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Larvicidal Activities of Leaf Extracts of *Adansonia digitata* L. (Malvales: Malvaceae) and *Ficus sur* Forssk (Rosales: Moraceae) against *Culex quinquefasciatus* Mosquito (Diptera: Culicidae)

Olayemi Israel Kayode [1], Samuel Oyiza Mariah [1], Ukubuiwe Azubuike Christian [1] ✉, Ande Adeolu Taiwo [2], Adeniyi Kamoru Abdulazeez [1], Shittu Kudirat Oluwatosin [3]

1 Applied Entomology and Parasitology Research Unit, Department of Biological Sciences, Federal University of Technology, Minna, Nigeria

2 Department of Zoology, University of Ilorin, Ilorin, Nigeria

3 Department of Biochemistry, Federal University of Technology, Minna, Nigeria

✉ Corresponding author email: a.ukubuiwe@futminna.edu.ng

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Abstract Due to the ineffectiveness of synthetic insecticides for sustainable control of Mosquito vectors, whose transmitted diseases are the major causes of morbidity and mortality in the world today, attention has been directed towards insecticide formulations of plant origin. This study was, therefore, carried out to evaluate the larvicidal potential of the methanolic and n-hexane crude extracts of leaves of *Adansonia digitata* and *Ficus sur* against fourth larval instar of *Culex quinquefasciatus* mosquito. The leaves of the plants were collected from Minna, Nigeria, pulverised, extracted and evaporated using Sohxlet apparatus, with methanol and n-hexane as solvents of extraction. The crude extracts of the leaves were screened for phytochemical constituents following standard methods. The larvae were obtained from a Laboratory colony of mosquitoes raised following standard protocols. Test concentrations of 0.0125, 0.025, and 0.05 mg/L of n-hexane and 0.1, 0.25 and 0.5 mg/L of the methanolic extracts were prepared and tested for larvicidal activities against the mosquito following the WHO standard protocols. Larval Mortality was recorded after 24 hours of exposure and mean mortalities computed. Lethal concentration values (LC_{50} and LC_{90}) of the extracts were determined using Probit regression analysis. Phytochemical screening revealed the presence of Flavanoids, Tannin, Saponin, Alkaloids, Steroids, Terpenoid, Cardiac glycosides and Anthraquinone, whose presence were solvent- and plant-species-dependent. There were significant differences in the recorded mortality between the various concentrations of each extracts, the solvents types and plant species. The n-hexane extracts of both plants showed significantly higher larvicidal efficacy against the larvae than their methanolic counterpart. While the n-hexane extract of *A. digitata* was more potent than its *F. sur* counterpart, the latter's methanolic extract was more potent than the former. The median (LC_{50}) and upper (LC_{90}) Lethal concentration of methanolic and n-hexane crude extracts of *A. digitata* leaf were 0.15 and 0.008 mg/L, and 1.21 and 0.22 mg/L, respectively, while these values for methanolic and n-hexane crude extracts of *F. sur* were 0.13 and 0.015 mg/L, and 2.64 and 0.15 mg/L, respectively. The plants extracts also elicited dose dependent mortality. The findings of this study suggest that *A. digitata* and *F. sur* are promising sources of botanical lead agents in the development of sustainable potent larvicides, for integrated control programmes against mosquito-borne diseases.

Keywords Bio-assay; Botanicals; Lethal Concentration; Methanol; n-hexane; Phytochemicals

Background

In most developing countries of the tropical and subtropical regions of the world, mosquitoes constitute foremost vectors of several debilitating diseases affecting humans and domestic animals (Reuda, 2008; Olayemi et al., 2014a). These diseases have not only caused very high level of morbidity and mortality, but also loss of manpower, man-hours and economic loss (Omalu et al., 2012; Olayemi et al., 2014b). *Culex quinquefasciatus* mosquitoes, belonging to one of the disease transmitting mosquito genera, is among the most abundant mosquitoes in Africa (Adeleke et al., 2008) and a major vector of lymphatic filariasis; a disease responsible for deaths of millions of people every year in developing countries (James, 1992; Ukubuiwe et al., 2013).

Mosquito vector control for the reduction of diseases transmitted has been principally through a single approach, the use of synthetic insecticides. This strategy, though, effective in reducing the burdens of mosquito-borne diseases, its successes are often not sustainable, as it is associated with a number of environmental, ecological, entomological, and economic short-comings. Some of these include cases of resurgence and resistance in target species (Tikar et al., 2008), disruption of the ecosystem (Tehri and Singh, 2015), destruction of non-target beneficial fauna and biomagnification of active ingredients (Ghosh et al., 2012), and some attendant human health concerns (Olayemi et al., 2011).

These and other pitfalls have compelled Scientists to advocate for a refocus on Botanicals, in Integrated Mosquito Management (IMM) protocols. This is predicated on the fact that plants have co-evolved with insects, and, overtime, have been equipped with a superfluity of chemical defences (secondary metabolites) such as alkaloids, terpenoids, essential oils, steroids, phenols, saponnins, glycosides and tannins (Pratheeba et al., 2015), which constitute some of the defence line system against predatory insects and herbivore (Shaalan et al., 2005; Remia and Logaswamy, 2009; Rattan, 2010). These compounds are known to play vital roles in the physiology of plant (Katie and Thorington, 2006; Arivoli et al., 2012). Furthermore, these bioactive compounds have been used in local medicine (Gonzalez et al., 2002; Svobodová et al., 2003; Akinpelu and Onakoya, 2006; Ehimwenma and Osagie, 2007; Egunyomi et al., 2009; Friedman, 2007; Yisa, 2009; Manner et al., 2013) and against micro-organisms of medical importance (Cazarolli et al., 2008; Oyeleke et al., 2008; Abalaka et al., 2011; Cushnie and Lamb, 2011; Arun et al., 2012).

With these bioactive chemicals, significant reduction in chances of development of resistance by pests has been reported, as the substances strongly act on both behavioural and physiological processes of insects (Ghosh et al., 2012). They are also relatively cheap, readily available and affordable (Kumar and Maneemegalai, 2008), non-toxic to mammals (Sukhthankar et al., 2014), biodegradable (Gomathi et al., 2014) and more environmentally friendly (Kamaraj et al., 2011). To this end, many herbal products have been evaluated and used as natural insecticides (De Caluwé et al., 2009; Zewdneh et al., 2011), even before the discovery of synthetic insecticides (Shahi et al., 2010). In addition, to constituting potent sources of oviposition deterrents, attractants, repellents, anti-feedants, and anti-moulting hormones (Prabu, 2011; Meenakshi and Jayaprakash, 2014); botanicals also act as larvicides (Choochote et al., 2009; Olayemi et al., 2014b), growth inhibitors and juvenile hormone mimics (Olayemi et al., 2013).

The potency of plant extracts depends on plant species (Das et al., 2007; Kishore et al., 2011; Shooshtaari et al., 2012), plant part used (Arivoli et al., 2012; Kazembe and Chaibya, 2012; Amrutha et al., 2013), age of plant parts (Bream et al., 2009; Pushpalatha et al., 2014) mosquito species targeted or used as model (Kamaraj et al., 2011; Zewdneh et al., 2011), geographical varieties of the plant or insect species (Singh and Mittal, 2013; Tyagi et al., 2013), extraction procedure (Bagavan and Rahuman, 2010; Maragathavalli et al., 2012) and the polarity of solvents of extraction (Tennyson et al., 2012; Shivakumar et al., 2013; Malik et al., 2014).

In Africa, *Adansonia digitata* (Baobab) (Malvaceae), a large iconic indigenous tree, is symbolic, culturally important and physically majestic sub-tropical tree (Wickens and Lowe, 2008). In the past decades, it has attracted the interest of several pharmaceutical companies and researchers due to its medicinal, nutritional and cosmetic usages (Codjia, 2001). Almost all parts of the tree are used in traditional medicine in Africa as a panacea for disease, but specific documented uses include the treatment of malaria fever and tuberculosis (Nguta et al., 2010), microbial infections (Sidibe and Williams, 2002), diarrhoea (Shukla et al., 2001), anaemia, dysentery, toothache, and internal pains, diseases of the urinary tract, otitis, and arthritis (Wickens and Lowe, 2008), as a tonic and for insect bites and Guinea worms, against excessive sweating and as a stringent (De Caluwé et al., 2009) and as an insect repellent (Denloye et al., 2006).

Also, *Ficus sur* Forssk belonging to the family Moraceae, is a medium size tree, which grows cylindrically up to 6-9 metres with brown bark with small scales (Keay, 1989). *Ficus sur* is used to treat diarrhoea, epilepsy and anaemia as well as sexually transmitted diseases (Adeshina et al., 2010). Methanolic extracts from the roots has

been reported effective against chloroquine-resistant malaria (Lansky and Paavilainen, 2011). Previous phytochemical screening of some species belonging to the genus *Ficus* have led to the isolation of tannins and saponins (Stary, 1998).

However, despite the confirmed significant medicinal and insecticidal activities of these plants species, there is a dearth of information on their mosquito larvicidal potentials. This study, was, therefore, carried out to elucidate the mosquito-larvicidal activities of *A. digitata* and *F. sur,* using *Cx. quinquefasciatus* as model vector.

1 Materials and Methods

1.1 Collection and processing of plant materials

Fresh leaves of *A. digitata* and *F. sur* were collected from a suburb of Minna metropolis (Lat. 9° 27 ´N and Long. 6° 33 ´E) in North Central Nigeria. The leaves were authenticated by a Botanist in the Department of Biological Sciences, Federal University of Technology (FUT), Minna, Nigeria; where voucher specimens were deposited in the Herbarium. The leaves were air-dried in the Laboratory at room temperature (28.00±2.00°C) for a period of two weeks. The dried leaves were pulverised using a milling machine (Model no: QASA QLB – 20L40).

1.2 Extraction process

Methanolic and n-hexane crude extracts of the leaves were prepared using Sohxlet's apparatus, following the methods of Koyel (2011). This involved 50 g of the milled plant material wrapped in a filter paper. The weighed powdered leaves were placed in the extracting flask of the Sohxlet's apparatus, after which 300ml of the solvent was poured into the round bottom flask of the setup, with the extracting chamber attached to the condenser, and the temperature set at 60°C and 30°C for methanol and n-hexane, respectively. Subsequently, the leaves were changed after exhaustive extraction, and the whole process was repeated until sufficient crude extract of the leaves were obtained. The crude extracts were dried in a rotary evaporator and preserved in a refrigerator at 4°C before use.

1.3 Source and maintenance of mosquito larvae

The *Cx. quinquefasciatus* mosquitoes used for this experiment came from a colony maintained in the Laboratory of the Department of Biological Sciences, FUT, and Minna. The mosquitoes were maintained following standard protocols (Ukubuiwe et al., 2012).

1.4 Phytochemical screening of plant extracts

Phytochemical screening of the plant extracts was carried out using the methods described by Sofowara (1993) and Harborne (1998).

1.5 Preparation of stock and working solutions of extracts

Stock solutions of the plant extracts were prepared according to World Health Organisation (WHO) protocols (WHO, 2005) with slight modification. Briefly, for stock solution of methanolic extracts of the plants, 5 g of the extracts were dissolved in 50 ml of methanol while that of n-hexane had 1 g of the extract in 10 mls of solvent. Working solution was, thereafter, prepared by adding 1 ml of stock solution to 99 mls of distilled water. Test concentrations of 0.0125, 0.025 and 0.05 mg/L and 0.1, 0.25, and 0.5 mg/L, respectively, of the n-hexane and methanolic extracts were prepared by respectively adding 0.125, 0.25, 0.5, 1, 2.5, and 5 ml of working solution to 99.875, 99.75, 99.95, 99, 97.5, and 95 ml of distilled water.

1.6 Bioassay of plant extracts against Fourth instar larvae of *Culex quinquefasciatus*

The mosquitoes were exposed to the extracts following standard World Health Organisation's Protocols for testing the efficacy of insecticides (WHO, 2005), with slight modifications. For the methanolic extracts, batches of 4th instar larvae of *Cx. quinquefasciatus* were separately exposed to 0.10, 0.30, and 0.50 mg/L of the extracts in 250 ml capacity bowls. There were two Controls namely, positive and negative, containing 1% methanol-distilled water, and only distilled water (i.e., neither extract nor solvent), respectively. Each test concentration and Controls had five replicates. The n-hexane extract bio-assay had the same set-up as the methanolic extract counterpart, except that the test concentrations were 0.0125, 0.025 and 0.05 mg/L, and the positive control was 1%

n-hexane-distilled water. The experiments were maintained in the Laboratory at ambient conditions of 28.00±1.00°C, 70.20±2.63 % RH, and 12:12 hours (L: D). The larvae were monitored and mortality recorded after post-24 hours of exposure.

1.7 Statistical analysis

The effects of extracts concentrations on larval mortality were subjected to Analysis of Variance (ANOVA), and differences in means were separated using Duncan Multiple Range Test (DMRT) at p = 0.05 level of significance, using the Statistical Packages for Social Sciences (SPSS), 16.0. The larval mortality data were subjected to Probit analysis for calculating LC_{50}, and LC_{90} (Finney, 1971).

2 Results

2.1 Phytochemical components of *Adansonia digitata* and *Ficus sur*

The phytochemical components of methanolic and n-hexane leaf extracts of *A. digitata* and *F. sur* are shown in Table 1. It revealed the presence of bioactive components in all extracts. These components include Flavonoid, Tannins, Saponnin, Alkaloids, Steroid, Cardiac glycosides, and Anthraquinones. However, there was disparity in their presence in the two different solvent extracts of a plant, as well as between plant species. While Flavonoid, Cardiac glycosides and steroids were present in all four plant extracts, Tannins and Anthraquinones were present in all extracts except n-hexane extract of *F. sur* and methanolic extract of *F. sur,* respectively. Terpenoid was conspicuously absent in all plant extracts except n-hexane extract of *A. digitata*, while, Saponnin and Alkaloids were found, exclusively, in methanolic and n-hexane extracts of the plants species, respectively.

There was a comparative difference in the presence of phytochemicals between the solvent type and species' extracts of the plants; as five (5) and seven (7) phytochemical constituents were present in methanolic and n-hexane extracts of the leaves of *A. digitata*, respectively, while those of *F. sur* were six (6) and five (5), respectively (Table 1).

Table 1 Qualitative Phytochemical constituents of *Adansonia digitata* and *Ficus sur* leaf extracts, from Minna, Niger State, Nigeria

Phytochemicals	***A. digitata***		***F. sur***	
	Solvent of Extraction		**Solvent of Extraction**	
	Methanol	**n-hexane**	**Methanol**	**n-hexane**
Flavonoids	+	+	+	+
Tannins	+	+	+	-
Saponnin	+	-	+	-
Alkaloids	-	+	-	+
Steroids	+	+	+	+
Terpenoid	-	+	-	-
Cardiac glycosides	+	+	+	+
Anthraquinones	-	+	+	+
Aggregate	5	7	6	5

Note: Key: + = present, - = absent

2.2 Larvicidal activities of extracts of against *Cx. quinquefasciatus*

The leaf extracts of *A. digitata* and *F. sur* demonstrated significant ($p<0.05$) toxicity against 4th instar larvae of *Cx. quinquefasciatus* under Laboratory conditions (Table 2). Though, both the methanolic and n-hexane extracts of the plants achieved 100% mortality against the mosquitoes, the later were considerably more toxic than the former, even, at very low concentrations. While, it took 0.5 mg/L to achieve 100% larval mortality in the methanolic leaf extracts of both plant species, similar results were attained with one-tenth (i.e., 0.05 mg/L) of that concentration in the n-hexane extracts.

Characteristically, the toxicity of the extracts was concentration- and plant species-dependent. Although, n-hexane extracts of both plant types produced the highest mortality (100.00%) at 0.05 mg/L, those of *A. digitata* were significantly ($p<0.05$) and consistently more toxic than the *F. sur* counterparts at the lower concentrations of

0.0125 and 0.025 mg/L. The reverse was, however, the case with methanolic extracts of the plant species, where *F. sur* was significantly ($p<0.05$) more toxic than *A. digitata* at lower concentrations of 0.1 and 0.25 mg/L, although, both extracts elicited absolute mortality (100.00%) at 0.5 mg/L.

Table 2 Larvicidal effects (%) of Methanolic and n-hexane leaf-extracts of *Adansonia digitata* and *Ficus sur* against 4th instar larvae of *Culex quinquefasciatus* mosquito

Extract Concentration (mg/L)	*A. digitata*		*F. sur*	
	Mortality (%) due to Solvent of Extraction		Mortality (%) due to Solvent of Extraction	
	Methanol	n-hexane	Methanol	n-hexane
0.0125	-*	$75.00 \pm 0.83^{b}_{b}$	-	$45.00 \pm 5.76^{b}_{a}$
0.025	-	$86.00 \pm 1.33^{b}_{b}$	-	$68.30 \pm 2.86^{c}_{a}$
0.05	-	$100.00 \pm 0.00^{c}_{a}$	-	$100.00 \pm 0.00^{d}_{a}$
0.1	$23.33 \pm 3.03^{b**}_{a***}$	-	$46.70 \pm 4.06^{b}_{b}$	-
0.25	$65.00 \pm 6.30^{c}_{a}$	-	$91.70 \pm 1.00^{c}_{b}$	-
0.5	$100.00 \pm 0.00^{d}_{a}$	-	$100.00 \pm 0.00^{d}_{a}$	-
Negative Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Positive Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Note: *Not Applicable; **Values followed by same superscript alphabets, in a column within an extract type are not significantly different at $p>0.05$; ***Values followed by same subscript alphabets, in a row for the same solvent type are not significantly different at $p>0.05$; Values are represented by percentage ± standard error of mean of four replicates

Generally, among all extract types of both plant species, the lethal concentrations (LC) of n-hexane extracts against the larvae were lower than those of the methanol extracts. Between the methanolic extracts, *F. sur* had considerably lower LC_{50}, and the reverse was the case for LC_{90} values. While, between n-hexane extracts, *A. digitata* had the lower LC_{50}, while the reverse was for LC_{90}. The LC_{50} and LC_{90} of the methanolic extracts of *A. digitata* and *F. sur* were, respectively, 0.15 and 1.21 mg/L, and 0.13 and 2.64 mg/L. While those of n-hexane extracts of *A. digitata* and *F. sur*, were respectively, 0.008 and 0.22 mg/L, and 0.015 and 0.15 mg/L, respectively (Table 3).

Table 3 Lethal concentrations (mg/L) of leaf-extracts of *Adansonia digitata* and *Ficus sur* against 4th instar larvae of *Culex quinquefasciatus* mosquitoes

Plant Species	Solvents	LC_{50}	LC_{90}	R_2	Regression equation
A. digitata	Methanol	0.15	1.21	0.9379	**Y=2.7667x +10.803**
	n-hexane	0.008	0.22	0.9999	**Y=4.3772x +8.6322**
F. sur	Methanol	0.13	2.64	0.7231	**Y=3.0395x +7.7207**
	n-hexane	0.015	0.15	0.9196	**Y=4.1x +12.45**

3 Discussion

Plant serves as a reservoir of metabolically active substances that can be harnessed in the production of insecticidal lead agents (Vasudevan et al., 2009). The results of this study showed presence of such inherent bio-active phytochemicals in leaf extracts of *A. digitata* and *Ficus sur.* The phytochemical constituents detected in the leaves of these plants namely Flavanoids, Tannin, Saponin, Alkaloids, Steroids, Terpenoid, Cardiac glycosides and Anthraquinone, have been demonstrated to have larvicidal activities against mosquitoes (Kamalakannan et al., 2015), and further expands the toxico-physiological scope of the plants. There was variation in the presence or absence of phytochemical constituents among the plant and solvent types; this could be responsible for the differential larvicidal activities of the extracts of these plants, as earlier observed by Olayemi et al. (2013).

In the present study, irrespective of plant source, n-hexane extracts were more effective as larvicidal agents against *Cx. quinquefasciatus* than methanolic extracts as they elicited relatively higher mortality than their methanolic counterparts. This could be due to the presence of Alkaloids, present only in the n-hexane extracts, which have been reported to possess enormous insecticidal potentials (Quevedo et al., 2011). Between the

n-hexane extracts, *A. digitata* was more potent than its *F. sur* counterpart. However, a different result was obtained between the methanolic extracts, where the reverse was the case, as extract of *F. sur* was more toxic than its *A. digitata* counterpart. The uneven distribution of phytochemical components may be responsible for the differential larvicidal activities observed in the plant species and solvent types.

The particularly high toxicity of the n-hexane extract of *A. digitata* may be attributed to the relatively higher numbers of phytochemical constituents; with the exclusive presence of Terpenoids in this extract type. Its superiority over the *F. sur* n-hexane extract could be ascribed to the presence of Tannins and Terpenoids in the latter and absence in the former. Studies have shown that these two phytochemical components have immense mosquito larvicidal potentials (Kumar and Maneemegalai, 2008). The lower toxicity of methanolic extract of *A. digitata* could be due to the absence of Anthraquinones, which was conspicuously present in all other extract type. Anthraquinone have been reported to have significant insecticidal potencies (Shalaan et al., 2005).

The presence of bioactive components could be responsible for the activities of the extracts of *A. digitata* and *F. sur* as larvicides, as shown in this study. Bioactivity of *A. digitata* as an insecticide and as a repellent, against *Anopheles gambiae* and *Musca domestica* has been reported earlier (Denloye et al., 2006). Further, larvicidal activity of its crude extracts from different solvents (benzene, chloroform, hexane and methanol) has been reported and showed increased mortality with increasing concentration (Klempner and Unnasch, 2007). These agree with the bioactivity of the plant in the present study.

Generally, the extracts, though effective, their activities were dose-, solvent-, and plant species- dependent. Though, the tendency of n-hexane extracts been more biologically effective has been reported in previous studies (Ghosh et al., 2012). The LC_{50} of both extracts of *A. digitata* in this study were much lower than that of methanolic leaf extract of another member of the family, Malvaceae, *Pavonia zeylanica* (22.14 mg/L) (Vahitha et al., 2002), while that of *F. sur* was lower than that of petroleum ether leaf extract of *Cannabis sativa* (3.77 mg/L), a member of the same family, Moraceae (Maurya et al., 2007).

The LC_{50} and LC_{90} values reported in this study for methanolic extracts of the leaf of the plants in the present study were lower than that reported for methanolic extracts of *Azadirachta indica Momordica charantia, Trichosanthes anguina, Luffa acutangula, Benincasa cerifera, Citrullus vulgaris* and *Vitex negundo* (Prabakar and Jebanesan, 2004). They were, also, lower than those reported for *V. trifolia, V. peduncularis*, and *V. altissima* (Krishnan et al., 2007), *Pavonia zeylanica* and *Acacia ferruginea* (Vahitha et al., 2002), *Coccinia indica* and *Cucumus sativus*, (Rahuman and Venkatesan, 2008). Further, the LC_{50} and LC_{90} of n-Hexane leaf extracts were lower than that reported for Hexane fruit extracts of *Momordica charantia* (Singh et al., 2006), Rhizome of *Kaempferia galanga* (Choochote et al., 1999), *Khaya senegalensis* and *Daucus carota* (Shaalan et al., 2005), *Curcuma aromatic* (Choochate et al., 2005) and n-hexane leaf extract of *Eucalyptus citriodora* (Singh et al., 2007).

Therefore, the relatively high toxicities of extracts of *A. digitata* and *F. sur* stand these plant species as promising sources of potent insecticidal lead-agent for mosquito vector control.

4 Conclusions

The outcomes of this study on the potency of *A. digitata* and *F. sur* leaf extracts for larvicidal efficacy against the fourth instar larvae of *Culex quinquefasciatus* mosquito opens new frontier of possibilities for a lead potential agent for combating mosquitoes. These extracts, judging by the relatively low lethal concentrations, could be environmentally friendly, and hence show great promise as source of sustainable efficacious mosquito larvicides. However, further studies are advocated to determine larvicidal activities of fractions of the extracts, evaluate mammalian toxicities and also efficacies of other flora part of the plants *vis-à-vis* other solvents of extraction.

Authors' contributions

Conceived and designed the experiment: OIK, AAT and UAC. Analysed the data: UAC and AKA. Wrote the first draft of the manuscript: OIK, SOM, and UAC. Contributed to writing of the manuscript: AKA and SKO. Agree with manuscript results and

conclusion: all authors. Made critical revisions and approved final version: all authors. All authors reviewed and approved the final manuscript.

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