



## Research Article

# Phytochemical Evaluation and Acute Oral Toxicity of Aqueous leaf Extract of *Ficus abutilifolia* (Miq) in Mice

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### ABSTRACT

Several species of the genus *Ficus* have been reported to exhibit antimicrobial and pharmacological effects. Extracts from the leaves of *Ficus abutilifolia* are used traditionally to treat various infectious diseases by indigenous communities of Nigeria with little or no data on the acute oral toxicity. Hence, the aim of this study was to conduct qualitative phytochemical screening and evaluate the acute oral toxicity of *Ficus abutilifolia* (Miq) aqueous leaves extract *in mice*. Fresh leaves of *Ficus abutilifolia* were collected from the field at Anguwan Daudu Tsauni, Giwa LGA., Kaduna State. The plant was authenticated and the leaves air dried and milled into powder. The powdered sample was macerated with water and allowed to stand for 24 hours. The qualitative phytochemical evaluation and LD<sub>50</sub> was carried out, also, haematology and serum biochemistry parameters were conducted using standard techniques. Results of phytochemical constituents showed the presence of flavonoids, cardiac glycosides, phenolic compounds, alkaloids, tannins, steroids, carbohydrates, saponins, and terpenoids. The LD<sub>50</sub> was found to be > 2000 mg/kg with no obvious signs of toxicity and death over the 14 days period. Significant ( $p < 0.05$ ) elevation was observed in the TWBC of the mice administered *Ficus abutilifolia* (Miq). There was no significant ( $p > 0.05$ ) increase in the AST, ALP, BUN and creatinine levels when compared to the normal control. In conclusion, LD<sub>50</sub> of *Ficus abutilifolia* (Miq) was shown to be greater than 2000mg/kg with no observed toxicity sign; hence, the plant possesses a wide margin of safety.

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## INTRODUCTION

*Ficus Abutilifolia* (*F. abutilifolia*) which belong to the family Moraceae, is a small to medium-sized tree (5 m in height) and encountered along streams (Hamed, 2011). It is called "Waa" in Hausa language, "Lapalapa" in Yoruba language and "Agbu inu" in Igbo language. Several species of the genus *Ficus* have been reported to exhibit antimicrobial as well as pharmacological effects (Taiwo *et al.*, 2016). The plant has a yellowish-white, smooth and flaking bark and its trunk is usually twisted. Broadly ovate and cordate at base, the leaves usually measure 7.5 - 20.0 × 6.5 - 18.0 cm. The smooth or slightly hairy fruits are 1.5 - 2.5 cm in diameter usually borne singly or in pairs at the leaf axils (Danmallam *et al.*, 2012). Nature has been a great source of ethnobotanical agents since time immemorial, and quite a number of drugs was isolated from these traditional medicines (Ribeiro *et al.*, 2020). Plants continue to play an essential role in health care of many populations (Ribeiro *et al.*, 2020). According to World Health Organization, about 80% of the world inhabitants depend mainly on traditional medicines for their primary health care (Doughari *et al.*, 2008). Plants contain several secondary metabolites which exert biological effects against microorganisms (Taiwo *et al.*, 2016). Safety continues to be paramount with regards to the use of medicinal plants; conduct toxicity study is an important step in ascertaining their safety profile. Therefore, evaluating the toxicological effects of any medicinal plant extract intended to be used in animals or humans is an important aspect of its assessment for potential toxicity (Chinenye *et al.*, 2019). Extracts from the leaves of *F. abutilifolia* are used traditionally to treat various infectious diseases such as typhoid fever, dysentery, malaria and other gastro-intestinal infections by indigenous communities of Nigeria (Ukwulibe, 2014). Most of these plants are injurious to health if not administered at the

proper dose. *F. abutilifolia* is being utilized by communities but little or no data is known with regards to the safest dose to take. This present study, aimed to evaluate the phytochemical constituents and toxicity profile of aqueous leaf extract of *Ficus abutilifolia* in mice.

## MATERIALS AND METHODS

### Collection, Identification, and Processing

Fresh leaves of *Ficus abutilifolia* were collected from the field at Anguwan Daudu Tsauni village (11.280164°N and 7.417857°E), Giwa local Government Kaduna state Nigeria. The sample was authenticated at the herbarium of the Dept of Botany, ABU Zaria. Fresh leaf sample was washed using distilled water and it was further air-dried at room temperature for two weeks. The sample was grounded using a sterile mortar and pestle and further pulverize to powder using an electric blender (Marlex Electrolyn IS:4250) until required for extraction.

### Preparation of Aqueous leaf Extract of *Ficus abutilifolia*

Five hundred (500) g of the powdered plant was soaked into 2 L of distilled water and the mixture was mixed thoroughly for 2 h at 65°C using a constant temperature stirrer type IKA-MAG RCT and filtered through Whatman (No 1) Filter paper. The extract was then obtained by evaporation in a hot air oven (memmert) at 50°C and stored at 4°C until required.

### Phytochemical Screening of Aqueous leaf Extract of *Ficus abutilifolia*

The leaf extract was screened for its phytochemical constituents using standard methods as described Faleye and Dada (2016).

### Source of Experimental Animals

Adult male albino mice, weighing 14-16 g, were sourced from the Animal House of Veterinary Pharmacology and Toxicology Department, Ahmadu Bello University in Zaria. The experimental mice were maintained at room temperature in 15 × 30 cm cages with metal tops that were kept cleaned. Sawdust was used as the bedding materials which were changed at least

twice in a week. The experimental animals were allowed to acclimatize for at least 1 week prior to beginning the experiment (Jang-Gi *et al.*, 2011). They were fed with commercial feed and allowed access clean water *ad libitum*.

#### **Acute Toxicity study**

The acute oral toxicity of *Ficus abutilifolia* Miq. aqueous extract was carried out using the 'Up-and-Down' method of testing in mice in accordance with the OECD guideline (OECD, 2008). Five (5) male albino mice, aging 8–12 weeks was used and the mice were allowed to fast (food) overnight before and 2 h after the extract administration at a single dose of 2000 mg/kg per mice. The animals were then monitored for 24 h for instant death, possible signs of toxicity, and death. The mice were further monitored for 48 h for short-term effect and subsequently 14 days for any delayed toxic effects.

#### **Haematological Assessment**

Blood samples of mice from both control and the treated groups were collected for the determination of haematological parameters such packed cells volume (PCV), haemoglobin concentration (HC), red and total white blood cell counts (RBC and TWBC) using standard procedures as described by Dacie and Lewis (1991).

#### **Serum Biochemistry**

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase activity (ALP), Albumin (ALB), total protein levels (TP), blood urea nitrogen (BUN) ranges were determined using the Randox assay kit (Randox Laboratories Ltd, Ardmore, Antrim, UK) according to the manufacturer's instructions.

#### **DATA ANALYSIS**

The quantitative data was presented in the form of a mean  $\pm$  standard deviation (SD) using tables. Independent sample t-test was used to analyze the data using GraphPad InStat. Version

3.1. *P* value less than 0.05 was considered statistically significant.

## **RESULTS**

### **Phytochemical Screening**

The phytochemical analysis revealed the presence of nine (9) secondary metabolites including flavonoids, cardiac glycosides, phenolic compounds, alkaloids, tannins, steroids, carbohydrates, terpenoids, and saponins in the aqueous extracts of *Ficus abutilifolia* Miq (Table 1). However, anthraquinones were not detected in the extract.

### **Acute Oral Toxicity Study**

The acute oral toxicity revealed that at 2000 mg/kg of the aqueous leaf extract of *Ficus abutilifolia*, no mortality was recorded in the first 24 hours and after 14 days post extract administration in mice. Similarly, no change in behavioural and or clinical signs was observed (Table 2).

### **Haematology**

There was an observed increase in the Total White Blood Count (TWBC) in the treated group (Table 3). The mean TWBC in the treated group was significant ( $p < 0.05$ ) when compared to the mean of the control group. Changes in the mean of other parameters in the group were observed to be non-significant ( $p > 0.05$ ).

### **Serum Biochemical Analysis**

There were no significant ( $p > 0.05$ ) changes in the serum alkaline phosphatase, aspartate aminotransferase, blood urea nitrogen, alanine aminotransferase, and creatinine in the treated group in comparison to the normal control (Table 4).

## **DISCUSSION**

The presence of phytochemicals in medicinal plants has been reported to exert several prevention activities against diseases (Asaduzzaman and Asao, 2018). These plant-based compounds possess significant anti-inflammatory, antidiabetic, antiaging, antimicrobial, antiparasitic, antidepressant, anticancer, antioxidant activities (Bahramsoltani

*et al.*, 2014). In this present study, flavonoids, cardiac glycosides, phenolic compounds, alkaloids, tannins, steroids, carbohydrates, terpenoids, and saponins were present in the aqueous leaf extract. The presence of these metabolites was reported in previous studies (Sandabe *et al.*, 2006; Rodriguez-Fragoso *et al.*, 2008). The absence of anthraquinones in this study could be due to the difference in the extraction process as anthraquinones are mostly extracted through organic solvent media (Mohanasundari *et al.*, 2007). Toxicological testing in experimental animal studies is crucial in providing relevant information on the hazardous effects of a chemical agents, the nature of these effects as well as the safe exposure levels (Ribeiro *et al.*, 2020). The LD<sub>50</sub> of *Ficus abutilifolia* (Miq) was observed to be > 2000 mg/kg with no toxicological or behavioral changes, thus ascribing the extract to be considered relatively non-toxic. This is in accordance with the observations made by Danmalam (2012). This toxicity study also includes a number of significant biochemical markers. The kidney and liver are considered as the principal organs that are vulnerable to the harmful effects of drugs. Increases in total red blood cells, white blood cell and PCV imply that the extracts may increase the populations of red blood cells production from the bone marrow, as well as increase in white blood cell of the whole blood because of the increased number of red blood cells in the blood (Fatemi *et al.*, 2007). The decrease in haemoglobin concentration in a test group compared to the control group may as a result of poor affinity of oxygen to hemoglobin molecules the tendency of hemoglobin to bind to oxygen enhances blood flow to the tissue (Ganong, 2005). From the results obtained from this study, the Total red blood cells, White blood cell and PCV were observed to have increased significantly. This indicated that the *F. abutilifolia* aqueous extract is not toxic because decrease in PCV and red blood concentration

shows toxicity to the red blood cells. This agreed with Nebedum *et al.* (2010) who reported on *Ficus* species as an excellent hematopoiesis enhancer.

The levels of serum creatinine, blood urea nitrogen, urea, albumin and total protein were tested to examine renal function, while the levels of AST, ALT, and ALP were evaluated to determine liver function. In this study, there was no observed significant ( $p > 0.05$ ) increase in the serum alkaline phosphatase, aspartate aminotransferase, blood urea nitrogen, alanine aminotransferase, and creatinine group when compared to normal control. This suggests that the extract did not possess any hepatotoxic and kidney effect in mice. ALT, AST and ALP are a major marker of liver injury while Blood urea and creatinine is a marker of renal injury. No significant difference in the level of AST, ALT, and ALP in treated animals was seen when compared with control group and this suggested no leakage of liver enzymes due to hepatic assault, hence, the muscle-protective activity of the leaf of *F. abutilifolia* being established. The result of this study agreed with the work of Prince *et al.* (2008) on the cardio protective effects of polyherbal formulation in wistar rats. However, it is contrary to the reports of earlier studies who recorded no significant difference in the levels of ALP and ALT in mice (El-Habbak *et al.*, 1989; Rokaya *et al.*, 2010)

## CONCLUSION

In this present study, flavonoids, cardiac glycosides, phenolic compounds, alkaloids, tannins, steroids, carbohydrates, terpenoids, and saponins were present in the aqueous leaf extract *Ficus abutilifolia* (Miq). The extract is relatively safe with an LD<sub>50</sub> greater than 2000mg/kg.

## ACKNOWLEDGEMENT

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### CONFLICT OF INTEREST

There is none to be declared.

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**Table 1:** Qualitative Phytochemical screening of aqueous leaf extract of *Ficus abutilifolia* (Miq)

| S/No. | Phytoconstituents  | Test                     | Inference |
|-------|--------------------|--------------------------|-----------|
| 1.    | Alkaloids          | Dragendorff test         | +         |
| 2.    | Cardiac Glycosides | Keller-Kiliani test      | +         |
| 3.    | Saponins           | Frothing test            | +         |
| 4.    | Phenolic compounds | Lead acetate test        | +         |
| 5.    | Tannins            | Ferric chloride test     | +         |
| 6.    | Steroids           | Salkowski test           | +         |
| 7.    | Carbohydrates      | Molisch test             | +         |
| 8.    | Flavonoids         | Shinoda test             | +         |
| 9.    | Terpenoids         | Liebermann Burchard test | +         |
| 10.   | Anthraquinones     | Borntrager's test        | -         |

+ present; - absent

**Table 2:** Acute oral toxicity of aqueous leaf Extract of *Ficus abutilifolia* (Miq) in mice

| Experimental mice | Mice label | Dose (mg/kg) | Short-term effect (24 hrs) | Delayed effect (14 days) |
|-------------------|------------|--------------|----------------------------|--------------------------|
| M1                | I          | 2000         | Survive                    | No death                 |
| M2                | II         | 2000         | Survive                    | No death                 |
| M3                | III        | 2000         | Survive                    | No death                 |
| M4                | IV         | 2000         | Survive                    | No death                 |
| M5                | V          | 2000         | Survive                    | No death                 |

**Table 3:** Effect of the aqueous leaf Extract of *Ficus abutilifolia* (Miq) on haematological parameters in the limit dose test study

| Parameters               | Test             | Control           |
|--------------------------|------------------|-------------------|
| TWBC ( $\times 10^9/l$ ) | $5.35 \pm 0.05$  | $6.45 \pm 0.35^a$ |
| TRBC ( $\times 10^9/l$ ) | $7.42 \pm 0.04$  | $7.24 \pm 0.06$   |
| PCV (%)                  | $53.5 \pm 0.5$   | $51.0 \pm 1.0$    |
| HGB(g/dl)                | $17.18 \pm 0.51$ | $18.25 \pm 0.15$  |
| NEUT (%)                 | $18.5 \pm 0.5$   | $29.0 \pm 1.0^a$  |
| LYMPHO (%)               | $72.5 \pm 2.5$   | $65.0 \pm 2.0^a$  |
| MONO (%)                 | $6.5 \pm 0.5$    | $3.5 \pm 0.5^a$   |
| EOSINO (%)               | $2.5 \pm 0.5$    | $1.5 \pm 0.5$     |
| MCH (%)                  | $23.92 \pm 0.18$ | $25.05 \pm 0.15$  |
| MCV (%)                  | $72.89 \pm 0.41$ | $70.09 \pm 0.45$  |
| MCHC (%)                 | $33.22 \pm 0.46$ | $36.81 \pm 0.61$  |

Data are expressed as mean  $\pm$  SD, n=5. <sup>a</sup>Means with different superscript across each row are statistically significant ( $p < 0.05$ ) when compared to control

**Table 4:** Effect of the methanol extract of aqueous leaf Extract of *Ficus abutilifolia* (Miq) on blood serum biochemistry in the limit dose test study

| Parameters    | Test           | Control          |
|---------------|----------------|------------------|
| AST (u/l)     | $22.0 \pm 1$   | $17.8 \pm 1.3$   |
| ALT (u/l)     | $17.5 \pm 2.1$ | $10.0 \pm 1.0^a$ |
| ALP (u/l)     | $37.5 \pm 2.1$ | $28.5 \pm 1.5^a$ |
| BUN (mg/dl)   | $33.0 \pm 1.0$ | $27.5 \pm 0.7$   |
| CREAT (mg/dl) | $77.0 \pm 1.0$ | $62.0 \pm 1.0^a$ |
| TP (g/l)      | $7.2 \pm 0.2$  | $7.5 \pm 0.1$    |
| ALB (g/l)     | $4.8 \pm 0.3$  | $5.0 \pm 0.1$    |

Data are expressed as mean  $\pm$  SD, n=5. <sup>a</sup>Means with different superscript across each row are statistically significant ( $p < 0.05$ ) when compared to control