

Research Article



Evaluation of Some Nigerian Savannah Plants for Antioxidant Activity, and Total Phenolic and Flavonoid Contents

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ABSTRACT

The plant leaves of *Nauclea diderrichii* (Merr) (**ND**), *Crossopteryx febrifuga* (Afzel) Benth (**CF**), *Lannea kerstingii* Engl. and K. Krause (**LK**), *Uapaca guineensis* (**UG**) and *Terminalia macroptera* (**TM**) which were obtained from the savannah region in Nigeria were evaluated for antioxidant property and total phenolic and flavonoid contents. The plants leaves were extracted with non polar and polar solvents. The extracts obtained were analyzed for the presence or absence of certain phytochemicals. DPPH radical was used as an in-vitro model for evaluating the antioxidant activity of the methanolic extracts. Spectrophotometric methods were used to determine both total phenolic and flavonoid contents. TLC analysis was carried out to identify and quantify individual flavonoids using specific standard flavonoids as control. Phytochemical screening of the plants leaves gave the following: **ND** and **LK** gave positive test for tannins, flavonoids and saponins while steroids, alkaloids, terpenoids, cardiac glycosides and anthraquinone glycosides were negative for the methanolic extracts; all phytochemicals screened were absent in the n-hexane extracts. **CF** showed presence of flavonoids, saponins, steroids and cardiac glycosides while tannins, alkaloids, terpenoids and anthraquinone glycosides were absent in the methanol extract; all phytochemicals screened were absent in the n-hexane extract. The n-hexane extract of **UG** exhibited presence of steroids, alkaloids and terpenoids while flavonoids, saponins, cardiac and anthraquinone glycosides were observed to be absent. Its methanolic extract showed presence of steroids, alkaloids, terpenoids and cardiac glycosides while saponins, flavonoids, tannins and anthraquinone glycosides were absent. Only steroids were observed to be present in the n-hexane extract of **TM**. Tannins, flavonoids, saponins, steroids, terpenoids and cardiac glycosides were present in the methanolic extract while alkaloids and anthraquinone glycosides were absent. The plants leaves extracts gave the following total phenolic (mg GAE/mg extract) and flavonoid (mg quercetin equivalent/mg extract) contents and antioxidant activity respectively: **ND** 49.43±0.15, 73.00±0.03, 37.42±0.04; **CF** 63.71±0.02, 81.58±0.06, 48.23±0.03; **LK** 58.00±0.02, 56.08±0.03, 43.91±0.02; **TM** 64.29±0.02, 146.25±0.02, 48.67±0.05. **UG** did not possess polyphenolics and flavonoids so did not exhibit any antioxidant activity. In the flavonoid distribution **TM** had the highest number of flavonoid types and percent type present (20.94%, kaempferol) while **LK** had the lowest percent type (2.78%, myricetin). The leaves of these plants can serve as source of bioactive compounds and antioxidant agents. Only **UG** possessed neither polyphenolic nor flavonoid compounds.

Keywords: Polyphenolics, flavonoids, antioxidants, leaves, savannah plants, Nigeria.

INTRODUCTION

Plants and humans have co-existed from the beginning of civilization. Man has been able to utilize plants in various ways so as to preserve life. Plants are used for food, religious purposes, and drugs. At the advent of synthetic drugs the use of plants and plant parts as drugs diminished. But as the use of synthetic drugs increased, there was also an increase in resistance to the synthetic drugs by disease causing microorganisms, coupled with the toxic nature of the drugs and in that people react to some of these drugs differently. These demerits of synthetic drugs caused man to turn back to his original source of drugs (plants) for his primary healthcare delivery. Researches into the use of plants for treatment of sicknesses and diseases have shown that some plants do possess compounds known as phytochemicals which possess biological and pharmacological activities^{1,2}. Examples of such important phytochemicals are tannins, polyphenolics and flavonoids. The presence of polyhydroxy groups in the structure of most flavonoids made some authors to refer

to them as polyphenolics³. These classes of compounds have been shown to exhibit antioxidant, antimalarial, anti-inflammatory, etc properties⁴⁻⁷. Some of these compounds have been shown to have the ability to reduce the risk of contracting diseases such as cancer, heart related diseases, etc⁸⁻¹⁴. Flavonoids possess a regulatory behavior that is similar to those of lipid-soluble vitamins. They serve as communicators with the environment¹⁵⁻¹⁷, can improve blood circulation and lower blood pressure¹⁸. They act as phytoalexins in microbial attacks¹⁹. Researchers have shown that some polyphenolics and flavonoids isolated from plants and plant parts possess antioxidant property²⁰. When plants or plant parts that contain these phytochemicals are ingested by humans, these compounds have the ability to reduce the free radicals which are generated during metabolic processes. Such free radicals include RO[•], O₂^{•-}, HO[•], ROO[•] and NO[•]. Some of these free radicals have been shown to possess very damaging effects on protein tissues, DNA and cell membranes²¹ and if not checked could lead to serious health issues such as cancer, heart



diseases and congestive dysfunction. The savannah region in Nigeria is rich in plants which the traditional health practitioners use for the treatment of different kinds of ailments (Table 1).

Most of these traditional health practitioners use the root, leaves and stem barks for treatment of ailments. Therefore, in our continued search for bioactive compounds in medicinal plants, this research work was designed to investigate the antioxidant potential, and also to determine the total phenolic and flavonoid contents of leaves of some medicinal plants found in the savannah region in Nigeria.

MATERIALS AND METHODS

Chemicals

Methanol, Ethanol, hexane, sodium carbonate, sodium hydroxide, aluminum hydroxide, sodium nitrite and Folin-Ciocalteu reagent were obtained from E. Merck (Darmstadt, Germany). Gallic acid, quercetin, rutin, vitexin, catechin, kaempferol, apigenin, myricetin and luteolin were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Equipment

Genesys 10S vl. 200 217H311008 spectrophotometer was used for absorbance measurements.

Plant material and extraction

Fresh plant leaves were collected by traditional health practitioners from Omu aran, Kwara State, Nigeria. These were taken to the Department of Biological Sciences, Landmark University, Omu aran, Kwara State, Nigeria for identification. Voucher specimens were deposited at the Department of Biological Sciences herbarium. The leaves were pulverized into fine powder and stored in a container. 500 g of the pulverized leaves was extracted with solvents of different polarities (n-hexane and methanol) using a soxhlet extractor. The extracts were concentrated under reduced pressure to give the crude extracts.

Phytochemical screening

Phytochemical screening was carried out to determine the presence or absence of certain plant constituents such as alkaloids, flavonoids, saponins, steroids, cardiac glycosides, tannins, anthraquinone glycosides. The method described by Harborne, 1993³³ was used for the identification of these classes of compounds in the extracts.

Determination of total phenolics

A mixture of 2.5 ml of diluted Folin-Ciocalteu reagent and 2.0 ml of Na₂CO₃ (7.5%) was added to a dilute solution of the methanolic extract and incubated for 30 mins at 40°C. It was later transferred to a UV spectrophotometer and the absorbance measured at 760 nm. The total phenolic content was calculated from the calibration curve for Gallic acid³⁴.

Determination of total flavonoid content

0.7 ml of 5% (w/w) NaNO₂ and 10 ml of 30% (v/v) ethanol were combined and mixed with a dilute solution of the extract (1mg/ml). This mixture was stirred for 5 mins and 0.7 ml of 10% AlCl₃ (w/w) added. The mixture was stirred for 6 mins, then 5 ml of 1N NaOH added. The mixture was diluted with 30% (v/v) ethanol and left standing for 10 mins. The mixture was placed in a UV spectrophotometer and the absorbance reading taken at a wavelength of 500 nm. The total flavonoid content was obtained from the calibration curve of quercetin and expressed as quercetin equivalent in mg/mg extract³⁵.

Thin layer chromatography

Both qualitative and quantitative TLC analyses were carried out for the identification and quantification of the individual flavonoids present in the leaf extracts. The quantification analysis was based on the method described by Bate-Smith (1977)³⁶. For the qualitative analysis, commercial flavonoids were obtained and used as standards. The commercial flavonoids used are kaempferol, apigenin, luteolin, quercetin, myricetin, rutin, vitexin and catechin. 1.0 g of each methanolic extract was further extracted with the solvent mixture methanol-water-acetic acid (14:5:1; v/v) in order to obtain the flavonoid soluble fraction. Merck silica gel plates (60 F 254) were spotted with the methanolic extract, the extracted flavonoid solution and each standard. The plates were introduced into a TLC tank and developed with the solvent mixture Butanol-acetic acid-water (4:1:5; v/v). After development of the spots, the plates were air dried and then sprayed with flavonoid detection reagents (1% ethanolic 2-aminoethyl diphenyl borinate solution (reagent A) and ethanolic solution of polyethylene glycol-400 (reagent B)). The plates were then placed under UV lamp and viewed at wavelengths of 254 and 365 nm. The visualized spots were marked and used to determine the R_f values of the various identified chemical spots.

Antioxidant activity

Determination of DPPH radical scavenging activity

Different concentrations of the methanolic extract were prepared and 2 ml of prepared DPPH in methanol was added to each dilution and left in the dark for 20 mins for the colour to develop. The control used was a mixture of 2 ml methanol and 2 ml DPPH solution. The absorbance readings were recorded at a wavelength of 517 nm from a UV spectrophotometer. Ascorbic acid was used as the standard. The free radical scavenging activity of the sample was calculated according to the formula:

$$\% \text{ of DPPH scavenging} = [(A_0 - A_1)/A_0] \times 100\%$$

Where A₀ was the absorbance of the control reaction and A₁ was the absorbance in the presence of the sample³⁷.

Statistical analysis

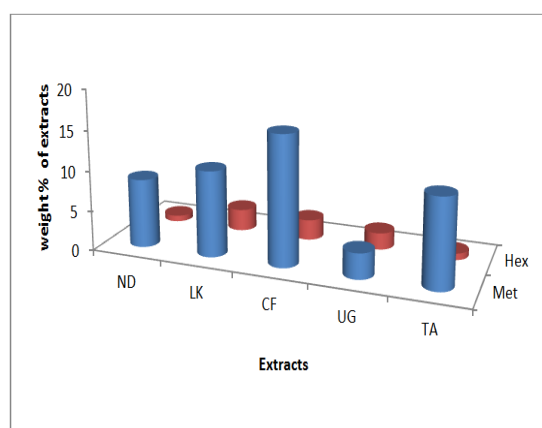
All data are presented as mean of standard deviation of quadruplicate results. The linear regression between total



phenolics and those of total flavonoids with antioxidant activity was carried out using Excel 2007.

RESULTS

- a. **Extract yield:** The crude extract yield as a percent of the weight of the plants leaf extracted are shown in Fig. 1. Extraction in methanol polar solvent gave more yield than in n-hexane. In n-hexane extraction, the percentage extract yield (by weight) was highest from LK and lowest from TM. In methanolic extraction, CF had the highest yield while UG gave the lowest.
- b. **Phyto-constituents:** The results obtained for the phytochemical screening are given in Table 2. Some of the leaf extracts exhibited the presence of some the screened phyto-constituents. None of the plants exhibited the full profile of the screened phytochemicals. Steroids were the most frequently occurring among the screened plants followed by flavonoids and saponins. Alkaloids were detected in only two of them. Anthraquinone glycoside was not detected in any of the extracts.
- c. **Of the hexane extracts:** ND, CF and LK lacked any of the screened phytochemicals. Only steroids were present in TM while UG showed presence of steroids, alkaloids and terpenoids.
- d. **Of the methanolic extracts:** ND showed presence of three secondary metabolites. CF had four, LK had three, UG had four while TM had six.
- e. **Presence:** Strong presence (+++) of the following were obtained (in methanolic extracts)- tannins in TM, flavonoids in CF and TM; and steroids in UG.
- f. **Total phenolic and flavonoid contents:** As shown in Tables 4 and 5 TM yielded the highest value for both total phenolic and flavonoid contents.
- g. **Flavonoid distribution:** The flavonoid distribution in the selected plants leaf extracts is as shown in Table 6. Rutin, apigenin and luteolin were absent in all the plants leaf extracts. Vitexin was present in TM; catechin in ND; Kaempferol in CF and TM; quercetin in ND, LK and TM; and myricetin in CF and LK. TM had the highest number of flavonoid types. The highest percentage of flavonoid was found in TM (kaempferol, 20.94%).
- h. **Antioxidant activity:** The results obtained for the antioxidant activity is as shown in Table 5. Only UG did not exhibit antioxidant activity among the screened plants.



Key: ND - *N. diderrichii* ; LK - *L. kerstingii* ; CF - *C. febrifuga*; UG - *U. guineensis*; TA - *T. macroptera*; Met – Methanol extract; Hex – hexane extract

Figure 1: Leaf extracts yields (as % by weight)

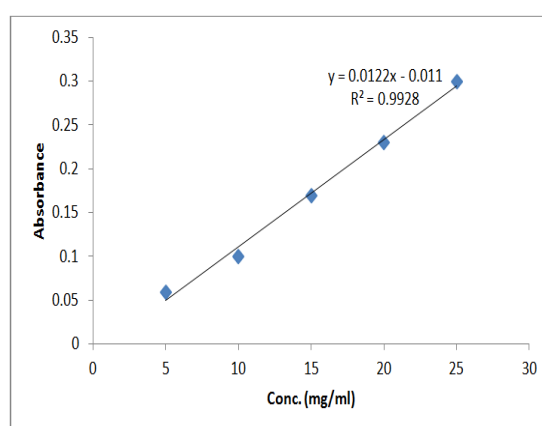


Figure 2: standard curve for quercetin

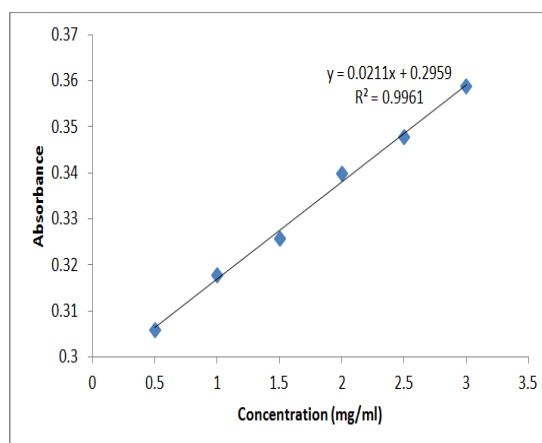


Figure 3: Standard curve for Gallic acid

Table 1: Plants studied and their systemic names, families and traditional uses

Biological name	Family	Uses	Plant part	Reference
<i>Nauclea diderrichii</i> (Merr)	Rubiaceae	Anaemia, stomach ache, indigestion, jaundice, gonorrhoea, measles	Bark, leaves	Nwodo and Agbo, 2010 ²²
<i>Crossopteryx febrifuga</i> (Afzel) Benth	Rubiaceae	Dry cough, fever, dysentery, pain, malaria	Stem bark	Salawu, 2011 ²³



<i>Lannea kerstingii</i> Engl. and K. Krause	Anacardiaceae	Anaemia, malaria, buruli ulcer, diarrhea, anthelmintic	Leaves, stem bark	Diallo, 2009; Adoum, 2009; Yemou, 2008; Maidou, 2005; Atawodi, 2003; Njinga, 2013 ²⁴⁻²⁹
<i>Uapaca guineensis</i>	Euphorbiaceae	Leprosy, epilepsy, abortion, oedema, sterility, toothache, rheumatism, pile, aphrodisiac, sexual asthenia, pain, fever, inflammation	Stem and root barks	Ndebia, 2007 ³⁰
<i>Terminalia macroptera</i>	Combretaceae	Headaches, pains, anxiety, wounds, depression, sores, agitation, asthma, cold, cough, fever, hepatitis, gastritis, colic, syphilis		Pham, 2011; Zou, 2014 ^{31,32}

Table 2: Phytochemical screening of plants leaves extracts

Plants	Extracts	Tannins	Flavonoids	Saponins	Steroids	Alkaloids	Terpenoids	Cardiac glycosides	Anthraquinone glycosides
<i>N. diderrichii</i>	hexane	-	-	-	-	-	-	-	-
	methanol	+	++	+	-	-	-	-	-
<i>C. febrifuga</i>	hexane	-	-	-	-	-	-	-	-
	methanol	-	+++	++	++	-	-	+	-
<i>L. kerstingii</i>	hexane	-	-	-	-	-	-	-	-
	methanol	++	++	+	-	-	-	-	-
<i>U. guineensis</i>	hexane	-	-	-	++	++	+	-	-
	methanol	-	-	-	+++	+	+	+	-
<i>T. macroptera</i>	hexane	-	-	-	+	-	-	-	-
	methanol	+++	+++	++	+	-	+	+	-

NOTE: + -weakly present; ++ - moderately present; +++ - strongly present; - -absent

Table 3: Total phenolic content (TPC) of methanolic leaf extracts of the plants expressed in mg Gallic acid equivalent/mg extract

Extracts	<i>N. diderrichii</i>	<i>C. febrifuga</i>	<i>L. kerstingii</i>	<i>U. guineensis</i>	<i>T. macroptera</i>
TPC	49.43 ± 0.15	63.71 ± 0.02	58.00 ± 0.02	-	64.29 ± 0.02

Table 4: Total flavonoid content (TFC) of methanolic leaf extracts of plants expressed in mg quercetin equivalent/mg extract

Extracts	<i>N. diderrichii</i>	<i>C. febrifuga</i>	<i>L. kerstingii</i>	<i>U. guineensis</i>	<i>T. macroptera</i>
TFC	73±0.03	81.58±0.06	56.08 ± 0.03	-	146.25±0.02

Table 5: DPPH radical scavenging activity of methanolic leaf extracts of plants expressed in %

Extracts	<i>N. diderrichii</i>	<i>C. febrifuga</i>	<i>L. kerstingii</i>	<i>U. guineensis</i>	<i>T. macroptera</i>	Ascorbic acid
Activity	37.0.03±0.04	48.23±0.03	43.91±0.05	-	48.67±0.05	11.42±3.17

Table 6: Flavonoid distribution in methanolic leaf extracts of selected medicinal plants (%)

Flavonoids	<i>N. diderrichii</i>	<i>C. febrifuga</i>	<i>L. kerstingii</i>	<i>T. macroptera</i>
Rutin	-	-	-	-
Vitexin	-	-	-	5.62
Catechin	9.2	-	-	-
Kaempferol	-	6.96	-	20.94
Quercetin	8.51	-	4.16	12.18
Apigenin	-	-	-	-
Myricetin	-	3.21	2.78	-
luteolin	-	-	-	-

Table 8: R_f values and colours exhibited by standards under UV lamp (365 nm)

Flavonoids	R _f	Reagent A	Reagent B
Rutin	0.41	Fluorescent yellow	Orange
Vitexin	0.66	Light green	Light green
Catechin	0.75	Dark green	Dark brown
Kaempferol	0.82	Light green	Light green
Quercetin	0.88	Yellow	Orange
Apigenin	0.86	Light green	Light green
Myricetin	0.73	Orange	Dark green
luteolin	0.91	Fluorescent yellow	Light green

DISCUSSION

Plants from four different families (Rubiaceae, Anacardiaceae, Euphorbiaceae and Combretaceae) were studied (Table 1). These plants were obtained from the savannah region in Nigeria. The plants were extracted with both highly polar and non polar solvents. The weight percent of the plants leaves extracts is shown in Fig. 1. *L. kerstingii* had the highest while *T. macroptera* had the lowest weight percent for n-hexane extracts while *C. febrifuga* had the highest and *U. guineensis* had the lowest weight percent for methanolic extracts. The plant extracts were screened for presence and absence of plant secondary metabolites. The result obtained for the phytochemical screening is given in Table 2. Some of the plants leaves extracts exhibited presence of some of the screened phyto-constituents but anthraquinone glycoside was observed to be absent in all the extracts. The n-hexane extracts of *N. diderrichii*, *C. febrifuga* and *L. kerstingii* showed absence of all the screened phytochemicals, *T. macroptera* had only steroids present while *U. guineensis* showed presence of steroids, alkaloids and terpenoids. The methanolic extract of *N. diderrichii* showed presence of three secondary metabolites, *C. febrifuga* had four, *L. kerstingii* had three, *U. guineensis* had four while *T. macroptera* had six. *T. macroptera* showed the strongest presence of tannins while flavonoids were strongest in *C. febrifuga* and *T. macroptera* as given in Table 2. The phytochemical screening carried out by Nwodo and Agbo, 2010²² on *N. diderrichii* showed presence of alkaloids, terpenoids and glycosides; also Njinga, 2013²⁹ identified alkaloids, anthraquinone glycosides and steroids in *L. kerstingii*. These constituents were absent in our sample extracts. This could be as a result of ecophenotypic factors such as soil and environmental factors which affect the areas where the samples were collected. Researches into the use of medicinal plants have revealed that phenolics and flavonoids are widely distributed in plants. These plant constituents have been shown to exhibit both biological and physiological properties^{38,39}. Results for antioxidant, total phenolic and flavonoid contents are shown in Tables 3-5. All the plants leaves screened possess high antioxidant property, and phenolic and flavonoid contents except *C. febrifuga*. The total phenolic content was determined using Folin-Ciocalteu reagent and Gallic

acid as the standard phenolic compound. The molybdotungstate in the Folin-Ciocalteu reagent is what oxidizes the phenolic compounds in the extract and yields a coloured compound which has a maximum absorption wavelength at around 745-750 nm⁴⁰. The Gallic acid calibration curve ($y = 0.021x + 0.295$) is in the range of 0.5-3.5 mg/ml with a coefficient of determination (R^2) value of 0.996 (Fig. 3). Tawaha, 2007⁴¹ noted that total phenolic content does not necessarily include all the antioxidants present in that particular extract. The results for the total phenolic contents of the plants leaves extracts are presented in Table 3. *T. macroptera* gave the highest value (64.29 ± 0.02 mg GAE/g DW) while *N. diderrichii* had the lowest value (49.43 ± 0.15 mg DAE/g DW). The total flavonoid content was determined using the aluminum colorimetric assay and quercetin as the standard flavonoid compound. The quercetin calibration curve ($y = 0.012x - 0.011$) is in the range of 5-25 mg/ml. It has a coefficient of determination (R^2) of 0.992 as shown in fig. 2. The total flavonoid contents obtained for the screened plants leaves are shown in Table 2. *T. macroptera* gave the highest content (146.25 ± 0.02 mg quercetin equivalent/g DW) while *L. kerstingii* gave the lowest content (56.08 ± 0.03 mg quercetin equivalent/g DW). *U. guineensis* does not possess any phenolic or flavonoid compounds. Oxidants such as superoxide, hydrogen peroxide, alkoxy, hydroxide radicals, nitric oxide, peroxy nitrite anion, nitrogen dioxide and dinitrogen dioxide radicals have been shown to possess deleterious effects on DNA, proteinous substances in the body. The presence of antioxidants in the body helps to put in check the activities of the oxidants thereby helping to prolong lifespan of oxidizable body constituents. The result of the antioxidant activity of the plants leaves extracts against DPPH free radical showed that they possess antioxidant activity. The activity of the control (Ascorbic acid) was observed to be higher than those of the plant leaves extracts. Among the plants leaves screened that of *N. diderrichii* was the highest while *T. macroptera* was the lowest. In the flavonoid distribution as shown in Table 7, quercetin was present in *L. kerstingii*, *N. diderrichii* and *T. macroptera*; catechin was present in only *N. diderrichii*. *T. macroptera* and CF had kaempferol; vitexin was found in *T. macroptera* while myricetin was present in *C. febrifuga* and *L. kerstingii*. *T. macroptera*



was observed to have the highest number (3) of flavonoid types. The flavonoids rutin, apigenin and luteolin were absent in all the extracts. *T. macroptera* had the highest percentage distribution of flavonoid types (kaempferol, 20.94%) while *L. kerstingii* had the lowest (myricetin 2.78%). The R_f values and the colours exhibited by the standard flavonoids under UV lamp (365 nm) are given in Table 8. This was used as a comparison tool for the selected plants leaf extracts.

CONCLUSION

With the exception of *U. guineensis*, all the other screened plants leaves possess high levels of phenolics and flavonoids; therefore can serve as potential antioxidant agents. Further work is ongoing to isolate and characterize the identified flavonoid types.

List of Abbreviations

LK	<i>L. kerstingii</i>
TM	<i>T. macroptera</i>
CF	<i>C. febrifuga</i>
UG	<i>U. guineensis</i>
ND	<i>N. diderrichii</i>

Author's Contributions

Edewor T. I: Carried out the extractions, phytochemical screening and the drafting of the manuscript.

Oluyori P: Determined the total phenolic and flavonoid contents.

Owa S.O: Obtained the plant materials, carried out their identification and edited the manuscript.

All authors read and approved the final manuscript.

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