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PRELIMINARY PHYTOCHEMICAL CONSTITUENTS AND PHYTOTOXIC EFFECT OF *Albizia lebbeck* (L.) BENTH ON *Sorghum bicolor*

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ABSTRACT
The preliminary Phytochemical investigations and phytotoxic effects of aqueous leaf extracts of Albizia lebbeck on Sorghum bicolor was assessed. The result from phytochemical screening revealed that all the allelochemicals tested where found present except steroids and phlobatannins when petroleum ether, methanol and water extracts were used. Petroleum ether extract show the presence of amino acids, protein and glycosides while methanol and water extracts showed their absence. Alkaloids, anthraquinones and acids were found present when methanol was used and absent using petroleum ether and water extracts. These allelochemicals are all important allelopathic sources. The leaf extract slowed down the rate of growth of the radicle and plumule of Sorghum bicolor seeds when compared to control. But these inhibitions were not significantly different at 5% level. The degree of inhibition increased with increase of concentration of the extracts hence inhibitions were prominent at extract of higher concentrations.
Keywords: Phytochemical constituents, Phytotoxic effects, Albizia lebbeck, Sorghum bicolor

INTRODUCTION

The use of herbicides during the last 50 years has raised pronounced doubts about the safety from their continuous use (Altland *et al*. 2003). Allelopathy is a natural and environmental friendly technique which may prove to be a tool for weed management and thereby increase crop yield (Rice, 1984). The main principle in allelopathy arises from the fact that plants produces thousands of chemicals; and many of these chemicals are released by leaching, exudation or decomposition processes (Einhelling, 2002). Subsequently some of these compounds which are known as allelochemicals alter the growth or physiological functions of receiving species. The most commonly found allelochemicals include cinnamic and benzoic acids, flavonoids, alkaloids, glycosides, saponins, tannins, steroids and various terpenes. These compounds are known to be phytotoxic (Einhelling, 2002).

Albizia lebbeck (L.) Benth. (Mimosaceae) is a medium- sized deciduous tree that reaches 30m in height (Prinsen, 1988). In Nigeria, *A. lebbeck* is planted in road sides as shade trees, in village, forests for fuel wood production and in front of schools or college premises as ornamental tree. It has been reported that it has some suppressive effects on some agricultural crops which might have been caused by secondary metabolites (allelochemicals) (Mohammed *et al*, 2007). The purpose of present study is to investigate the phytochemical constituent and phytotoxic effect of *A. lebbeck* on plumule and radicle elongation of *Sorghum bicolor* seeds.

MATERIALS AND METHODS

Leaves of *Albizia lebbeck* were collected and identified from College of Arts, Science and Remedial Studies Kano, campus. Located between (Lat. 12^0 03'N; Long. 8^0 32'E) in the Sudan Savanna eco-region of Nigeria (Olofin, 1987). The leaves of the plant were washed and air dried at room temperature for two weeks. When dried to a constant weight, the leaves were ground into fine powder in a mortar and pestle. About 40g of the powdered sample was taken for phytochemical screening in Pharmacognosy Department Faculty of Pharmaceutical Sciences Ahmadu Bello University, Samaru, Zaria. The seeds of *Sorghum bicolor* were used to test the phytotoxicity of this plant.

Extraction Processes:
The maceration method of extraction was employed. About 30g portions of ground powdered leaves was placed in round bottom flask containing 200cm^3 of methanol and mixed. The round bottom flask was placed on a flask shaker and agitated for four hours and left to stand overnight. The residue was stirred and filtered using Whatman No. 1 filter paper and glass funnel into a beaker. The process was repeated using petroleum ether and water as the extracting solvent with the same quantity of powdered leaves and solvent. The phytochemical tests were conducted to qualitatively verify the presence or absence of each of the following classes of secondary metabolites.

Phytochemical Screening Test:
The phytochemical tests were conducted according to the methods outlined by Evans and Trease (1999) as well as Cannel (2000). For Alkaloids, Dragendorff's test was carried out, Carbohydrates test was carried out using Molisch's test, for Protein Biuret's test was used, for Amino acids Ninhydrin test was employed, for Glycosides Borntrager test was employed, Form test for Saponnin, Salkowski test for Steroids while for Tannin and Phlobatannin Ferric Chloride test was used. Shibata test for Flavonoids, Bontrager test for Anthraquinones, Wagner test for Rasins while Ammonium and concentrated hydrochloric acid test was employed for Acids.

Preparation of Aqueous Leaf Extract
About 10g of the leaf powder was soaked in 1000cm^3 of water for 24h and filtered. The extract was prepared to get concentrations of 5, 10, and 15% using the procedure as described by Heisey, (1990). The seeds were surfaced sterilized with 1% mercuric chloride for 1 minute to remove the fungal spores on the seeds. Then the seeds were also washed with distilled water for several times to remove the mercuric chloride (Jayakumar and Manikandan, 2008).

Experimental Design and Growth records
The experiment was conducted in the laboratory of Biological Science Department, College of Arts, Science and Remedial Studies, Kano. The growth experiment was carried out in sterile petridishes of 9cm in diametre placing a Whatman No.1 filter paper on the petridishes. The extract of each concentration was added to each petridish of respective treatment daily and control was treated with distilled water. Five (5) seeds of *S. bicolor* were placed in the petridish replicating three times and were arranged in complete randomized design. The petridishes were set at a room temperature ranging from 28-30^{0}C. Elongation measurements were recorded at 24 hour intervals for six days. The percentage of inhibitory effect on the radicle and plumule growth of treatment plants to control was calculated as formula described by (Jayakumar and Manikandan, 2008).

$$I = 100 - (E_2 \times 100/E_1)$$

Where,

I = % inhibition.
E_1 = Response of control plant.
E_2 = Response of treatment plant.

The data obtained from the experiment were compared statistically (t-test at 5% level) to those obtained from the control.

RESULTS AND DISCUSSION
The result of phytochemical screening of aqueous leaf extracts of *Albizia lebbeck* revealed that all the phytochemicals tested where found present except steroids and phlobatannins when petroleum ether, methanol and water extracts were used. Petroleum ether extract show the presence of amino acids, protein and glycosides while methanol and water extracts showed their absence. Alkaloids, anthraquinones and acids were found present when methanol was used and absent using petroleum ether and water extracts (Table 1). Einhelling (2002) reported that presence in isolation or combination of two or more of these allelochemicals identified are all important allelopathic sources. In other reports by Chon *et al*. (2002) and Fischer *et al*. (1994) allelochemicals such as caumarines, tannins, saponins, flavonoids, resins, phenols and many alkaloids were shown to inhibit cell division, changes in phytohormones and their balance, water uptake, and germination of seeds. Other effects include stomatal movement, pigment synthesis, nitrogen fixation, photosynthesis and perhaps others.

Table 1. Phytochemical Screening of *Albizia lebbeck* Aqueous Leaf Extract.

	Extracts		
Phytochemicals	**Petroleum ether**	**Methanol**	**Water**
Alkaloids	_	+	_
Glycosides	+	_	_
Tannins	+	+	+
Saponins	+	+	+
Steroids	_	_	_
Flavonoids	+	+	+
Phlobatannins	_	_	_
Acids	_	+	_
Resins	+	+	+
Anthraquinones	_	+	_
Carbohydrates	+	+	+
Amino acids	+	_	_
Proteins	+	_	_

Key: + Presense of constituents - Absence of constituents

The effect of the different aqueous leaf extracts of *A. lebbeck* on the radicle length of *S. bicolor* are shown in Table 2. The extracts slow down the rate of growth of the radicle when the results from the extracts treated seeds were compared to the control. The degree of inhibition of the radicle increased with the increased in the concentration of the extracts. Highest inhibitory effect (75.0%) was from 15% extract concentrations at 72 hours while the lowest (10.5%) was from 5% extract concentration at 48 hours. The % decrease from the control was 27.2, 63.1 and 67.6% in the 5, 10 and 15% extract concentrations respectively. These inhibitions were not significantly different (P>0.05) from the results obtained in the control at 5% level.

Table 2. Effects of Different Aqueous Leaf Extract of *A. lebbeck* on Radicle Length (cm) in *Sorghum bicolor*

	Experimental Time (hour)						
Conc. (%)	**24**	**48**	**72**	**96**	**120**	**144**	**Average % decrease**
0	0.8	3.8	9.2	16.5	20.0	25.5	
5	0.7 (12.5)	3.4 (10.5)	7.0 (23.9)	10.4 (37.0)	12.1 (39.5)	13.6 (39.6)	(27.2)
10	0.4 (50.0)	2.0 (47.4)	2.6 (68.8)	4.2 (74.5)	5.5 (72.5)	7.8 (65.3)	(63.1)
15	0.4 (50.0)	1.3 (65.8)	2.3 (75.0)	4.3 (74.0)	5.1 (74.5)	7.6 (66.2)	(67.6)

Figures in brackets represent % decrease from the control

Table 3. Effects of Different Aqueous Leaf Extract of *A. lebbeck* on Plumule Length (cm) in *Sorghum bicolor*

	Experimental Time (hour)						
Conc. (%)	**24**	**48**	**72**	**96**	**120**	**144**	**Average % decrease**
0	_	_	1.2	3.2	6.0	7.6	
5	_	_	1.1 (0.3)	2.9 (9.4)	4..0 (33.3)	5.5 (27.6)	(17.7)
10	_	_	_	1.2 (62.5)	2.4 (60.0)	3.2 (57.9)	(70.1)
15	_	_	_	1.3 (59.4)	1.6 (73.3)	2.8 (63.2)	(74.0)

Figures in brackets represent % decrease from the control

Similar effects were demonstrated on the plumule where complete inhibition of plumule was observed at all extract concentrations at 24 and 48 hours more so, at 10 and 15% extract concentration at 72 hours. The highest inhibition recorded (73.3%) was from 15% concentration at 120 hours while the lowest (0.3%) from 5% extract at 72 hours. The % decrease from the control was 17.7, 70.1 and 74.0 at 5, 10 and 15% extract concentrations respectively (Table 2). Also the result obtained from the extract treated seeds were not significantly different from those obtained from the control experiments ($P>0.05$). Thus the results from Tables 1 and 2 showed that the degree of inhibition of both the radicle and plumule appeared to be more prominent at the extract of higher concentrations. These results are in agreement with one obtained by El-Rokiek *et al*. (2010) who reported the reduction of plumule and radicle elongation and other growth parameters of purple nutsedge (*Cyperus rotundus* L.) using mango aqueous leaf extracts. Moreso, the results from this study does not vary from those previously obtained by Oladele (1990) who reported the inhibitory effect of *Terminalia superba* and *Parkia biglobosa* fruits, leaves and bark aqueous extract on germination and seedlings growth of *Amaranthus viridis*.

CONCLUSION

This study indicated that the presence of these allelochemicals in the extract of *A.lebbeck* leaves could be the reason for the reduction in plumule and radicle elongations of *Sorghum bicolor* seeds. The reduction effect was concentration dependent and the inhibition was more on plumule than radicle length. Though laboratory bioassay in allelopathic researches are of great importance, long term field studies must be recommended to carry out before incorporating *A. lebbeck* in any agroforestry system.

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