex mixtures of secondary compounds in plant extracts has been reported to contribute to a great deal for synergism, which enhances the joint action of active compounds against insect and reduces the rate of resistance development [35].

4. Conclusion

The results of antifeedant bioassay has clearly indicated that the two layers, epicarp and mesocarp, of the *Balanites aegyptiaca* seed possess dose dependent antifeedant activity with the mesocarp exhibiting the highest activity. The methanolic extracts of both layers of the seeds have shown to contain the phytochemicals alkaloids, flavonoids, steroids, terpenoids and saponins. The presence of these secondary metabolites may be responsible for the observed antifeedant activities. Moreover, mixtures of secondary metabolites in plant extracts in most cases contribute to a great deal for synergism, thereby enhancing the joint action of active compounds against insect and sometimes reduces the rate of resistance development.

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