Research Article

Evaluation of Allelopathic Action of *Adansonia digitata* L. Root Extract on the Germination and Growth of *Lettuce*, *Hibiscus* and *Sorghum*

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ABSTRACT

The present study is aimed at evaluating the allelopathic effect of *Adansonia digitata* L. on seed germination and seedling growth of *Lactuca sativa* L., *Hibiscus sabdariffa* L. and *Sorghum bicolor*. Three root samples of *A. digitata* were collected, extracted by ethanol and subjected to qualitative phytochemical analysis. Each extract, in three concentrations (0.1, 0.25 and 0.5 mg/mL) was determined for inhibitory effects on germination and seedlings growth of the three test plant species. Results revealed that all root extracts of *A. digitata* caused significant inhibitory effect on germination, shoot and root lengths of seedlings of the test plant species. The inhibition effects increased with increasing extract concentrations. The study also revealed a variation in inhibition effects due to different location of root sample. The inhibitory effect on germination and seedlings growth was more pronounced in *L. sativa* and *Sorghum* rather than *Hibiscus*. Roots of all test plants were more sensitive to the extract than their shoots. The results suggest that the inhibitory and stimulatory effect potency of *A. digitata* may be due to the presence of the allelochemicals like; flavonoids, saponins, tannins, alkaloids and terpenoids in the root extract, and the plant may be a candidate for isolation and identification of allelopathic compounds.

Keywords: *Adansonia digitata*, allelopathic activity, root extracts, phytochemical screening.

INTRODUCTION

Allelopathy is a novel approach for environment safety and development of sustainable agriculture.[1] Incorporating allelopathy into natural and agricultural management systems may reduce the use of herbicides, insecticides, and other pesticides and diminish autotoxicity hazards.[2] Plants produce secondary metabolites that are generally non-essential for the basic metabolic processes of the plant. Among these secondary plant metabolites, some are known as allelochemicals that improved defense against other plant competition, and most of them are phenolic acids, flavonoids, terpenoids, steroids, alkaloids and organic cyanides.[3] Allelochemicals act through direct interference with physiological functions of 'receiver' such as seed germination, root growth, shoot growth, stem growth and symbiotic effectiveness.[4]

Baobab Tree

Baobab, *Adansonia digitata* L. (Malvaceae), is one of the oldest living tree species. It is a deciduous, massive and majestic tree up to 25 m high that grows principally in Africa and can live up to 1000 years.[5] The baobab is easily distinguishable by its huge trunk. It has an extensive lateral root system, relatively shallow but spread out to a distance greater than the height of the trees.[6-7] The baobab trees bear leaves only for three months per year. During the leafless period physiological processes such as photosynthesis take place in the trunk and branches, utilizing water stored in the trunk.[8] In Sudan, the baobab tree is called *Tebaldi*, and it is most frequently found on sandy soils and by seasonal streams in short grass savannas. It forms belts throughout Central Sudan, Kordofan, Darfur and Blue Nile regions.[9]

A. digitata has been widely used in traditional medicine.[8,10-12] More than three hundred traditional uses have collectively been documented in Africa.[13]

The plant parts (leaves, bark, roots and fruit pulp), have traditionally been used to treat various ailments such as diarrhea, dysentery, malaria, microbial infections, inflammations, analgesic and insect repellent and as pesticides.[14,15]

The chemical constituents of *A. digitata* have been investigated extensively, and several classes of compounds have been identified from various parts of baobab including terpenoids, flavonoids, sterols, vitamins, amino acids, carbohydrates and lipids.[16,17]

The alkaloid 'adansonin' and the terpenoids friedelin, lupeol and baurenol were identified in the bark of baobab.[18]

It has been observed that in places where baobab trees dominate, the vegetation beneath the tree is poor. It was hypothesized that certain substances excreted by the plant roots may have allelopathic action in that they inhibit the growth of other plants.

However, no bioactive compound directed at plants has been reported in this species. Keeping in mind the above facts the present study was formulated to investigate possible allelopathic activity of *A. digitata* root extracts

and to determine their growth inhibitory activity on monocotyledonous and dicotyledonous species.

MATERIALS AND METHODS

All chemicals, solvents and reagents used were of analytical grade and most of them were supplied by Fisher Scientific (Springfield, NJ), British Drug Houses (England) and Sigma (Germany), and were of the purest grade available.

Study Area

This study was conducted in North Kordofan region, located in central of the Sudan and lies between latitude 12˚ 43ˉ - 13˚ 42ˉ N and longitude 30˚ 14ˉ - 31˚ 55ˉ E.

It is characterized by a dry, hot climate, typically tropical continental with a relatively short rainy season. The plant samples were collected from three locations around El Obeid city (Capital of the region); Khor tagat which was located about 10 kilometers north east the city (labeled C_1), Japal Kordofan (12 kilometers, south east, C_2) and Um semayma (15 kilometers, west, C_3).

In all the three locations the type of the soil is sandy loam and recorded species neighboring to baobab trees are: *Boscia senegalensis*, *Balanites aegyptiaca*, *Calotropis procera*, and *Ziziphus spina-christi*.

Plant Materials

Roots were collected from mature baobab trees in the three sites.

The plant species was identified by Mr. Monir ELyas, a botanist at the Department of Forestry and Range Sciences, University of Kordofan, Sudan. Collection was done before the rainy season between January and March 2014, when the trees were completely leafless. Root samples were washed several times to get rid of soil particles and were chipped to small pieces, air-dried under shade then pulverized into powdered forms and stored for further use.

Target Plants

Lettuce (*Lactuca sativa* L., Asteraceae) a dicotyledonous species was chosen as test plant because of their known seedling growth behavior and sensitivity to allelochemicals.[19,20] Two monocotyledonous species were chosen; Hibiscus (*Hibiscus sabdariffa* L., Malvaceae) a common herbaceous species in study area and Sorghum (*Sorghum bicolor*, Poaceae) a potent allelopathic crop with documented inhibitory effects.[21] All seeds were purchased from The Sudanese Company for Propagation of Seeds (El Obeid, Sudan).

Preparation of the Extracts by Maceration Method

Hundred grams of root sample was extracted by soaking in ethanol at room temperature for 3 days, protected from sunlight, and mixed several times daily with a sterile glass rod. Extract was obtained by removing the solvent under reduced pressures. The above method was repeated for the three roots samples and result in three crude extracts labeled as C_1, C_2 and C_3. The dried extracts were kept in a refrigerator till used.

Preliminary Phytochemical Screening

Root extract was subjected to qualitative phytochemical analysis using modification of the methods described by Trease and Evans, and Harborne.[22,23] The screening covered mainly alkaloids, saponins, flavonoids, tannins, sterols and / or triterpenes, and anthraquinones.

Germination Bioassays

Three dilutions of the root extract 0.5, 0.25 and 0.1 mg/mL were prepared from stock solution (5%, w/v) for the three different root extracts (C_1, C_2 and C_3). Two growth experiments were conducted under laboratory conditions.

Experiment A

The objective of this experiment was to test whether the root extracts of *A. digitata* exerted any effect on germination of lettuce, Hibiscus and Sorghum seedlings. The bioassay was conducted in a growth chamber with a photoperiod of 16h light/8h dark and temperature alternating between 25 and 27 °C. In each Petri dishes (9 cm diameter), the filter paper was moistened with 6.0 ml of each extract at 0.1, 0.25 and 0.5 mg/mL. Controls were obtained using distilled water. After solvent evaporation, in a flux chamber for 12 h, the filter papers were moistened with 6.0 mL of distilled water, maintaining the initial concentrations and seeds were arranged on the filter paper in the plate. The number of seeds in each Petri dishes depended on the seed size. Twenty to twenty eight seeds were used per plate. Petri dishes were sealed with Parafilm to ensure closed-system models. Three replicates were maintained for each treatment. The seed was considered as germinated when radicle emerged visibly protruded from the seed coat. Germinated seeds were counted at 24 h intervals during 7 days and average percent germination over control in each treatment was calculated using the following equation:

$$Germination\ (\%\ of\ control) = \frac{GT}{G0} \times 100$$

Where:

GT = average number of germinated seed in the treatment in each time of measurements.

G0 = average number of germinated seed in the control at the same time of measurements.

Experiment B

This experiment aimed to evaluate the effect of different concentrations of the root extracts on shoot and root lengths of the test plants. Thus, test plant seeds were germinated in Petri dishes, under the same conditions described in experiment A, though hydrated only with distilled water. Following germination, 6 plant seedlings were transferred to similar plates containing water

solutions of the root extracts at the same concentrations as experiment A. These plates were sealed and incubated in a growth chamber at 26±1°C using a photoperiod of 16 h light/8 h dark for another 4 days. After this period, the shoot lengths and root lengths of test plants seedlings were measured and compared to the length of control seedlings.

The bioassay was repeated twice with three replications in each case.

Statistical Analyses

All the experiments were conducted with triple replicated and repeat twice. The data for seed germination percentage were statistically analyzed using analysis of variance of SPSS version 16 and means of parameters measured were tested using Least Significant Difference.

RESULTS AND DISCUSSION

Phytochemical Screening

Preliminary phytochemical screening of the root extract of *A. digitata* revealed the presence of alkaloids, saponins, tannins, flavonoids and terpenoids with different concentrations, but the highest concentration was observed for flavonoids (Table 1).

The isolation of some flavonoids in root of *A. digitata* has been previously described.[16,17]

Table 1: Results of phytochemical screening of the root extract.

Screened plant	Part used	Phytochemicals					
		Alkaloids	Saponins	Flavonoids	Tannins	Anthraquinones	Terpenoids
Adansonia digitata L.	Root	+	++	+++	++	-	+

* The number of positive signs indicated the intensity of reactions that reflects the quantity present; * (-) = not detected.

Germination Bioassays

The results of experimental (A) and (B) were determined by counting the number of germinated seeds, measuring the average lengths of seedlings in centimeter. Results indicated that, root extracts had inhibitory activity in seeds germination and in terms of root and shoot lengths and the inhibitory effects were increased with increasing of concentrations from 0.1 to 0.5 mg/mL.

Experiment A

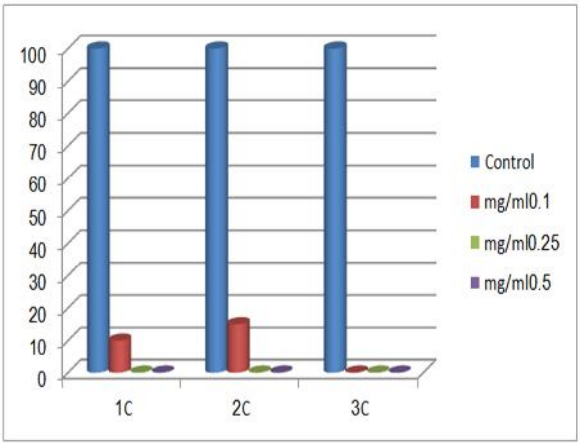

Figure 1: Effect of root extracts of *A. digitata* on germination percentage of lettuce.

In the seed germination bioassay, the effect of *A. digitata* root extracts on germination varied according to test species, concentration of extract and the root samples. Results ranged from stimulation to inhibition and the recorded percent of germination were decreased with increasing extracts concentrations. By the 7thday of incubation, all lettuce seeds in the water control had germinated (100%). However, with the exception of 0.1 mg/mL concentration of C_1 and C_2, all other concentrations of C_1, C_2 and C_3 root extracts were reduced germination percentage to zero, thereby completely inhibiting lettuce germination and the only seeds germinated in C_1 and C_2 extracts (0.1 mg/mL) were germinated by 10% and 15% respectively, compared to control (Figure 1).

Germination percentage of Sorghum was reduced significantly by higher concentrations of the three root extracts of *A. digitata* as compared to control (Figure 2). The C_2 extract was much more effective in reducing the germination percentage than C_1 and C_3. The lowest germination percentages were recorded in C_2 treatment at 0.25 and 0.5 mg/mL concentrations, which were 9.09 and 13.95% respectively, compared to 94.74% of the control.

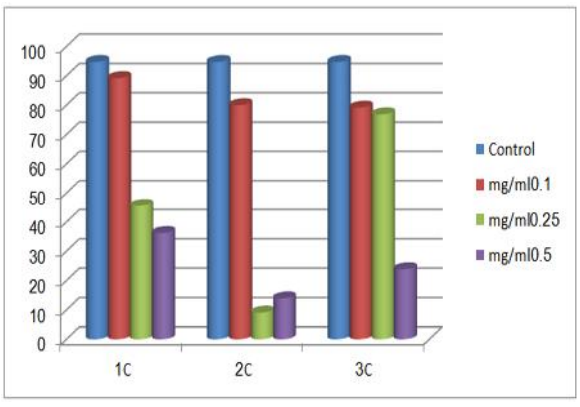

Figure 2: Effect of root extracts of *A. digitata* on germination percentage of Sorghum.

For Hibiscus, the observed effects of root extracts of *A. digitata* on germination percentage were low comparing with that on lettuce and Sorghum. The highest inhibition was observed with 0.5 mg/mL concentration of C_3 root extract (52.3%), followed by C_1 extract as 56.66% compared with 100% for the control (Figure 3).

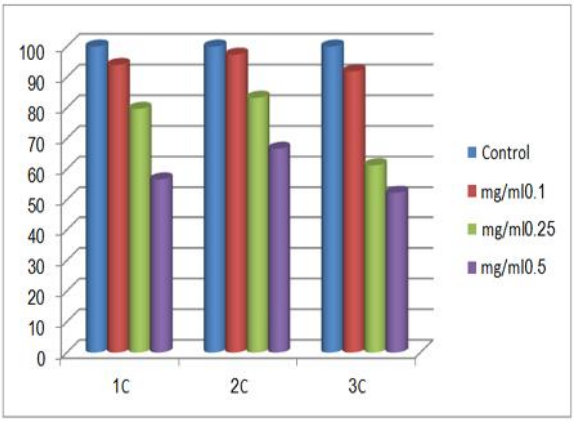

Figure 3: Effect of root extracts of *A. digitata* on germination percentage of Hibiscus.

Experiment B

Radicle lengths of lettuce, Hibiscus and Sorghum seedlings were significantly reduced by all root extracts of *A. digitata* in a concentration-dependent manner and the degree of inhibition was gradually alleviated with increasing days of incubation. For lettuce the effects of different concentrations of root extracts were very clear, almost all seedlings were dead after 7 days, but they were still a life in the controls. However, with the exception of C_2 extract, C_1 and C_3 root extracts in 0.1 mg/mL concentration completely inhibited shoot and root growth of lettuce, whereas for C_2 the average lengths recorded for roots and shoots were 0.25 cm, 0.25 cm respectively, compared to 1.17 cm and 0.52 cm of the control (Figure 4). Thus roots growth of lettuce showed a significant inhibition of 79% in presence of the C_2 extract at 0.1 mg/mL however, aerial parts which were more resistant, showed an inhibition of 52% at the same conditions.

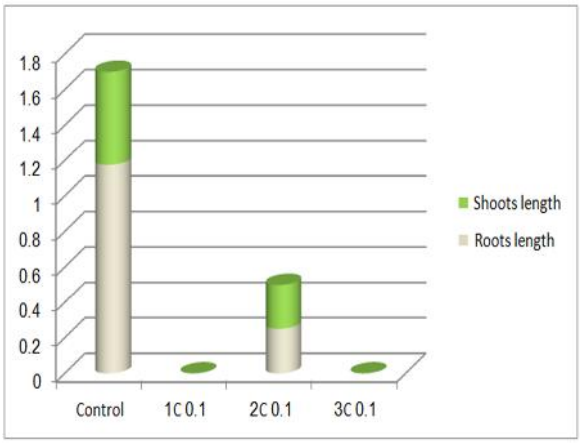

Figure 4: Average lengths of root and shoot in Lettuce at 0.1 mg/mL.

For *Sorghum*, the highest concentration (0.5 mg/mL) of C_1 and C_2 root extracts of *A. digitata* completely inhibited root and shoots growth, and most of seedlings were dead after 7 days of incubation. Whereas, in 0.1 and 0.25 mg/mL concentrations of C_1 extract, root length was decreased from 6.05 cm under control to 0.25 and 0.20 cm respectively, and similar reduction was observed in C_2 extract. The root length was decreased with increasing concentration from 6.05 cm under control to 0.90, 0.53 and 0.38 cm in 0.1, 0.25 and 0.5 mg/mL concentrations of C_3 respectively, while shoot lengths reduced to 1.23, 0.63 and 0.25 cm in 0.1, 0.25 and 0.5 mg/mL respectively, compared to 2.55 cm of control (Figure 5).

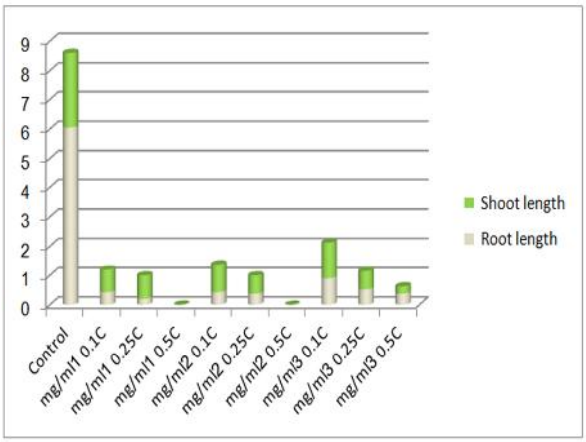

Figure 5: Average lengths of root and shoot in Sorghum at different concentrations.

Inhibition in root length of Hibiscus seedling depicted the highest trend as observed in case of shoot length with increasing concentration compared with a control. The lowest root length (0.03 cm) was recorded in C_2 extract at concentration of 0.5 mg/mL. The root lengths in C_3 extract were differed from 4.03 cm under control to 1.83, 1.10 and 0.40 cm at concentrations of 0.1, 0.25 and 0.5 mg/mL respectively. The shoot length was also decreased significantly in dose-dependent manner with maximum reduction from 4.48 cm under control to 0.98, 1.06 and 1.43 cm in 0.5 mg/mL concentration of C_2, C_1 and C_3 respectively (Figure 6).

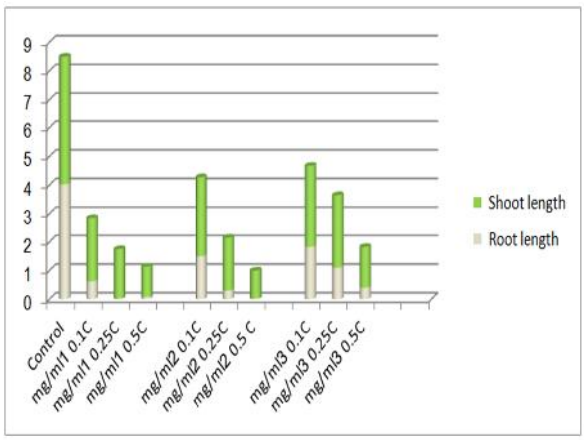

Figure 6: Average lengths of root and shoot in Hibiscus at different concentrations.

The overall results of this study showed significant allelopathic effects of *A. digitata* on germination, root and shoot growth of both dicotyledonous plants and monocotyledonous plants. Allelopathic activity varies depending on the concentration of the extract, target species and root sample. The results showed that all the three root extracts (C_1, C_2 and C_3) of *A. digitata* were exhibited significant inhibition on germination of lettuce, Sorghum and Hibiscus at all concentrations. But the inhibition effects was varied according to root extracts, for example for lettuce the degree of seed germination inhibition was in the following order $C_3>C_1>C_2$, whereas for Sorghum the order is $C_2>C_1>C_3$. This may be due to difference in sensitivity of the test plants and the difference in levels of allelochemicals present in each root samples of *A. digitata*, which in line with the fact that the quantity of allelochemicals released or those created by plants or microbes varies by species, chemical composition, and environmental conditions.[24] In addition, the three test species showed different responses to root extracts and those inhibitions increased with increasing of extract concentrations, suggesting that inhibitory exhibitions were species specific and concentration dependent.[25] Also result revealed that lettuce is the most affected by *A. digitata* root extracts and this was agreeing with the fact that seeds of lettuce are very sensitive to any allelopathic.[20] Furthermore, it was observed that there were stronger inhibitory effects of all the three root extracts of *A. digitata* on seedling roots than the shoots of the tested plant species. This difference in the sensitivity might be due to the fact that roots were in direct contact with the extract; hence, they are the first organ to absorb allelochemicals from the environment.[26,27]

The concentration dependent inhibitory activities of the root extracts on the germination and seedling growth of the test species suggest that *A. digitata* may possess allelopathic activity and contain allelopathically active substances. The presence of flavonoids in *A. digitata* root may give support to its observed allelopathic effect especially; as flavonoids are known to possess allelopathic activities and have been reported widely as highly potent inhibiting substances on seed germination and shoot growth or seedling root elongation.[28-31]

CONCLUSION

Natural products research offers a good chance of discovering new allelopathic compounds that can be used as natural herbicides. One common effect of phytotoxic compounds is the inhibition of seed germination and the resulting abnormalities in seedling development. In the present study, root extracts of *A. digitata* were tested for allelopathic potential on lettuce, Hibiscus and Sorghum. The germination and seedlings growth of all test species were significantly inhibited by the extracts at concentrations tested. Moreover, the effectiveness of *A. digitata* root extracts was different among test species, and the inhibition effect of the extracts was concentration dependent. These results suggest that the root extract of *A. digitata* may possess allelochemicals which are responsible for their inhibitory activity and could be the main reason for the restricted growth of other plant species near baobab trees. Isolation and identification of those allelochemicals could be act as lead for the development of new natural herbicides for sustainable weed management strategies. However, more research is needed to further confirm the allelopathic potentiality of *A. digitata* under field conditions to make these predictions more accurate.

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