# Antibacterial Efficacy of Adamsonia Digitata Stem Bark Crude Extracts Against Staphylococcus Aureus and Shigella Dysenteriae

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DOI: 10.56201/ijhpr.v8.no3.2023.pg136.147

#### ABSTRACT

This research work on the antibacterial efficacy of Adamsonia digitata crude stem bark extracts against Staphylococcus aureus and Shigella dysenteriae, was conducted using Disc diffusion method, on the ethanol and aqueous crude extracts of Adamsonia digitata stem bark against Staphylococcus aureus and Shigella dysenteriae. The crude extracts were effective at all the concentrations (10, 30, 50 and 70 mg/ml) used on the test bacteria. The ethanol crude extract indicates high antibacterial efficacy of 6.0, 12.0, 16,0, 16.0mm and 10.0, 16.0, 16.0 and 16.0mm with all the concentrations used against Staphylococcus aureus and Shigella dysenteriae respectively while the aqueous crude extract also indicates activity of 6.0, 12.0, 16,0, 20.0mm and 12.0, 16.0, 16.0 and 16.0mm all all the concentrations used against Staphylococcus aureus and Shigella dysenteriae. Ciprofloxacin impregnanted antibiotic disc was used as positive control which indicates activity of 18.0 and 20.0 against Staphylococcus aureus and Shigella dysenteriae. The minimum inhibitory concentration (MIC) and the minimum bactericidal study of ethanol and aqueous stem bark crude extracts of Adamsonia digitata against Staphylococcus aureus and Shigella dysenteriae indicates that the plant part used is potent against the test bacteria under study. The phytochhemical screening of the stem bark crude extracts of the plant showed the present of alkanoids, saponins, tannins, flavonoids, terpeniods, glycoside and Anthraquinose. This research work is pointer of the possible used of Adamsonia digitata stem bark crude extracts as remedy for the treatment of infections or disorders cause by these bacteria under study.

*Keywords:* Adamsonia digitata, antibacterial, efficacy, Staphylococcus aureus, Shigella dysenteriae, diffusion, determine.

#### **INTRODUCTION**

*Adamsonia digitata,* 'the Baobab' belongs to family Malvaceae and it is the most widespread tree species in the genus Adamsonia. The baobabs, The plant is native to Africa and typically present in dry, hot grasslands of sub-Saharan Africa. The tree is massive and grow up to 25m high, deciduous in nature which may survive for hundreds of years an used for medicinal purpose (Anani *et al.*, 2000).

The plant's lead infusions are important in the treatment of several diseases such as diarrhea, fever, inflammation, kidney disease, and asthma. The leaf is also a good source of proteins. The antibacterial efficacy of A. digitata may be attributed to the availability of secondary metabolites it contained. Several studies conducted on its phytochemistry revealed the presence of important bioactive constituents. (Yusha'u et al., 2010) found that the back extracts of A digitata contain alkaloid, flavonoid, tannin, reducing sugar and steroid which were active against some pathogenic bacteria; E.coli, S.aureus, Klebsiella spp, Proteus morabilis and Shigella dysentariae. (Kubmarawa et al., 2007). Reported that phytochemicals such as Alkaloid, Saponins, Flavonoids, tannins and terpenoids are chemical bioactive components that could be responsible for antibacterial activities in the plant. Findings on antibacterial studies indicated that ethanolic, methanolic and aqueous stem bark extracts of A. digitata demonstrated antibacterial activity against some pathogenic bacteria such as E. coil, Bacillus subtilis, Staphylococcus aureus, Mycobacterium phlei and Streptococcus faecalis. The stem bark extracts of the plant contain secondary metabolites which are responsible for antimicrobial activity of the crude aqueous and ethanol extracts of the plant contain secondary metabolites which are responsible for antimicrobial activity of the crude aqueous and ethanol extracts. Vitexdoniana is used by traditional healers alone or in a combination with stem bark of A. digitata to treat diarrhea, leprosy and dysentery. (Yusha'u et al., 2010). It was also found that extract of lead and root bark possessed antiviral properties due to the presence of sterol, Saponins and teriterpenes. The lead of the plant is used for treatment bladder and kidney diseases, general fatigue, diarrhea, asthma, insects bite, inflammations and guinea worm. A decoction of the bark deteriorates rapidly due to the mucilaginous substances present Baobab bark is used in Europe as a febrifuge (antipyretic). In some Africa countries such as Ghana, the stem bark extract of A.digitata is used for curing malaria fever in place of quinine. In Indian medicine, Baobab bark is used internally as a refrigerant, antiperiodic. In Nigeria, the bark of the plant is certainly used for the treatment of fever. The antibacterial activity of A. digitata may be attributed to the presence of secondary metabolites it contained. The present study was aimed to screen for phytochemical constituents and antibacterial efficacy of leaf and stem bark extract of A. digitata against clinical isolates of E. coil and S. dysentriae (Abdullahi et al., 2016).

#### Statement of the problem

In an attempt to combat the various forms of disease that have continued to plague humans from time immemorial to this day, different types of antimicrobial agents have been developed to fight the pathogens responsible for these diseases. Antimicrobial agents, which are substances that kill or inhibit the growth of microorganisms, could be in the form of antibiotics, which are products of microorganisms or synthesized derivatives (Cowan, 2002), antimicrobial peptides produced by complex organisms as well as some microbes (Jenssen *et al.*, 2006) and medicinal plants, which

appear to be the focus of mainstream medicine today (Cowan,2002). They are widely employed to reduce the microbial load or in the cure of disease associated with microorganisms mostly bacterial. The action of these agents could either irreversibly kill the bacteria or reversibly inhibit the growth of a microorganism due to continuous contact with the agent (Rajesh and Rattan, 2008). Drug reaction and side effects, increase the risk of malignancy, fake and adulterated drugs have added to the problem of antibiotic resistance (Green, 2007). The emergence of pathogens resistant to antibiotics as a result of their excessive use in clinical applications represents a serious public health concern (Keymanesh *et al.*,2009). The search for plants with antibacterial properties has gained increasing importance in recent years due to the development of resistance. (Fhogartaigh and Edgeworth, 2009). This research evaluate the antibacterial efficacy of *Adamsonia digitata* stem bark crude extracts against some selected clinical isolates (*Staphylococcus aureus and Shigella dysentiae*) from community acquired infections, for ethnomedicinal use.

# METHODOLOGY

## Sample collection

The stem bark of baobab (*Adamsonia digitata*) was collected in Birnin Kebbi Local Government Area of Kebbi State Nigeria and taking to the Department of Science Technology, Waziri Umaru Federal Polytechnic, Birnin Kebbi, for identification and processing.

#### Sample processing

The sample was washed thoroughly on tap water and air dried in a shade for two weeks, and then cut into smaller piece and grinded into powder using a sterile pestle and mortar under laboratory condition. The powder was then kept in air tight condition for further use. (Mukhtar and Tukur,2016).

## **Extraction of the processed Sample**

Adamsonia digitata stem bark plant extract were prepared by cold maceration method. 100g of the powdered extract was weighed and poured in a concial flask, 250ml of ethanol was then poured into the conical flasks that contain the extract and was allowed to stay for three days. This was then sieved (Filtered) using whatman No1 filter paper and the extract was concentrated in water bath at  $40^{\circ}$ C for 4 days to obtain the ethanol crude extract. The same process was repeated for the aqueous extract using warm water to obtain the aqueous extract. (Al-Basri *et al.*, 2005).

#### Formulation of varying concentrations of extracts

Exactly 1g, of the ethanol extract was measured using weighing balance and poured into 10ml of distilled water in different beakers. The same procedure was repeated for 3g, 5g, and 7g to obtain varying concentration of 10mg/ml, 30mg/ml, 50mg/ml, and 70mg/ml respectively.

#### **Media preparation**

All the media used in this research, were prepared according to the manufacturers instruction as contained on the label of the media container. The media used are nutrient agar, nutrient broth, S-S agar and EMB agar.

#### **Confirmation of Bacteria**

The clinical isolates obtain from the hospital were *Staphylococcus aureus* and *Shigella dysenteriae*. The isolates were transported to microbiology laboratory of Department of Science Technology, Waziri Umaru Federal Polytechnic Birnin Kebbi, Kebbi State. The isolates were subjected to a confirmatory test by sub-culturing the colonies, Gram staining and to specific biochemical test.

## Gram's Staining

A smear of the isolates were prepared from the colonies that developed from the medium on glass slide separately, which were then stained with crystal violet solution and were allowed for 60 seconds, after which each slide was washed with distilled water and was well-drained to avoid diluting the mordant. The slides were flooded with iodine solution (mordant) and allowed for 60 second and was washed with water. The slides were then decolorized with acetone and were washed immediately with water. The slides were counter stained with neutral red and were allowed to stay for 1-2 minutes and were washed and allowed to dry. All slides were examined under the oil immersion objectives lens (Cheesbrough 2000).

# **Biochemical Test**

The biochemical test performed include; catalase test, coagulase test (Slide test), indole test, methylered voges-proskauer test (MR-VP) for bacterial characterization and identification. (Parija *et al.*, 2006).

## **Catalase Test**

A drop of hydrogen peroxide was placed on a clean grease-free slide separately and emulsified with the test organism picked using a sterile wire loop then observed for able formation. (Oyeleke and Manga, 2008).

## Coagulase Yeast (Slide Test).

Clean glass slides were marked into two halves by a marker. Two drops of sterile saline were added on two halve of the glass slides. Colonies of S. Aureus to be tested were picked up from agar culture and gently emulsify with drops of saline. A drop of undiluted plasma was added to the bacterial suspension and mixed with a wire loop. Another drop of saline was added in another half of the slide as a control. The slides were rock back and forth, and observed for the prompt clumping of the bacterial suspension within 10-15 minutes seconds (Parija *et al.*, 2006).

## Indole test

The organisms were grown in 5 ml of peptone water for 24 hours. After 24 hours of incubation Kovac's indole reagent of about three was indicated by the development of a red colour in the reagent surface layer above within 10 minutes while negative reaction retained its yellow colour (Parija *et al.*, 2006).

## Methyl-Red Vogues- Proskauer Test (Mr-Vp)

Five milliliters of MR-VP broth were inoculated with the isolates and incubated at 37<sup>o</sup>C for 48 hours. After incubation, 1ml of the broth each were transferred to a small test tube separately and about 2-3 drops of methyl-red were added. A red colour on addition of the indicator signified a positive test while a yellow colour signified negative test. To the rest of the broth in the original tube 5 drops of 40% potassium hydroxide followed by 15 drops of 5% naptthol; in ethanol were

added. It was shaken and the cap of the tube was loosened and the tubes were placed in a sloping position. The development of a pink colour starting from the liquid air interface within 1 hour indicated a VP positive test, no colour change occurs in a VP negative test (Parija *et al.*, 2006).

# H<sub>2</sub>S Test

The sample was inoculated in a labeled test tube by means of stab inoculation in SIM (Sulfide, Indole, and Motility) medium. The inoculated tubes were incubated at  $37^{0}$ C for 24 -48 hours. Observe for the formation of black precipitate on the medium (Parija *et al.*, 2006)

## **Citrate Test:**

Simmons citrate agar was inoculated with isolates, using sterile wire loop and incubate for 47hours at  $37^{0}$ c a deep blue color indicate a positive result. (Parija *et al.*, 2006).

## Glucose test

Exactly 0.5g of extract was dissolved in 3cm of 3.5cm<sup>3</sup> Feel<sub>3</sub> om g;acial acetic and left for minute. 1.5cm of H<sub>2</sub>SO<sub>4</sub> was then being pipette into it in order to run down the side of the test tube. A positive test will serve as a clear interphase with a blue layer (Parija *et al.*, 2006).

# **Oxidase Test:**

A piece of tissue paper was place in a clean petridish and 2-3 drops of freshly prepared oxidase reagent was added a colony of the test isolated was picked using a sterile glass rod and smear on the filter paper. Blue-purple colour was observed within few seconds. (Parija *et al.*, 2006).

## Sucrose test:

After incubating the test tube of sucrose + HCL for 20 minutes at  $37^{0}$ C degrees Celusius in an attempt to hydrolyze the sucrose. After the 20 minutes, you conduct a Benedicts test on the contents of the sucrose + HCL test tube and this is what you see ( the test tube on the left is you control; the test tube on the right is your sucrose + HCL. (Parija *et al.*,2006).

## Lactose Test

An inoculums from a pure culture is transferred aseptically to a sterile tube of phenol red lactose broth. The inoculated tube is incubated at  $35-37^{0}$ C for 24 hours and the results are determined. A positive test consists of a colour change from red to yellow indicating a pH change to acidic. (Parija *et al.*,2006).

## **Motility Test**

This contains low concentration of agar (0.2-5%) and motion organisms are able to move away from the line of inoculation through sloppy agar. (Parija *et al.*,2006).

## **Unease Test**

The prepared unease medium was inoculated with the test isolated and incubated at  $36^{\circ}$ C for 4-448 hours. A bright pink or red colour indicated a positive reaction. (Parija *et al.*,2006).

# **Triple Sugar Iron (TSI) Test:**

Colonies from fresh culture were picked and streaked on the surface of the slope and stabled on the bottomed by using sterile wire loop and incubated at 37<sup>0</sup>C for 48 hours. Tripe Sugar Iron

medium are composite media and several reaction can be read each 24 hours incubation. Gas information is determined by the appearance of one or several bubbles. These can results in crack in the butt or may be pushed from the bottom.

Formation of hydrogen Sulphate is determined by then blackening at the slant butter junction/Glucose information is indicated by butt becoming yellow in addition to glucose formation, lactose or sucrose or Bothe sugar are fermented in TSI both the butt and the start would become yellow. In TSI, it means that either glucose or lactose has been fermented or glucose has fermented or clucose has been fermented or glucose, lactose and sucrose has been fermented (Parija *et al.*,2006).

#### Antibacterial testing

Whatman's No1 filter paper was punched into 6 mm disc from, sterilized and dipped in crucible concentrations of extracts which are 5mg/ml for 24hours precautions were taken to prevent the flow of the solvent extract from the discs to the outer surface (Ahmed and Beg, 2001).

#### Sensitivity Test using disc Diffusion Method

Disc diffusion methods was employed in this research work. The disc were mixed into the various concentrations of the extract removed and allowed to dry. The organisms were starched on the plate that Muller Hinton agar. The dried discs were placed into the plated that contain the organisms (Staphylococcus aureus and Shigella dysenteriae this was incubated at 370C for 24 hours, after the incubation period, the zone of inhibition was then measure, recorded and expressed in millimeter, Ciprofloacin antibiotic impregnated disc was used as the control s(Ahmed and Beg, 2001).

## **Evalution of the MIC and MBC**

The minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the crude extracts on the test bacteria (Staphloccus aureus and Shigella dysenteriae) were determined according to the method purposed by Guarav'et al., (2005). Twelve sterilized test tube were used and 1ml of sterilized nutrient broth was dispensed from test tube 2 to test tube 12, a stock of solution of each of the stem bark extract of Adamsonia digitata (Ethanol and Aqueous) extracts was prepared i.e. 70mg of the crude extract was dissolved in 10ml of distilled water, 11 of the stock solution was dispensed aseptically into tube 1 and 1ml into test tube 2 from the content of the test tube 2, a double dilution was performed using 1 mkl transfer to test tube 10, leaving 11 and 12, 1 ml was taken out from test tube 10 and discarded. The concentration in each test tube 1 to 10 was 70, 35, 17, 5, 8, 75, 4, 38, 2.19, 1.09, 0.55, 0.27 and 0.14 respectively. Broth culture of each of the organism Staphylococcus aureus and Shigella dysenteia was prepared separately and 1ml of the prepared broth culture was dispensed into each test tube with exception of the test tube 11, and was then incubated at 37<sup>o</sup>C for 24 hours, the test tubes were examined for turbidity in order to determine the MIC and MBC. The MIC was the concentration in the test tube that failed to show evidence of growth (turbidity), just immediately after the last one that showed growth. MBC were the tubes that fail to show nay growth including the MIC and were cultured on nutrient agar. The absence of the growth after incubation indicated a positive result of MBC (Adeleke et al., 2006).

#### Phytochemical screening

Phytochemical screening of the extracts was done as described by Sofowora (2008).

#### Test for alkaloids

A fraction of the extract of 2ml was treated with 2ml Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml distilled water) and observed for the formation of reddish brown coloured precipitate. (Sofowora, 2008).

#### **Test for Flavonoids**

Sulphuric acid test. A fraction of the extract of 2ml was treated with 2ml concentrated Sulphuric acid and observed for the formation of orange color. (Sofowora, 2008).

#### **Test for Terpenoild**

To 1ml of chloroform, was added to 2ml extract and were mixed, and concentrated Sulphuric acid was added drop wise to form a layer. The presence of reddish brown coloration at the interface indicates the presence of terponoids. (Sofowora, 2008).

#### **Test for Saponins**

Twenty milliliters of distilled water was added to 1 ml of the extract in a graduate cylinder and shaken gently for 15 minutes. The formation of 1cm layer of foam indicates the presence of saponins (Sofowora, 2008).

#### **Test for Tannins**

One percent gelating solution containing sodium chloride was added to extract. The formation of white precipitate indicates the presence of tannins. (Sofowora, 2008).

#### Test for steroids (Salkowski's Test)

Exactly 0.2g of the extracts was dissolved in 2ml of chiroform, concentrated sulphuric acid was carefully added to form a lower layer. A reddish-brown colour at the interphase indicated the deoxy sugar characteristics of cardenolides. (Sofowora, 2008).

#### **Test for Anthraanuinones**

Exactly 2.0g of the extract was shaken with 4ml of benzene. The mixture was filtered and 2ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of pink. (Sofowora, 2008).

#### **Test for Glycoside**

The extract was hydrolyzed with HCL solution and neutralized with NAOH solution. A few drops of fellings solution A and B were added. Red precipitate indicates the presence of glycosides (Sofowora, 2008).

## **RESULTS AND DISCUSSIONS.**

All the results of this research work are presented on tables.

**Table1:** Shows the confirmed clinical bacteria isolates

	<b>Confirmed Bacteria isolates</b>		
Gram Rxn/Biochem. Test	S. aureus	S. dysentariae	
Gram Rxn	+veCocci	-veRod	
Catalase	+ve	+ve	
Coagulase	+ve	+ve	
Indole	+ve	-ve	
Citrate	+ve	+ve	
Urease	+ve	+ve	
Methyl red	+ve	+ve	
Voges Praisker	-ve	-ve	
Glucose	-ve	-ve	
Sucrose	-ve	-ve	
H <sup>2</sup> S	+ve	-ve	
Motility	-ve	-ve Key:Rxn= Reactio	

Ve= Positive, -ve = Negative (Cheesbrough 2000).

Table2: Shows mean zone of inhibition of Adamsonia digitata stem bark crude extracts against the test bacteria

	Test Bacteria/Mean zone of Inhibition (mm)			
Extracts	Conc. (mg/ml)	S. aureus	S. dyconteriae	
Ethanol extract	10	6.0	10.0	
	30	12.0	16.0	
	50	16.0	16.0	
	70	16.0	16.0	
Aqueous extract	10	6.0	12.0	
	30	12.0	16.0	
	50	16.0	16.0	
	70	20.0	16.0	
Cipro tab control		18.0	20.0	

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Key: mm=millimeter, mg/ml=milligram per ml (Ahmed and Beg,2001).

 Table3: Shows the minimum inhibitory Concentration (MIC) and Minimum bacteriacidal

 Concentration (MBC) results of the extracts Clinical against the test bacteria

Extracts / Test Bacteria	MIC Values	MBC Values	
			Ethano
S aureus	8.75	17.5	
S dysenteriae	8.75	17.5	
Aqueous Extract			
S aureus	4.375	8.75	
S dysenteriae 17.5	8.75		

**Key:** mg/ml = Milligram/ml, mm = Millimeter

**Table4:** Shows the Phytochemical Constituentspresent Adansonia digitata stem bark Extractscrude Extract.

Level of presence		
Ethanol extracts	Aqueous extract	
++	++	
++	++	
+	++	
++	++	
-	-	
++	++	
++	++	
+	+	
	Ethanol extracts ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++	

Key: ++=highly present, +=Slightly present, -= Absent (Abdullahi *et al.*, 2007).

#### Discussion

The use of plants as remedy to cure diseases, has been extensively practice in indigenous folk medicine from time immemorial and the literature survey reveals, that medicanal plants are of great potential for therapeutic treatments, in spite of the fact that they have not been completely investigated for maintaining health, especially in the last decade, with more intensive studies for natural therapies in the search for antibacterial compound mainly from plants, which continue to be a major source of biological active matabolites that may provide lead structure for the development of new drugs.(Green,2007).

The result of the antibacterial efficacy study of both the ethanol crude extract and aqueous extract of *Adansonia digitata* stem bark was carried using disc diffusion methods at various concentration (10, 30, 50, and 70 mg/ml) against the test bacteria isolated (*Staphylococcus aureus* and *Shigella dysenteriae*) which are clinical isolates obtained from the hospital. The results indicates that the *Adansonia digitata* stem bark crude extract has good activities against the test bacteria.

Table1, indicates the results of the confirmatory test of the clinical bacteria isolates that was obtain from the hospital to confirm the claim of the hospital laboratory. Similarly from Table2, indicates the results of the efficacy of ethanol crude extract of *Adansonia digitata* stem barck, which indicates activity of 6.0, 12.0, 16.0, 16.0 and 7.0; 16.0, 16.0, and 20mm against *Staphylococcus aureus* and *Shigella dysenteriae*, on the various concentrations of 10, 30, 50, abd 70 mg/ml used respectively. Followed by aqueous crude extract of *Adansionia digitata* setm bark, which also show the high efficacy of 6.0, 12.0, 16.0, 20.0, 18.0 and 7.0, 6.0, 16.0, 16.0 and 20.0mm against *Staphylococcus aureus* and *Shigella dysenteriae*, on same concentration used. This finding shows that the activities of extracts increases with increase in concentrations of the extracts against the test bacteria. The finding of this studies agree with that of Green, (2007) who reported that the *Adansonia digitata* have wide range of spectrum against microbes.

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extract against the test isolates in presented in the table3 from the table, the result showed that the extracts were able to inhibit the growth of the isolates at concentration ranged between 10-70mg.ml. Table4 indicates the preliminary phytochemical secreening of *A. digitata* stem bark crude extracts, which indicates the presence of alkaloids, flavnoids, tannin, terpenoids, sapanins glycodide and Anthroquinose. This finding is inline with that of (Ali and Yahaya, 2016), who screened for the phytochemical constituents of *A. digitata* stem bark using different solvent such as aqueous, chloroform and ethanol, and result showed the present of ternoid alkaloids and fatty acids.

The findings of this research work agrees with that of Yusha'u *et al.*, 2010), who found that the bark extract of *A. digitata* contain alkanoids, flavonoid, termins reducing sugar and steroid. The result of antibacterials activity of *A. digitata* extract against *Staphylcuccus aureus* and *Shigella dysentriae* that the ethanol extracts is more effective against the test bacteria than the aqueous extract. *Staphylcocccus aureus* was found to be more susceptible to the extract in compared to the *Shigella dysenteriae* each with average zone of inhibition of 14.97 and 13.84mm respectively. The result of antibacterial efficacy of *A. digitata* in this study is in conformity with the study conducted by many researchers and the efficacy of the extracts was expected, due to the presence of compounds such as alkalnoids, flavornoids and tannins.

The Minimum Inhibitory concentration of the extract against the isolates revealed that the extract were able to inhibit the growth of the bacteria at the concentrations used, the ethanol stem bark crude extract were more effective with low MIC when compared to the aqueous extracts.

#### Conclusion

The present investigation envisages that the crude extract of *A. digitata* contains potential antibacterial compounds that have great activity against the bacteria under study. The phytochemical screening of the stem bark crude extract of *Adansonia digitata* shows the presence of important bioactive compounds. The Minimum Inhibitory Concentration (MIC) of the extracts shows the tendency of the extracts to inhibit the test bacteria.

#### Recommendation

The following recommendations were made:

- Further research work should be carried out on the roots, seeds, fruits pulp and aerial part to be able to ascertain which of these parts is more effective on the bacteria studied.
- Further characterization and isolation of bioactive constituent(s) of the extracts of this plant should be carried out.
- Also, toxicity study should be carried out on the plant

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