



Anti-Diabetic Efficacy and Phytochemical Screening of Methanolic Leaf Extract of Pawpaw (*Carica papaya*) Grown in North Central Nigeria.

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Abstract: *Carica papaya* leaf samples (Green) were freshly harvested and dried for six consecutive days. The leaves were extracted with methanol; the resulting extracts were screened for the phytochemical constituents using a standard procedure. Phytochemical screening revealed the presence of bioactive compounds such as tannins, saponins, terpenoids, glycosides, and alkaloids. The *in vitro* anti-diabetic potential of the plant was also determined so as to justify the traditional usage of the plant in treating diabetes. The result of the present study confirmed that the methanolic extract of *C. papaya* leaves possess significant anti-diabetic activity *in vitro*, this shows that the leaves has the potential for the development of drugs in combating diabetes.

Keywords: *Carica papaya* leaves; phytochemical screening; bioactive substances; anti-diabetic property; *in-vitro* analysis.

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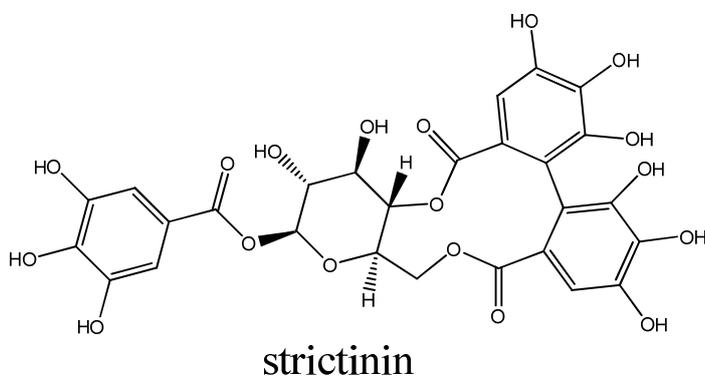
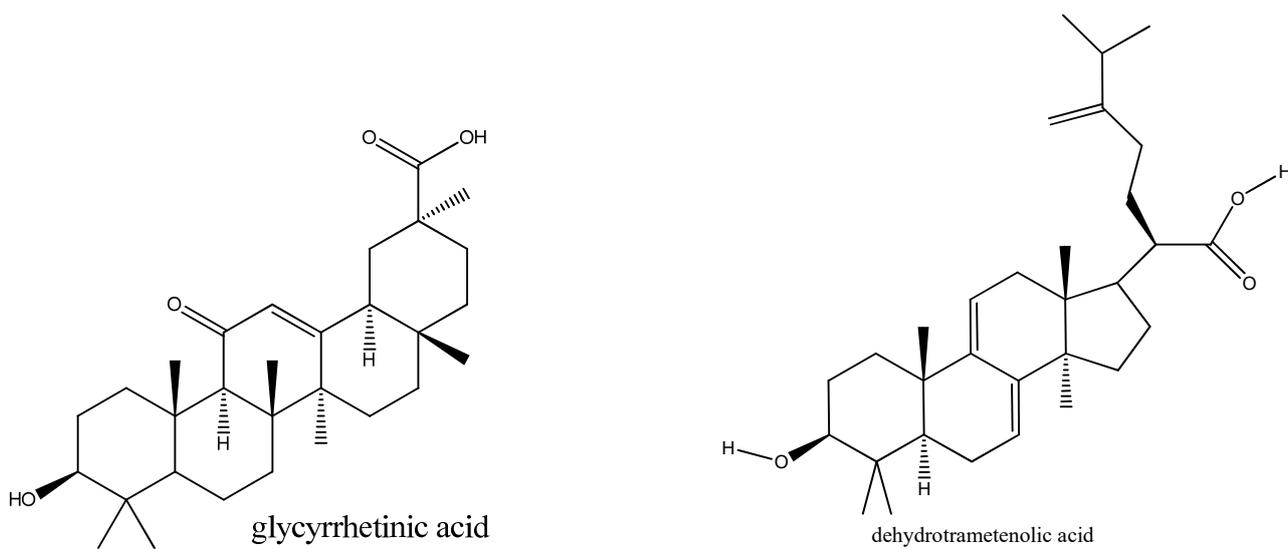
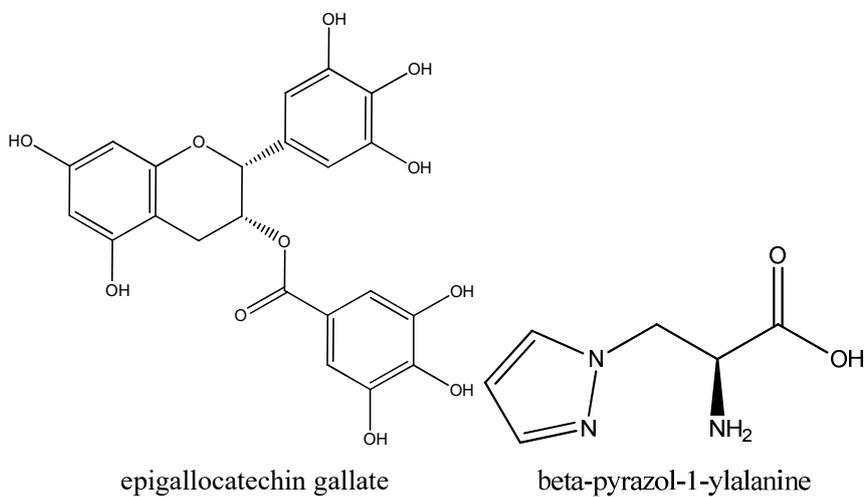
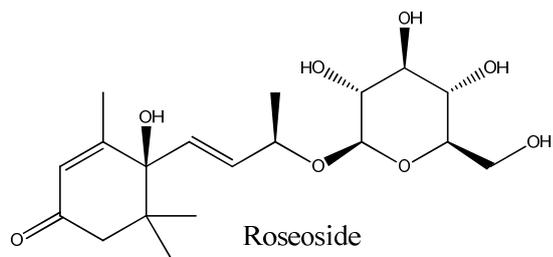
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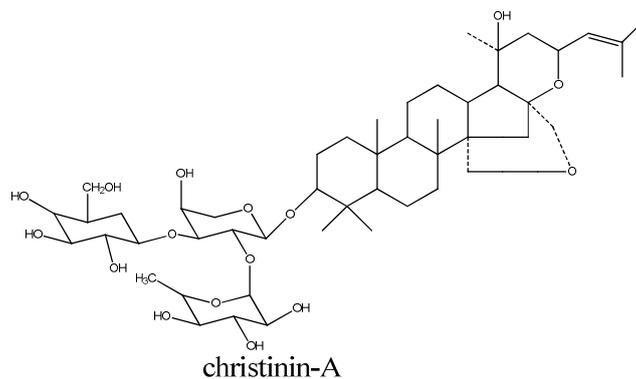
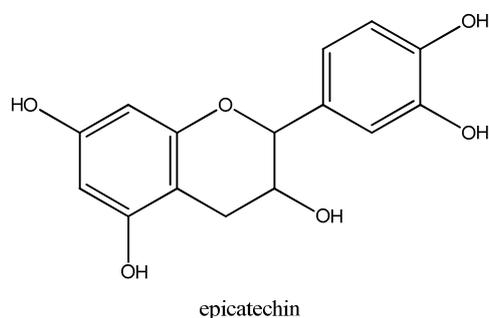
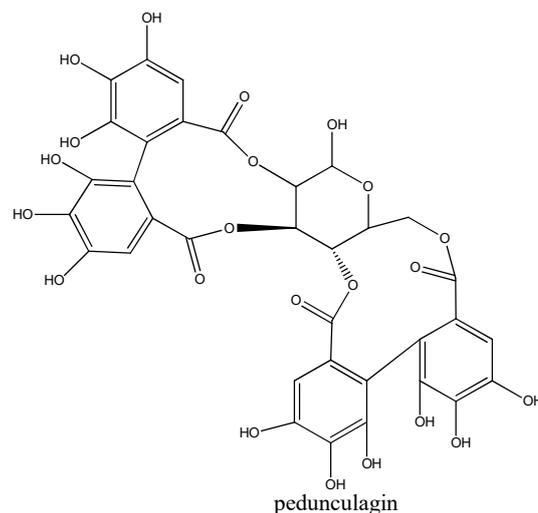
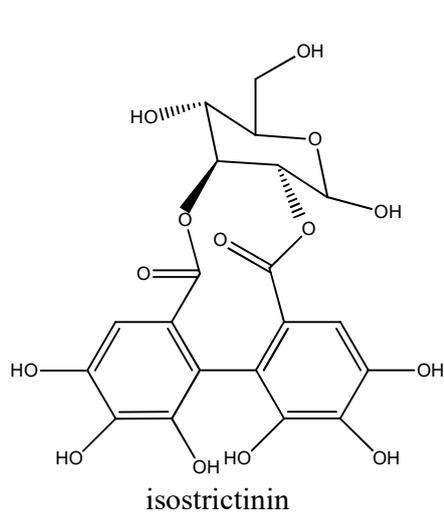
INTRODUCTION

Human beings have been plagued with diverse types of diseases such as HIV/AIDS, cancer, cholera, hepatitis, leprosy, measles, and diabetes among others. Diabetes mellitus is among the leading causes of death worldwide and it is prevalent in developing nations which may be partly due to the nature of the diets in this part of the world and other factors such as heredity and environmental factors. (1). This disease is caused by an unusual high concentration of sugar in the blood which might be a direct consequence of deficiency of insulin (2). The control or the cure of diabetes without side effects is a serious challenge militating against the development of anti-diabetic therapeutics over the centuries. Of core importance to the development of an anti-diabetic therapeutic is controlling hyperglycaemia, which is the milestone of diabetes and its complications. Thus, controlling excess sugar in the blood is the most crucial aim in reducing the risks associated with diabetes and its complications (3). One of the major means of treating the disease includes, but not limited to, insulin and various oral anti-diabetic agents such as sulfonylureas, biguanides and glinides. However, these agents are not devoid of adverse effects as such, there has been renewed search for hypoglycaemic agents with far lesser or no effect whatsoever.

Diabetes mellitus is an age-long disease and medicinal plant extracts have remained rich repository of therapeutically useful phytoconstituents and viable alternative in combating various diseases plaguing the mankind. There are some plants which possess bioactive compounds which have been reported to be used in combating diabetes worldwide and have been used in treating diabetes. Anti-hyperglycaemic properties of the plants are in direct relationship with their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes (4).

Structures of some anti-diabetic compounds that have been successfully isolated from different plants are given below:





Plants are normally made use of in folk medicine to combat different ailments among which diabetes belong to, such plants include; *Allium cepa* (Onions), *Cynodon dactylon*, *Clerodendron phlomoides*, *Cinnamomum tamala*, *Coccinia indica*, *Enicostemma littorale*, *Ficus bengalensis*, *Gymnema sylvestre* leaves (Gurnar), *Momordica charantia* (Bitter melon), *Pterocarpus marsupium*, *Syzygium cumini* (6,7), *Vernonia amygdalina*, *Pandanus amaryfollius* (Pandanus), *Ocimum basilicum* L. (*Lamiaceae*), *Phyllanthus niruri* L. (*Euphorbiaceae*), *Viscum album* L. (*Viscaceae* or *Loranthaceae*) (8), *Allium sativum* (Garlic), *Aloe vera*, *Cinnamomum cassie*, *Catharanthus roseus* (Madagascar periwinkle), *Murraya komingii*, *Ocimum santum*, *panax ginseng*, *Trigonella foenum-graecum* (Fenugreek), *Pterocarpus marsupium* (Indian kino) and *Syzygium cumini* (9-11). The isolated compounds from these plants are currently undergoing clinical trials so as to ascertain their efficacy and conformability to the standard.

Medicinal plants that have been confirmed to possess anti-diabetic properties in Nigeria are- *Vinca major* L, *Azadirachta indica*, *Carica papaya*, *Gongronema latifolium*, *Securridaca lanpedunculata*, *Sclerocarya birrea* [CA. Rich], *Momordica balsamina* L, *Hypoxis*

hemerocallidea (Africa potato), *Harpagophyllum procumbens* DC, *Cluseria anisata* (wild), *Cissampelos capensis*, *Brachylaena discolor*, (12-17).

Quite a number of researches have been conducted on various parts of *Carica papaya* family Caricaceae. The plant is home to various types of bioactive compounds (18). The leaves of *Carica papaya* are used traditionally to treat numerous ailments such as malaria, dengue, jaundice, immunomodulatory, and antiviral activity (19). Other diseases that have been reported to be controlled by *Carica papaya* traditionally in Nigeria includes; abdominal discomfort, pain, malaria, diabetes, obesity, infections, and oral drug poisonings. Adeneye and Olagunju, 2009 and Ahmad *et al.*, 2011 reported the therapeutic potential of *Carica papaya* on dengue and malaria while Owoyele *et al.*, 2008 confirmed the anti-inflammatory potency of the plant, similarly, Aruoma reported the effect of *Carica papaya* fruits and leaves on the sugar reducing tendency of the plant (20-23).

Despite the enormous work which have been done to probe the anti-diabetic potency of various parts of *C. papaya* over the world, report is scanty on the efficacy of the leaves of this plant in lowering the abnormally high blood sugar usually associated with diabetes hence, the present study aimed at investigating the phytochemical profile and also evaluate *the in vitro anti-diabetic potency of C. papaya leaves*.

MATERIALS AND METHODS

Sample collection

Green leaves of *Carica papaya* were collected from Islamic village in Ilorin, Ilorin West Local Government, Kwara State, Nigeria. The plant was authenticated at the herbarium of Plant and Environmental Biology Department, Kwara State University Malete, Nigeria.

Preparation of Sample

The leaves were dried under ambient conditions for six consecutive days and subsequently pulverized. A 70-gram-sample of the pulverized leaves was extracted with methanol for 72 hours and the resultant crude extract obtained was filtered. The crude extract was concentrated under reduced pressure using rotary evaporator to obtain a solvent-free methanol extract and stored in an air-tight bottle for further analyses.

Phytochemical analysis

The qualitative phytochemical tests were carried out to determine the presence and absence of various phytochemical constituents such as of alkaloids, saponins, glycosides, terpenoids, and tannins using the standard method (24).

***In vitro* anti-diabetic activity test**

The *Carica papaya* leaf extract was subjected to non-enzymatic glycosylation of haemoglobin assay, and α -amylase inhibition assay. Anti-diabetic efficacy of *C. papaya* was carried out by estimating the degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically at 520 nm. Glucose (2%), haemoglobin (0.06%) and gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M and pH 7.4. 1 mL each of above solution was mixed. 1 mL of the concentrated extract was added to the mixture. It was incubated in dark at ambient condition for 72 hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520 nm. % inhibition was calculated using standard literature procedure (34). α -Tocopherol (Trolox) was used as a standard drug for assay (25).

Alpha amylase inhibition assay

A starch solution (0.1% w/v) was obtained by stirring 0.1 g of potato starch in 100 mL of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha amylase in 100 mL of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartrate solution and 3, 5-dinitrosalicylic acid solution at 96 mM. In alpha amylase inhibition method, 1 mL of substrate - potato starch (1% w/v), 1 mL of plant extract of four different concentrations (40, 60, 80, and 100 μ g/mL), 1 mL of alpha amylase enzyme (1% w/v) and 2 mL of acetate buffer (0.1 M, pH 7.2) were added. Both control and plant extracts were added with starch solution and left to react with α - amylase solution under alkaline conditions at 25°C. The reaction was monitored for 3 minutes. The generation of maltose was quantified by the reduction of 3, 5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid. This reaction is detectable at 540 nm (26).

Glucose uptake in yeast cells

Commercial baker's yeast was washed by repeated centrifugation (3000 g; 5 mins) with distilled water until the supernatant fluids were clean and 10 % (v/v) suspension was prepared in distilled water. Various concentrations of extracts (1-5 mg) were added to 1 mL of glucose solution (5, 10 and 25 mM) and incubated together for 10 mins at 37 °C. Reaction started by adding 100 mL of yeast suspension, vortexed and further incubated at 37 °C for 60 mins. After that time, the tubes were centrifuged (2500 g, 5 min) and glucose was estimated in the supernatant. Metronidazole was taken as standard drug (27). The increase in the percentage of glucose uptake by yeast cells was calculated based on standard literature procedure.

RESULTS AND DISCUSSION

Phytochemical analysis

The result of the qualitative phytochemical screening (secondary metabolites) present in the methanolic extract of *C. papaya* leaves is presented in Table 1. The screening revealed the presence of various bioactive constituents like polyphenol, tannins, saponins, terpenoids, flavonoids, glycosides and alkaloids. Anthocyanin was absent in the leaves studied. This result is in concordance with the reports (28-31). Various medicinal values of plants are functions of the phytochemicals that are present in such plant and may therefore be responsible for their anti-hyperglycemic properties.

Ayoola & Adeyeye reported (30) that saponins, cardiac glycosides, and alkaloids are the major bioactive components present in the leaves of *Carica papaya*. The presence of saponins supports the fact that pawpaw leaf has cytotoxic effects (32). The presence of alkaloids in the leaves shows that this plant can be effective anti-malarial, since alkaloids consist of quinine, which is an anti-malarial drug (33).

Table 1. Phytochemical screening of methanolic extracts of *Carica papaya* extract.

Phytochemical compounds	MTH/CP
Polyphenol & Tannins	+
Flavonoids	+
Saponins	+
Terpenoids	+
Anthocyanins	-
Glycosides	++
Alkaloids	+

Key terms: +=trace amount, ++= strongly present, -= absent, MTH/CP= Methanolic extract of *Carica papaya*

α -amylase inhibitory effect of methanolic *C.papaya* extract

Alpha-amylase is a digestive enzyme found in the secretions of the intestinal mucosa, pancreas, and the saliva. It is responsible for the breakdown of α -1, 4-glycosidic bonds in starch. Thus, the catalytic activities of the enzyme (especially in the small intestine), increase the availability of glucose in the blood, since the pH of the intestine is around 6.9, α -amylase has access to starch at this pH and catalyses the breakdown of this polysaccharide into monosaccharide and disaccharide. At a concentration of 100 $\mu\text{g/mL}$, *C. papaya* leaf shows the highest percentage of inhibition of 25.2%. There was a dose dependent increase in percentage inhibitory activity against α -amylase enzyme as shown in Table 2. From the data, the results suggest that the extract possesses significant inhibitory activity since the percentage inhibition varied from 19.63 to 25.2 in the concentration range of 80 $\mu\text{g/mL}$ - 100 $\mu\text{g/mL}$ which was higher when compared to that of the standard drug within the same range. The α -amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates (36).

Table 2. Effect of methanol extract of *Carica papaya* leaf on α -amylase inhibition.

Concentration ($\mu\text{g/mL}$)	Standard % Inhibition	Methanolic extract of <i>Carica papaya</i> leaf % Inhibition
20	5.65%	9.6%
40	9.58%	13.2%
60	13.20%	16.54%
80	13.20%	19.63%
100	22.50%	25.2%

Values are expressed as mean \pm SMD. Absorbance of blank= 0.217 \pm 0.00

Glucose uptake action of methanolic *C.papaya* extract

Glucose transport across yeast cellular membrane occurs via facilitated diffusion down concentration gradient. The rate of glucose transport across the membrane in yeast cell system at different glucose concentrations, *i.e.* 20, 40, 60, 80, and 100 $\mu\text{g/mL}$ respectively, is

presented in Table 3. The mechanism of glucose transport across the yeast cell membrane has been receiving attention as *in vitro* screening method for hypoglycaemic effect of various compounds present in medicinal plants. Recent studies on the transport of non metabolizable sugars and certain metabolizable glycosides suggest that sugar transport across the yeast cell membrane is mediated by stereospecific membrane carriers. This implies that the dose-dependent increase in the glucose uptake of yeast (Table 3) is a reflection of the extract's ability to enhance the utilization of glucose by yeast. This is because transport of glucose is only attained (and continued) if there is removal of intracellular glucose. The result obtained suggest that methanol extract of *C. papaya* leaves were capable of enhancing glucose uptake (utilization) thereby controlling the blood glucose level (25).

Table 3. Effect of methanolic extract of *Carica papaya* leaf on the uptake of glucose by yeast cells.

Concentration ($\mu\text{g/mL}$)	Methanolic extract of <i>Carica papaya</i> leaf
% Glucose uptake	
20	9.17%
40	16.2%
60	16.2%
80	19.3%
100	24.82%

Values are expressed as mean \pm SMD. Absorbance of blank=0.225 \pm 0.00

***In vitro* non-enzymatic glycosylation of haemoglobin**

The inhibitory effect of methanolic *C. papaya* samples on non-enzymatic glycosylation of haemoglobin is shown in Table 4. *C. papaya* exhibits a concentration-dependent increase in % inhibition of glycosylation, which suggests that the plant extract decreases the formation of the glucose haemoglobin complication and thus the amount of free haemoglobin increases. Interestingly, at a concentration of 100 $\mu\text{g/mL}$, *C. papaya* showed the highest percentage inhibition of 20.6% which compared favorably with that of the standard. Increased concentration of glucose in the blood leads to its binding to haemoglobin which may result in the formation of the reactive oxygen species (37).

Table 4. Effect of methanolic extract of *C. papaya* leaf extract on haemoglobin glycosylation.

Concentration ($\mu\text{g/mL}$)	Standard % Inhibition	Methanolic extract of <i>Carica papaya</i> leaf % Inhibition
20	5.38%	5.38%
40	8.8%	12.1%
60	17.8%	15.2%
80	23.1%	18%
100	29.7%	20.6%

Values are expressed as mean \pm SMD. Absorbance of blank= 0.246 ± 0.00

CONCLUSION

The result of the present study demonstrated that the qualitative phytochemical analysis of methanolic extract of *C. papaya* leaves confirmed the presence of the following phytoconstituents; polyphenol, tannins, saponins, terpenoids, flavonoids, glycosides, and alkaloids. The *in vitro* anti-diabetic potential of the plant extract was confirmed through α -amylase inhibition, glucose uptake by yeast cells and non-enzymatic glycosylation methods. The result of this research confirms the claim of Yoruba herbalists in using the leaves of the plant to control diabetes, however; further exploration of the various bioactive compounds should be isolated and developed into drugs to combat the disease. The result of the present study confirmed that the methanolic extract of *C. papaya* leaves possess significant anti-diabetic activity *in vitro*, this shows that the leaves has the potential for the development of drugs in combating diabetes.

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Türkçe Öz ve Anahtar Kelimeler

Anti-Diabetic Efficacy and Phytochemical Screening of Methanolic Leaf Extract of Pawpaw (*Carica papaya*) Grown in North Central Nigeria.

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Öz: *Carica papaya* yaprak örnekleri (Yeşil) taze iken toplanmış ve altı tam gün kurutulmuştur. Yapraklar metanolle ekstrakte edilmiştir; elde edilen ekstraktlar standart bir prosedür kullanarak fitokimyasal içerik bakımından gözden geçirilmiştir. Fitokimyasal görüntülemeye göre tanenler, saponinler, terpenoidler, glikozidler ve alkaloidler gibi biyoaktif maddelerin varlığı ortaya konmuştur. Bitkinin *in vitro* antidiyabetik potansiyeli, geleneksel kullanımı doğrulamak amacıyla belirlenmiştir. Bu çalışmanın sonucuna göre *C. papaya* yapraklarının *in vitro*'da belirgin antidiyabetik aktivite gösterdiği ortaya konmuştur, buna göre, bitkinin yaprakları diyabetle savaşta kullanılacak ilaçların geliştirilmesi için potansiyele sahiptir.

Anahtar kelimeler: *Carica papaya* yaprakları; fitokimyasal görüntüleme; biyoaktif maddeler; anti-diyabetik maddeler; *in-vitro* inceleme.

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