Original article

Phytochemical and Elemental Constituent of Methanolic Extract of Musa acuminata Flower: A Case Study on Median Lethal Dose in Albino Rats

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ABSTRACT

Background: Medicinal plants are widely used for therapeutic purposes, and the banana plant (Musa acuminata) has multiple traditional applications in Nigeria. However, its flower is often discarded despite potential medicinal value. This study aimed to evaluate the phytochemical constituents, elemental composition, and acute toxicity of the methanolic extract of Musa acuminata flower to provide scientific evidence supporting its usefulness.

Methods: Fresh banana flowers were collected, authenticated, air-dried, pulverized, and extracted using 98% methanol. Phytochemical screening was performed using standard qualitative procedures. Elemental analysis of the ashed sample was conducted using titrimetric and spectrophotometric methods to determine essential macro- and micro- elements. Acute toxicity testing was carried out in albino rats to estimate the median lethal dose (LD_{50}).

Results: Phytochemical evaluation revealed the presence of flavonoids, alkaloids, tannins, saponins, glycosides, terpenoids, anthraquinones, phlobatannins, and carbohydrates. Elemental analysis showed appreciable levels of potassium, calcium, magnesium, sodium, iron, copper, chloride, and trace amounts of lead. Acute toxicity assessment indicated that the extract was well tolerated at the administered doses, suggesting a high safety margin.

Conclusion: Musa acuminata flower contains diverse bioactive compounds and nutritionally relevant elements with potential therapeutic benefits. The observed safety profile and phytochemical richness highlight the value of the banana flower as a potentially useful medicinal resource rather than waste material.

Keywords: *Banana flower, Phytochemical screening, Elemental analysis, Methanolic extract, Acute toxicity.*

INTRODUCTION

Since ancient times, people have been exploring nature, particularly plants, in the search for new drugs and this has resulted in the use of many medicinal plants with curative properties to treat various diseases (Chidi et al., 2017). Banana flower has been reported to possess antidiabetic, antidiarrheal, antidysentery, anthelmintic, anti-ulcerative, antimicrobial, hypoglycemic, antihypertensive, diuretic, antilithiatic, wound healing, antimalarial, and anti-snake venom activities (Imam et al., 2011; Bhaskar et al., 2012; Ramith et al., 2015). In Nigeria, Banana flowers are wasted in farms due to ignorance on their medicinal values. Banana leaves and fruits medicinal, nutritive and toxicological value have been explored traditionally, researched and evaluated over time but the flower is often discarded as waste in many countries. Bioactive compounds such as alkaloids, anthocyanins, flavonoids, glycosides, phlobatannins, tannins, and terpenoids have been reported in banana peels, and these compounds have been shown to exert various biological and pharmacological effects (such as antibacterial, antihypertensive, antidiabetic, and anti-inflammatory activities). Musa acuminata fruit peels are used in the northern parts of Nigeria for the treatment of hypertension and other cardiovascular-related diseases because they are rich in fibers, flavonoids, polyphenols, and tannins (Al-Mqbali and Hossain, 2019). The leaves are traditionally used for treating some skin ailments such as eczema, wounds, irritation, rashes, dandruff, and sunburn as they possess a cooling effect on the skin (Kora, 2019). Studies has shown that the tannins present in banana leaves prevent serpentine woolly nematode eggs, providing parasite resistance (Kumari et al., 2023). Magnesium was found to be highest in banana leaves followed by potassium, sodium, phosphorus, and calcium. Variations in nutrient content may be due to fertilization, soil type, or banana species (Mat et al., 2020). Banana flowers have tremendous nutritional value and healthy effects. Banana blossom has some mineral nutrients; potassium (K) is the macro-element with the highest concentration, followed by calcium (Ca) and magnesium (Mg). On the other hand, banana inflorescence has a high level of iron (Fe) among its microelements (Ramu et al., 2017; Basumatary and Nath, 2018).

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans and animals further than those attributed to macronutrients and micronutrients and they accumulate in different parts of the plant, such as leaves, flowers, fruits, and seeds (Ahuja et al., 2017). Banana blossom contains a high amount of fiber and ash and also has good antioxidant activity. The flower extract of Banana showed promising therapeutic effects due to the presence of phytochemicals and minerals (Lakshmi et al., 2024). The aim of this study is to investigate the phytochemical and elemental constituent of Banana flower with the view of determining the median lethal dose of its methanolic extract in albino rat.

MATERIALS AND METHODS

Plant collection and identification

Fresh Banana flowers were collected from a garden in Dalori quarters, Konduga local government area of Borno State, Nigeria. The flowers were identified to be of Banana plant by a taxonomist from the Department of Biological Sciences, University of Maiduguri.

Methanolic extraction

The flowers were air dried at room temperature, and crushed using wooden mortar and pestle into fine particles. Three hundred and fifty grams (350 g) of the powdered flower was taken to Chemistry Department laboratory, University of Maiduguri, where it was weighed using an electric weighing balance. Three-hundred-gram (300g) of the plant material was exhaustively extracted with 98% methanol. The extraction method as described by Usman et al. (2009) was used. The methanol was reflux for about 3 hours. The extract was filtered to remove debris and concentrated to dryness at 40 to 50 oC on hot air oven. The solid obtained was weighed and kept in a desicator until required for use.

Preliminary phytochemical screening

The extract of the flower of Musa acuminata was screened qualitatively for phytochemical constituents using Standard Operating Procedures (Brain and Turner, 1975; Markham, 1987; Silva et al., 1998; Sofowora, 2008; Evans, 2009).

Test for carbohydrates

The powdered sample (4 g) was boiled in 50 ml of distilled water on a hot plate for 3 minutes. The mixture was filtered using Whatman filter paper No. 1, while hot and the filtrate was allowed to cool and used for the following tests:

Molisch's test for carbohydrates

Two (2) drops of Molisch's reagent were added to each of the filtrates followed by addition of 1 ml of concentrated sulphuric acid (H2SO4) by the side of the test tube. The mixtures were allowed to stand for 2 minutes and then diluted with 5 ml of distilled water. The formation of a red or dull violet colour at the interphase of the two layers was a positive test for carbohydrates (Sofowora, 2008).

Barfoed's test for monossacharides

The filtrate (1 ml) was mixed with 1 ml of Barfoed's reagent in a test tube and heated on a water bath for 2 minutes. A red precipitate of cuprous oxide was considered as a positive test for monosaccharides (Sofowora, 2008).

Fehling's test for free reducing sugars

Each filtrate (1 ml) was heated with 5 ml of equal volumes of Fehling's solutions A and B. The formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars (Sofowora, 2008).

Test for combined reducing sugars

Each filtrate was hydrolysed by boiling with 5 ml of dilute hydrochloric acid and the resulting solution neutralised with sodium hydroxide solution. To this, few drops of Fehling's solution were added and heated on a water bath for 2 minutes. The appearance of a reddish-brown precipitate of cuprous oxide indicated the presence of combined reducing sugars (Sofowora, 2008).

Seliwanoff's test (standard test for ketoses)

Crystals of resorcinol (0.5 g) and 2 ml of concentrated hydrochloric acid (HCl) were added to 2 ml of extract already prepared and boiled for 5 minutes. A reddish colouration indicated the presence of ketoses-like fructose (Evans, 2009).

Test for tannins

Each extract (0.5 g) to be tested was stirred with 10 ml of distilled water and filtered. The filtrate was used for the following tests as described by Evans (2009).

Ferric chloride test

To 2 ml of the filtrate, few drops of 1 % ferric chloride solution were added; occurrence of a blue-black precipitate showed the presence of tannins.

Lead acetate test

A mixture of equal volumes of 10 % lead ethanoate was added to 2 ml of the filtrate. The formation of white precipitate was an indication of the presence of tannins (Evans, 2009).

Test for cardiac glycosides Salkowski's test

The extract (0.5 g) was dissolved in 2 ml of chloroform. Sulphuric acid was carefully added to the mixture by the side of the test tube to form a lower layer. The appearance of a reddish-brown colour at the interphase was an indication of the presence of steroidal ring (i.e a glycone portion of cardiac glycoside) (Silva et al., 1998)

To 0.2 g of each portion, 2 ml of acetic acid was added; the solution was cooled well in ice followed by the addition of concentrated H2SO4 carefully. The colour development from violet to bluish green indicated the presence of a steroidal ring i.e., aglycone portion of cardiac glycosides (Sophomore, 2008).

Test for phlobatannins

Each extract (0.5 g) was boiled with distilled water and filtered; the filtrate was further boiled with 1 % hydrochloric acid. The appearance of a red precipitate showed the presence of phlobatannins (Evans, 2009).

Test for glycosides

Test for free anthraquinones: (Bontrager’s test)

Each extract (0.5 g) was shaken with 10 ml of benzene and filtered. Then 5 ml of 10% ammonia solution was added to the filtrate and the mixture was shaken. The appearance of violet colour in the ammoniacal (lower) phase was taken as the presence of free anthraquinones (Evans, 2009).

Test for combined anthraquinones

The extract (0.5 g) was shaken with 10 ml aqueous sulphuric acid (H2SO4) and then filtered while hot, the filtrate was shaken with 5 ml of benzene; the benzene layer separated and half its own volume of 10 % ammonia solution was added. The appearance of red colour in the ammoniacal (lower) phase was taken as the presence of combined anthraquinones (Evans, 2009).

Test for terpenoids

Each extract (0.5 g) was dissolved in ethanol and 1 ml of acetic anhydride was added, followed by the addition of

concentrated sulphuric acid (H2SO4). A color change from pink to violet showed the presence of terpenoids (Silva et al., 1998).

Test for saponins

The extract (1 g) was boiled with 5 ml of distilled water, filtered and the filtrate was divided in two portions. To the first portion, 3 ml of distilled water was added and then shaken for about five minutes. Frothing which persisted on warming was evidence of the presence of saponins (Sofowora, 2008). To the second portion, 2.5 ml of a mixture of equal volumes of Fehling's solutions A and B were added. Appearance of a brick-red precipitate indicated the presence of saponins (Vishnoi, 1979).

Test for flavonoids

Shinoda's method

Each extract (0.5g) was dissolved in ethanol then warmed and filtered. Three pieces of magnesium chips were then added to the filtrate, followed by a few drops of concentrated hydrochloric acid. A pink, orange to purple colouration was an indication of the presence of flavonoids (Markham et al., 1985).

Ferric chloride method

Each of the extract was boiled with distilled water and then filtered. To 2 ml of the filtrate, a few drops of 10 % ferric chloride solution were added. A blue-green coloration was an indication of the presence of a phenolic hydroxyl group (Evans, 2009)

Lead acetate method

A mixture of equal volumes of 10 % lead ethanoate was added to 2 ml of the filtrate. The formation of white precipitate was an indication of the presence of tannins (Evans, 2009).

Sodium hydroxide method

Each extract (0.5 g) was dissolved in distilled water and filtered. To the filtrate, 2 ml of 10 % aqueous sodium hydroxide was added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication of the presence of flavonoids (Evans, 2009).

Test for alkaloids

Each (0.5 g) of the extracts were stirred with 5 ml of 1 % aqueous hydrochloric acid on water bath and then filtered. The filtrate (3 ml) was divided into 3 portions in a test tube and was used for the test below:

Dragendroff's test

Two (2) drops of Dragendroff's reagent were added to the first portion of the filtrate; the occurrence of an orange-red precipitate indicated the presence of alkaloids.

Meyer's test

Ten (10) ml of methanol was added to the second portion of the filtrate and filtered. To 2 ml of the filtrate, 1 % hydrochloric acid was added; the mixture was then boiled and filtered. Meyer's reagent (6 drops) was added to 1 ml of the filtrate. A creamy, reddish-brown precipitate was an indication of the presence of alkaloids (Evans, 2009).

Identification of the elemental components in banana flower

Fifty grams (50 g) of the remaining powdered flower was used to determine some of the elements present. It was placed in aporcelain crucible and then put into muffle furnace. The temperature was raised to about 50oC. After the sample was ashed, it was removed and cooled in a dessicator, then stored in a refrigerator at 4oC until required. Half gram (0.5 g) of the ashed sample was transferred into 250 ml beaker, 10 ml of 6 molar hydrochloric acid was added again and swirled. Ten (10) ml of distilled water was added and was heated on hot plate to complete dissolution. After allowing it to cool, it was filtered with filter paper (Whatman No-541) into 100 ml volumetric flask up to the mark. The solution was then transferred into polyethylene bottle for elemental analysis.

Sodium (Na)

Part A: Bicarbonate and Carbonate Anions

Test-tube was filled to the 10 ml line with sample water, 3 drops of total alkalinity indicator was added, capped and swirled to mix. A direct reading titrator 0-500 range was filled with sulfuric acid 0.IN and the titrator tip was inserted in the center hole of the test-tube cap. While gently swirling the tube, the plunger was passed slowly to titrate until the blue - green colour changed to red - orange. The test result was read directly from the titrator in PPM CACO (HCO, and CO) and recorded as result A

Part B: Sulphate and Chloride anions

Resin column was attached to a test - tube by inserting the tip of the column in the center hole of the tube cap. The column cap was removed. Another test - tube was half filled with deionized water. One ml pipette was used to add 4 ml of deionized water to the column. Additional deionized water was added in 1.0 ml increments until the water draining from the column became clear. One to ten ml pipette was used to add 5.0 ml of sample water to the resin column. Then the water that passed through the column was discarded up to this point. Three drops of total alkalinity indicator were added, capped and mixed. The second direct reading titrator 0-500 range was filled with sodium hydroxide 0.1N and the tip was inserted into the cap, while gently swirling the tube, it was titrated until the colour changes from red or red - orange to blue or blue green. The result was read from the titrator in PPM CACO (SO, and CL) and recorded as result B.

Part C: Calcium and Magnesium cations

A test - tube was filled up to the 12.9 ml line with sample water. Five drops of hardness reagent 45 was added, capped and swirled to mix. One hardness reagent # 6 was added, capped and mixed. A direct reading titrator 0-200 range was filled with hardness reagent # 7 and the tip was inserted in the cap. While gently swirling the tube, it was titrated until the colour changed from red to clear blue. The test result was read from the titrator in PPM CACO, (Ca and Mg) and recorded as result C. The sodium content of the test sample was calculated from the formula: Result A + Result B- Result C] x 0.46 - PPM Sodium.

Chloride (Cl)

A test - tube was filled with sample water to 10 ml line. The pH was adjusted to read between 7 and 10 using a pH meter or indicator stick to determine pH. Then 3 drops of chloride reagent A were added, capped and swirled to mix. A yellow colour was observed. The direct reading titrator was filled with chloride reagent B. The titrator was inserted into the center hole of the test - tune cap. While gently swirling tube, the plunger was pressed slowly until the yellow colour changes to orange brown. The test result was read where the plunger tip met titrator scale in PPM chloride, when reading test result, the original amount of the reagent dispensed should be included.

Lead (Pb)

Part A

Five ml (5 ml) of the sample was taken, then 5 ml of ammonium chloride buffers was added. Three drops of sodium cyanide 10 % was added to the solution, 0.5 ml of stabilizing agent was added also, it was capped, and shaken to mix. Then 0.5 ml of PAR indicator was added, capped, shaken to mix. The blank was scanned using the lamotte smart spectrophotometer and the sample was also scanned using the lamotte smart spectrophotometer.

Part B

The already heated sample in part A was used, 3 drops of DDC reagent was added to the sample and scanned. Final lead content - Part A- Part B.NB - The blank is either 10 ml of unheated sample or 10 ml of distilled water .Instruments used were : lamotte lead kit , test - tubes and lamotte smart spectrophotometer .

Calcium (Ca)

Using a 1.0 ml pipette, about 3ml of sample water was put into a calcium used dose vial (UDV) . The vial was inverted to mix and was scanned

Copper (Cu)

One point zero (1.0 ml) pipette was used to put about 3 ml of sample water into a copper used dose vial (UDV). The vial was inverted to mix and was scanned.

Magnesium (Mg)

A test –tube was filled to the 10 ml line with sample water. Put 0.1 g of magnesium and add hydrochloric acid 0.1 $moldm^3$ and nutric acid 0.4 $moldm^3$. The pH was adjusted to read between 7.35 and 10.5 using a pH meter or indicator stick to determine a silvery white colour.

Zinc (Zn)

A test tube was filled to the 10 ml line with sample water, 0.1 g of sodium ascorbate powder was added to the measured sample water, 0.5 g of zinc buffer was added, 3 drop of sodium cyanide 10% was also added, 4 drops of formaldehyde solution 37% was also added. one (1) ml pipette was used to add 1 ml of dilute zinc indicator solution, capped and mixed. Then the treated sample was scanned using smart spectrophotometer.

Iron (Fe)

A test - tube was filled to the 10 ml line with sample water. Zero point five ml (0.5ml) pipette was used to add 0.5 ml of iron with hardness reagent 1, zero point one gram (0.1g) of iron with hardness reagent 2 reagent was also added, capped and mixed. It was allowed for 3 minutes for colour development. The solution was shaken and scanned using spectrophotometer.

Potassium (K)

A test - tube was filled to the 10 ml line with sample water. Four drops of sodium hydroxide 1 ml was added to the measured sample water, 0.05 g of tetraphenylborate reagent was also added, capped and mixed. The solution was allowed to stay for 5 minutes. Then it was scanned using smart spectrophotometer.

Manganese (Mn)

A test - tube was filled to the 10 ml line with sample water. Three drops of sodium cyanide 10 % was added, zero point five mi (0.5 ml) of manganese indicator was added using 0.5 ml pipette. It was capped, mixed and scanned using smart spectrophotometer.

Experimental animals

Thirty-two (32) albino rats were used for this study. Both sexes (male and female) rats weighing 120g to 200g were obtained from the Department of Biochemistry, University of Maiduguri. The rats were kept in aluminum cages and allowed to adjust to the laboratory environment for a period of three days before commencement of the experiment. They were fed with broiler's finisher (Ammo feed® Nig. L.T.D) and water was provided ad libitum.

Acute toxicity study (LD_{50})

The acute toxicity was determined as described by Lorke (1983). Twelve

(12) rats were used for acute toxicity study, using Lorke's method phase I and II. The rats were randomly selected for the experiment.

In phase I, nine (9) rats were weighed and labeled with picric acid on the head, back and tail (as required) as a mark of identification, the nine(9) rats were grouped into A, B, and C with three rats each. The extract was given orally at the dose rates of 10 mg/kg, 100 mg/kg, and 1000 mg/kg respectively. They were observed for 24 hours for signs of toxicity and mortality.

In phase II, three (3) rats were randomly selected weighed and labeled as A, B and C of one rat in each group and were given identification marks. The animals were given the extract at doses 1600 mg/kg, 2900 mg/kg and 5000 mg/kg respectively based on the result of phase[1]. The rats were allowed to access food and water ad libitum and were observed for 24 hours for signs of toxicity and death.

Experimental animals

Twelve (12) albino rats were used for this study. Both sexes (male and female) rats weighing 120g to 200g were obtained from the Department of Biochemistry, University of Maiduguri. The rats were kept in aluminum cages and allowed to adjust to the laboratory environment for a period of three days before commencement of the experiment. They were fed with broiler's finisher (Ammo feed® Nig. L.T.D) and water was provided ad libitum.

RESULTS

Phytochemical screening of methanolic extract of banana flowers

The qualitative phytochemical analysis of methanolic extract of banana flowers is presented in Table 4.1. The analysis revealed the presence of carbohydrate, cardiac glycosides, terpenoids, flavonoid, tannins and saponin glycoside, while alkaloid, cardenolide and anthraquinone were absent.

Elemental analysis of methanolic extract of banana flowers

The results of elemental analysis of methanolic extract of banana flowers are shown in Table 4.2. The results revealed the presence of potassium, calcium, manganese, chloride, zinc, lead, iron, sodium, copper, and magnesium in parts per million. Potassium had the highest concentration, followed by manganese and copper respectively.

ACUTE TOXICITY

In the acute toxicity test, rats of different groups (a and b) were orally administered methanolic extract of banana flowers at the dose of 10 mg/kg to 5000 mg/kg and the rats were monitored for behavioral changes, toxicity signs and

mortality but there was no mortality recorded, however some changes such as dizziness and weakness were observed as shown in the Table 4.3.

$LD_{50} = \sqrt{axb}$

Where a = least dose that showed zero mortaliy (10 mg/kg). b = highest dose that showed no mortality (>5000).

There was no mortality even at the highest Dose of 5000mg/kg.

RESULTS

Phytochemical	Test	Result
Alkaloids	Dragendroff's reagent	-
Alkaloids	Mayer's reagent	-
Flavonoids	Shinoda's	+
Flavonoids	Ferric chloride	+
Flavonoids	Lead acetate	-
Flavonoids	Sodium hydroxide	-
Cardiac glycoside	Salkowski's	+
Cardiac glycoside	Lieberman-Burchard	+
Cardenolide	Keller-Killiani	-
Terpenoid	Standard test	+
Tannin	Ferric chloride	+
Tannin	Lead acetate	-
Saponin glycoside	Frothing	+
Carbohydrates	Molisch's	+
Carbohydrates	Barfoed's (monosaccharide)	-
Carbohydrates	Fehling's (free reducing sugar)	+
Carbohydrates	Combined reducing sugar	+
Carbohydrates	Standard test for ketone	+
Anthraquinone	Combined anthraquinone	-

Table 4.1: Phytochemical constituents of methanolic extract of banana flower

Element	Concentration (ppm)
Potassium (K)	13.4
Calcium (Ca)	0.5
Manganese (Mn)	13
Chloride (Cl)	1
Zinc (Zn)	2.56
Lead (Pb)	2
Iron (Fe)	5.6
Sodium (Na)	8
Copper (Cu)	13
Magnesium (Mg)	4.8

Table 4.2: Elemental constituents of methanolic extract of banana flowers

Oral Routes

Phase 1	Dose (mg/kg)	Number of rats	Clinical signs	Mortality
1	10	3	Dizziness	0/3
1	100	3	Weakness	0/3
1	1000	3	Weakness	0/3
2	1600	1	Weakness	0/1
2	2900	1	Weakness	0/1
2	5000	1	Weakness	0/1

Table 4.3: Calculation of LD_{50} of the methanolic extract of banana flowers in albino rats

DISCUSSION

Plant consumption for their nutritional and medicinal values has been since the evolution of man. The phytochemicals obtained using methanolic extract in this study was in agreement with similar studies conducted by Dong et al (2016), Nascimento et al (2021), Oresanya et al

(2020) and Lakshmi et al (2024) which showed methanolic banana flower extract had carbohydrate, cardiac glycosides, terpenoids, flavonoid, tannins and saponin glycoside. Presence of these phytochemicals suggests that banana flower can be used therapeutictically as an antioxidant as well as an anticancer agent as they inhibit the growth of cancer cells. Saponins are known to lower the level of cholesterol. Some elemental contents obtained in this study, were also in agreement with the study conducted by Ramu et al (2017) and Basumatary and Daniel (2024) where potassium

(K) is the macro-element with the highest concentration, followed by calcium (Ca) and magnesium (Mg). However, in this study, potassium (K) had the highest concentration, followed by manganese (Mn) and copper (Cu). Potassium is an essential macronutrient that functions for both maintaining osmotic pressure of cells and as a neurotransmitter. Calcium on the other hand, is essential for bone development as well as muscle contraction. In addition, iron (fe) found in the methanolic banana flower extract in this study, indicate that it is essential for hematopoiesis.

The acute toxicity value obtained in this study (>5000mg/kg), is within the standard range of 500-5000

mg/kg body weight for non-toxicity of a substance (Lorke, 1983). Any substance with LD_{50} of 50-500 mg/kg body weight is regarded as highly toxic. Substances having an LD_{50} of 500-1000 mg/kg are regarded as moderately toxic. While those with LD_{50} above 1000 mg/kg are regarded as being of low toxicity and therefore relatively safe to be applied for treatment (Clarke and Clarke, 1977). Any compound with an oral LD_{50} between 500-5000 mg/kg is considered practically non- toxic (Hodge and Sterner, 2005). According to Hodge and Sterner toxicity scale, banana flower is considered safe for use.

STATEMENTS AND DECLARATIONS

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: EOA and AYG designed the research study. EOA, AYG, BU, DAT, and UAM collected the research data. YML and AYG analyzed the data. EOA, AYG, BU, DAT, UAM, and YML contributed to the writing and review of the manuscript. All authors have read and approved the final manuscript.

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