



Faculty of Medical and Health Sciences, University of Poonch Rawalakot

Journal of Pharma and Biomedics

ISSN: 3007-1984(online), 3007-1976 (Print)

<https://www.jpbsci.com/index.php/jpbs>

Toxicological Assessment of *Adansonia digitata* L. Reveals a Favorable Safety Profile in In-vivo and In-vitro Models

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ABSTRACT

Natural plants are considered among the safest remedies and have been used for the treatment of various ailments since ancient times. However, due to their continued use, it is essential to provide scientific evidence supporting their safety profiles. The objective of this study was to evaluate the toxicological effects of *Adansonia digitata* L. leaf extracts using standard in vivo and in vitro models. Several assays were conducted, including the acute toxicity test, brine shrimp lethality assay, larval hatching assay, phytotoxic assay, insecticidal activity test, rearing technique, and cytotoxicity assay. The results of all tests indicated a significantly safe profile. It is therefore concluded that *Adansonia digitata* L. exhibits a high safety margin, as demonstrated by both in vivo and in vitro evaluations, and may be effectively utilized as a natural remedy for various diseases.

Keywords: Natural plants, *Adansonia Digitata* L., Acute toxicity, in vivo and in vitro models.

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INTRODUCTION

Natural remedies have been used for the cure of illness since prehistoric times and their demand are increasing tremendously because of their beneficial potentials (Pandey et al., 2011). Commonly natural plants considered as safe and due to the part of the traditional system of medicine regarded as harmless (van Wyk and Prinsloo, 2020) but studies indicate that some plants contain active chemicals responsible for adverse reactions or their excessive intake or interactions with other medicine leads to serious issues (Ekor, 2014). Consequently, there is a need to explore and focus the toxic manifestations and risk

associated with the usage of medicinal plants along with herbal remedies to minimize fatal incidents.

Adansonia digitata L., or African baobab is one of the long-lived trees of the arid and semi-arid areas in sub-Saharan Africa. As a Malvaceae family it is known to have a giant trunk, food bearing fruit and widespread uses in traditional medicine. Numerous plant sections, leaves, the pulp of the fruits, the seeds, the barks have been used extensively by African ethnomedicines in treatment of various diseases conditions like its use in diarrhea, malaria, inflammations, microbial infections and febrile undertones (Adesina and Zhu, 2022). Phytochemical

studies have demonstrated that *Adansonia digitata* contained active constituents, such as flavonoids, phenolic acids, sterols, and vitamins as well as important minerals

(Eltahir and Elsayed, 2019) and possesses antioxidant, anti-inflammatory, antimicrobial and analgesic effects (Fatema et al., 2015).



Figure 1: *Adansonia digitata*.

Although, *Adansonia digitata* (AD) are mostly used in dietary patterns and traditional production of herbal medications, scientific evidence of its safety profile is limited. With the increase in the production of plant nutraceuticals and other types of therapeutical substances, the strict knowledge of the properties of their toxicology is a must. Natural products though by first sight are safe, the bioactive constituent that is employed in these products may in some cases have undesired effects either when ingested at high doses or upon chronic administration (Kaboré et al., 2011). There is need to conduct systematic toxicological within the body of work therefore to reiterate that *Adansonia digitata* leaf extracts are safe particularly when applied over a long period or in large doses.

However, the science behind the toxicological profile of *Adansonia digitata* is still scant and even contradictory despite the long history of its traditional use. Thus, strict toxicological testing, be it an acute or chronic one is necessary to approve its safety, set dosage patterns and guarantee its safe incorporation into the pharmacologic and food field. In this research work, the objective is to examine the toxicological effect of *Adansonia digitata* leaves extracts in standard in vivo and or in vitro models with a view to expanding the recognition of its toxicological safety profile.

MATERIALS AND METHODS

Chemicals

Absolute Ethanol (EtOH), Dimethyl sulfoxide, acetic

acid were obtained from Merck, Germany.

Preparation of plant extracts

The aerial part of the plant was cleaned with distilled water, dried under shade for 5 days. 10 kg of leaves were soaked in Ethanol (90%) at room temperature for 8-12 days. The percolate was filtered through Whatmann no.1 filter paper. Then remaining solvent was evaporated by rotary evaporator (Eyela, Japan) at 40°C under reduce pressure. This process was repeated thrice and then combine all three filtrates to obtain crude Ethanolic extract (550g) (Mumtaz et al., 2024).

Acute Toxicity

Acute toxicity of ethanolic extract i.e. AD-1 was analyzed by Swiss albino mice of either sex having body weight (20-25g). The mice were distributed into control and treated groups. A number of mice in each group were six. Control group received physiological solution while gradually increased doses of AD-1 (50, 100, 500, 1000, 2500 and 3000 mg/kg) given to treated groups by the oral route. Mice were monitored continuously for any toxic sign and symptoms behavioral responses and changes in motor activities for at least 4hrs with a gap of 15 minutes. The observation of mortality in all mice was made for 7 days (Zaoui et al., 2002).

Brine shrimp lethality assay

Artemia salina (leach) shrimp larvae are used to determine the toxic effect (LD₅₀) of bio-natural compounds. This lethality test is rapid, general and

inexpensive which has been examined for screening, fractionation and analysis of bioactive substances. The brine shrimp eggs should be stored at 4°C for the year and easy hatching is achieved within 48 hrs. Offering a large number of larval species (Carballo et al., 2002).

Hatching of Larvae

The collection of brine shrimp was done with the help of pasture pipette under illuminations. The hatching tray was rectangular dish (22×32cm) which was half filled with filtered larval solution. Then kept brine shrimp eggs 50mg on the tray and covered with the lid. Incubate the shrimp for 2 days at 27°C in growth chamber Three different concentrations (10, 100, 1000 µg/ml) of all experimental samples AD (1-5) was tested accordingly. In each vial, ten

shrimps and 5ml of sea water were taken and then incubated at 26°C for 24 hours. The cytotoxic lethality was performed by preparing three replicates of each concentration on the next day total no of alive shrimps were counted. In this assay, the standard cytotoxic drug Etoposide (LD50= 7.465µg/ml) was referred as positive control while solvent used as negative control. After initiation of hatching the larvae of brine shrimp should be used within 48-72 hours and they must be wasted after 72 hrs. When the process was completed. The data of all extracts can be analyzed by percentage calculation to determine degree of lethality (Carballo et al., 2002). According to Ali et al. the toxicity studies interpreted on the basis of the below mentioned criteria (Ali et al., 2014).

Table 1: Interpretation of level of toxicity.

Intensity of Lethality	Activity
30-40%	Low
50%	Moderate
60-70%	Good
Above 70%	Significant

Phytotoxic Assay

The crude extracts AD-1 along with various fractions AD (2-5) was examined for phytotoxic activity using Lemna bioassay protocol. First, the fronds were washed with distilled water. In organic E medium was prepared by mixing various ingredients with 100 ml water and PH was adjusted 5.5-6.5 by adding some amount of KOH in order to produce alkalinity thereafter, a medium was sterilized at 121°C for 15 minutes. The inoculation was done in three different flasks by taking plant extracts AD (1-5) in different concentrations i.e. 10,100 and 1000µg/ml separately. These flasks were left over night for evaporation of solvents. In each flask, 20 fronds and 20 ml of E medium were transferred. The flask had been placed in the growth chamber, at 27±1°C, 56±10 relative humidity and 12-hour day length for 7 days. The observation was carried out in each day during incubation. In this analysis, Paraquat was used as positive control while distilled water used as negative control (Itokawa et al., 1982; Lewis, 1995). Finally, on day 7 the no of fronds per flask was counted and their growth regulation was calculated by following formula (Itokawa et al., 1982; Lewis, 1995).

$$\text{Growth regulation \%} = \frac{100 - \text{No of fronds in test sample}}{\text{No of fronds in - ve control}} \times 100$$

Insecticidal activity

Insecticidal activity of all extracts of AD (1-5) was analyzed respectively by impregnated filter paper method also called as direct contact application by using filter paper. *Sitophilus oryzae*, *Rhyzopertha dominica*, and *Callosobruchus analis* were tested insects while Permethrin used as the standard insecticide. The test samples were accurately weighed and dissolved in the volatile solvent and applied on the filter paper of petri plate size were 9 cm or 90mm. On next day 10 alive and healthy insects were placed on each petri plate without food. In the growth chamber, these plates were incubated at 27 oC with relative humidity 50% for 24 hours. For mortality count the insects of same size and age were selected. The following formula was used to assess the mortality count of all tested extracts of AD (Pavela, 2004).

$$\% \text{ Mortality} = \frac{100 - \text{no of insects survived in test}}{\text{Number of insects survived in - ve control}} \times 100$$

Rearing Technique

The stored pests were incubated in the laboratory at 30±1 oC, 65% relative humidity, 12:12 hrs. Dark/light photoperiod which have been recommended for proper growth (Collins, 1998).

Cytotoxic Activity

The leaves extract of AD was examined for anticancer

(cytotoxic activity) by standard MTT colorimetric method in which flat bottom 96 –well microplates have been used. For this, 3T3 cell line of the mouse (Fibroblast) was cultured in modified Dulbecco's eagle's medium, while fetal bovine serum (FBS 5%), Penicillin (100 IU/ml) and Streptomycin (100 µg/mL) were supplemented in three 25 ml flask. After wards, these flasks were incubated in 5 % CO₂ incubator at 37°C. By diluting with particular medium exponentially growing cells were harvested and then counted with a hemocytometer. The prepared cell culture with the concentration of 1×10⁵ cells/mL was then taken into 96-well microplates, in each well 100 µl of culture was present. The incubation of plates was done overnight with an addition of 200 µl freshly prepared medium and different (1-100 µM) concentration of extracts.

After three days, 2mg/ml, 50µl of MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) was added to each well with 100 µl of DMSO and again incubated for 4 hrs. The calculation of the intensity of MTT declination with in cells with respect to formazan cells was measured at 570nm by means of ELISA reader. In case of 3T3 cells, cytotoxicity was noted at a concentration which causes 50% growth inhibition (Mosmann, 1983).

RESULT

Acute Toxicity

No toxicity has been experienced in ethanolic leaves extract of AD at the dose of (50, 100, 500, 1000, 2500, and 3000) mg/kg body weight and showed no lethal signs and mortality of tested animals (Table 2).

Table 2: Acute toxicity of Ethanolic leaves extract of *Adenonia digitate* L.

Treatments	Dose(mg/kg)	Gross behavior effect	No. of animal died (n=6)
Control (Normal saline)	-	No change	0
AD-1 (EtOH)	50	No change	0
	100	No change	0
	500	No change	0
	1000	No change	0
	2500	No change	0
	3000	No change	0

Larvicidal Activity

Lethality measures in terms of the death of larvae after 24 hrs with exposure of extracts. Leaves extracts of *Adenonia*

digitate showed insignificant activity except Aqueous extract (AD-5) which showed low activity at higher dose (Table 3).

Table 3: Brine shrimp bioassay of *Adenonia digitata* leaves extracts.

Treatments	Dose (µg/ml)	No of larvae in –ve control	No of larvae survive	% Mortality
AD-1 (EtOH)	1000	30	30	0
	100	30	30	0
	10	30	30	0
AD-2 (n-Hx)	1000	30	27	10
	100	30	29	3
	10	30	30	0
AD-3 (EtOAc)	1000	30	30	0
	100	30	30	0
	10	30	30	0
AD-4 (n-BuOH)	1000	30	29	3
	100	30	30	0
	10	30	30	0
AD-5 (Aq)	1000	30	20	33
	100	30	29	3
	10	30	30	0

Phytotoxicity Assay

Results indicates that extracts of AD showed dose dependent responses. Like at 1000µg/ml AD-3 (EtOAc) extract of AD

exhibited significant effects. Whereas, AD-4 (n-BuOH) produced moderate activity while AD-1(EtOH), AD-2(n-Hx) and AD-5(Aq) represents low toxicity (Table 4).

Table 4: Phytotoxic activity of leaves extracts of *A. digitata*.

Treatments	Dose (µg/ml)	No of fronds in test	No of fronds in (-) Control	% Frond inhibition
AD-1 (EtOH)	1000	24	37	35
	100	37	37	0
	10	37	37	0
AD-2 (n-Hx)	1000	24	37	35
	100	33	37	11
	10	37	37	0
AD-3 (EtOAc)	1000	00	37	100
	100	35	37	5
	10	37	37	0
AD-4 (n-BuOH)	1000	22	40	45
	100	40	40	0
	10	40	40	0
AD-5 (Aq)	1000	24	37	35
	100	31	37	16
	10	37	37	0

Standard drug (ParAquat) = 0.015µg/ml, Negative control = Distill water

Insecticidal activity

The insecticidal activity of AD extracts (EtOH, n-Hx, EtOAc, n-BuOH, Aq) were tested against three stored grain pests i.e. *Sitophilus oryzae*, *Rhyzopertha dominica* and *Callosbruchus analis* by using Permethrin as standard.

Each observations shows mean of three replicates i.e. 10 insects in each replication. It was observed that among all extracts, AD-2 (n- Hx) and AD-4 (n-BuOH) of AD revealed weak and moderate insecticidal activity respectively (Table 5).

Table 5: Insecticidal activity of *Adenсонia digitata* leaves extracts.

Name of Insects	% Mortality						
	AD-1 EtOH	AD-2 n-Hx	AD-3 EtOAc	AD-4 n-BuOH	AD-5 Aq	+ve control	-ve control
<i>Sitophilus oryzae</i>	0	20	0	20	0	100	0
<i>Rhyzopertha dominica</i>	0	0	0	60	0	100	0
<i>Callosbruchus analis</i>	0	40	0	60	0	100	0

Concentration of test sample =1019.10 µg/cm², Concentration of +ve control (Permethrin) = 239.5 µg/cm², -ve control = Organic solvent

Cytotoxic Activity

Cytotoxic potential of Ethanol leaves extract of AD were detected separately. According to data, both EtOH extracts

showed no cytotoxicity against 3T3 cell line and exhibited % inhibition values which reflects IC₅₀ values > 30 as shown in Table 6.

Table 6: Cytotoxicity (3T3) of *Adenсонia digitata*.

Sample	Conc.(µg/ml)	% inhibition	IC ₅₀ ± SD
AD-1 (EtOH)	30	11	> 30
Cyclohexamide (Standard)	30	71	0.8±0.2

DISCUSSION

Pakistan is an agriculture country having diversified medicinal flora, due to this it has long history of treatment by folklore and traditional system of medicine. The knowledge of regional plants has been transmitted from person to person (Hussain et al., 2012). These plants in the form of herbal remedies used to cure in a variety of illness range from minor to major diseases. The valuable knowledge of indigenous medicinal plants is still in danger of being lost in Pakistan (Mustafa, 2025). Therefore, proper documentation required to conserve these medicinal and nutraceutical herbs with proper utilization of agricultural and horticultural guidelines along with their toxic manifestations are important.

Adansonia digitata, or the baobab tree, is a long-lived, iconic tree species that occurs naturally in Africa. Its giant trunk, water storage capacity, and high nutritional quality of its fruit and leaves are notable characteristics. Baobab is an important element in ecosystems and local cultures, providing food, medicine, and shelter. Although *Adansonia digitata* leaves are mostly used in dietary patterns and traditional production of herbal medications, scientific evidence of its safety profile is limited (Adesina and Zhu, 2022).

In acute toxicity studies, no toxicity has been experienced in ethanolic leaves extract of AD at various doses (Table 2). *Artemia salina* toxicity assay of AD (AD ,1-5) were subjected to analyze by three different doses respectively. Only Aq (AD-5) extract at higher concentration (1000 µg/ml) showed low toxicity. Whereas, no lethal signs appeared in other extracts including (AD, 1-4) with low to high dose when Etoposide (LD50 7.4625 µg/ml) was used as standard cytotoxic drug. According to studies conducted by Nguta et al. the Aqueous leaves extract of *Adansonia digitata* (LC50<100µg/ml) showed strong cytotoxicity against *Artemia salina* when etoposide (LC50<6µg/ml) used as standard drug (Nguta, 2015). This might be due to agricultural malpractices or according to ontogenic variation the accumulation of comparatively toxic substances or some metabolic compounds contains certain type of cytotoxicity during metabolism in plants. Toxicity studies on extracts of Ghanaian plants revealed that ethanolic leaves extract has no cytotoxicity against brine larvae. (LC50> 1000µg/ml) (Tuani et al., 1994). These studies are in line with our results The overall

scenario depicted the safety profile or no cytotoxic effects of AD. This confirms the effective usage of drug in clinical settings as well as its safe consumptions for nutritional purposes.

Lemna minor (duck weed) belongs to family Lemnaceae, is an Aquatic thalloid monocot. Its structure consists of the filamentous root and oral frond in which two central daughter fronds is attached. Its cultivation requires optimum condition for 1-2 days. After washing in distilled water fronds transfer to a nutritional solution. The plant contains many primary metabolites and nutrients as compared to other vascular plants. The crude extracts AD-1 along with various fractions AD (2-5) was examined for phytotoxic activity using Lemna bioassay protocol. The in vitro herbicidal activity of leaves extracts of AD exhibited dose dependent bioactivities at different dose. At highest dose, AD-3 (EtOAc) extract possess significant bioactivity. While other extracts like (AD-4) BuOH showed moderate while AD-1 (EtOH), AD-2 (n-Hx) and AD-5 (Aq) extracts produced low phytotoxicity (Table-4). The results indicate organic extracts of AD seedling growth and germination of Aquatic plant. The dose dependent inhibitory action of these Plants extracts revealed phytotoxic activity. The literature confirms the usefulness of natural extracts as herbicidal agent produces inhibitory effects at early growth and germination (Ulukanli et al., 2014). Leaves extract of AD was analyzed against three stored pests by direct contact method. The moderate to weak insecticidal activity of AD (AD-4 and AD-2) were noticed against *Rhyzopertha dominica*, *Callosbruchus analis* and *Sitophilus oryzae* respectively at a dose of 1019.10 µg/cm². While 100% mortality produced by standard drug permethrin at concentration of 230.5 µg/cm². Cytotoxicity study with 3T3 cell line were carried out in which AD-1 extract exhibited no cytotoxic activity as compared to standard dug cyclohexamide which inhibited 71 % of 3T3 cell line.

CONCLUSION

It is concluded from this study that *Adansonia digitata* L. shows greater safety profiles as evidence by both in vivo and in vitro results and can be efficiently used as a natural remedy in various diseases.

CONFLICT OF INTEREST

The authors declares no conflict of interest.

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