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Anticonvulsant activity of the methanol root bark extract of *Ficus sycomorus* Linn. (Moraceae)

[Actividad anticonvulsivante del extracto metanólico de la corteza de la raíz de *Ficus sycomorus* Linn. (Moraceae)]

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Abstract

Context: *Ficus sycomorus* Linn. (Moraceae) is used in Northern Nigeria for the management of *grand mal* epilepsy without any scientific validation.

Aims: To evaluate the phytochemical constituents, acute toxicity study and anticonvulsant properties of the methanol root bark extract of *F. sycomorus*.

Methods: The basic phytochemical screening, Lorke's method and three models of epilepsy, which included the maximal electroshock test (in chicks), pentylenetetrazole and 4-aminopyridine induced seizures (in mice) were employed.

Results: The extract revealed the presence of tannins, saponins, carbohydrate, alkaloids, flavonoids, steroids, terpenoids and cardiac glycosides. The intraperitoneal LD₅₀ in mice was estimated to be 565.69 mg/kg body weight. The extract afforded a slight protection, 30% (highest dose used) to the laboratory animals against the maximal electroshock test. The duration of convulsion decreased, which was not statistically significant ($p > 0.05$) when compared to the untreated group. Also, the extract did not protect the animals against the chemically induced seizures by pentylenetetrazole and 4-aminopyridine.

Conclusions: The data suggest that the methanol root bark extract of *F. sycomorus*, at the doses tested and under the experimental conditions reported, may not contain psychoactive principles that are relevant to the management of *grand mal* epilepsy as claimed by the traditional medicine practitioners.

Keywords: 4-aminopyridine-induced seizures; *grand mal* epilepsy; maximal electroshock test; pentylenetetrazole-induced seizures; psychoactive principles; traditional medicine.

Resumen

Contexto: *Ficus sycomorus* Linn. (Moraceae) se utiliza en el norte de Nigeria para el manejo de la epilepsia *gran mal* sin ninguna validación científica.

Objetivos: Evaluar los componentes fitoquímicos, la toxicidad aguda y las propiedades anticonvulsivantes del extracto metanólico de corteza de la raíz de *F. sycomorus*.

Métodos: Se empleó el método de Lorke para realizar un tamizaje fitoquímico básico y tres modelos de epilepsia, que incluyeron el ensayo de electrochoque máximo (en pollo) y convulsiones inducidas por pentilentetrazol y 4-aminopiridina (en ratones).

Resultados: El extracto reveló la presencia de taninos, saponinas, hidratos de carbono, alcaloides, flavonoides, esteroides, terpenoides y glucósidos cardiacos. La DL₅₀ intraperitoneal en ratones se estimó en 565,69 mg/kg peso corporal. El extracto produjo una ligera protección, el 30% (dosis máxima utilizada) contra la prueba de electrochoque máximo. La duración de convulsión disminuyó, pero no fue estadísticamente significativa ($p > 0,05$) en comparación con el grupo no tratado. Además, el extracto no protegió a los animales contra las convulsiones inducidas químicamente por pentilentetrazol y 4-aminopiridina.

Conclusiones: Los datos sugieren que el extracto metanólico de corteza de la raíz de *F. sycomorus*, a las dosis ensayadas y en las condiciones experimentales reportadas, podrían no contener principios psicoactivos que relevantes para el manejo de la epilepsia *gran mal* según lo demandado por los practicantes de la medicina tradicional.

Palabras Clave: convulsiones inducidas por 4-aminopiridina; convulsiones inducidas por pentilentetrazol; epilepsia de gran mal; medicina tradicional; principios psicoactivos; prueba de electroshock máximo.

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INTRODUCTION

The practice of using plants to treat and prevent diseases has started since prehistoric times and flourishes today as the primary form of medicine especially in the African continent. Humankind for millennia has used medicinal plants, and their use is as old as humanity itself. The range of species used and their scope for healing is vast (Butler, 2004). It was reported by the World Health Organization (WHO) that over 80% of the world's population depend mainly on plants and plant extracts for health care (WHO, 2002). It has been estimated that over 250000 higher plant species occur on earth, more than 80000 species are reported to have at least some medicinal values, and around 5000 species have specific therapeutic value (Joy et al., 1998).

Epilepsy is one of the most common diseases of the brain, affecting at least 50 million persons globally (Scheuer and Pedley, 1990). It is a chronic disorder characterized by the periodic and unpredictable occurrence of epileptic seizures, which are caused by an abnormal discharge of cerebral neurons. Many different types of seizures can be identified because of their clinical phenomena (Löscher, 1998).

Seizures are fundamentally divided into two major groups: partial and generalized. Partial (focal, local) seizures are those in which clinical or electrographic evidence exists to suggest that the attacks have a localized onset in the brain, usually in a portion of one hemisphere, while generalized seizures are those in which evidence for a localized onset is lacking (CCTILE, 2003).

The current therapy of epilepsy with modern anti-epileptic drugs (AEDs) is associated with side effects, dose-related and chronic toxicity and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy. Therefore, there is need for new AEDs (especially from plants) with greater efficacy and novel mechanisms of action to serve as alternate therapy for the treatment of resistant epilepsy (Wickenden, 2002).

The discovery of novel antiepileptic drugs relies upon the preclinical employment of animal models to establish efficacy and safety before the introduction of the antiepileptic drugs in human volunteers (Löscher and Schmidt, 2006).

Ficus sycomorus belongs to the family Moraceae. It is commonly known among the Hausa people of Northern Nigeria as Farin Baure. The roots bark of this plant is traditionally used for the treatment of epilepsy, diarrhea, dysentery, painful urination and vaginal infections (Abubakar et al., 2015).

The *in vitro* antimicrobial screening of the methanol root bark extract of *F. sycomorus* revealed that the extract exhibited varying activity against *Enterococcus faecalis*, *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae* and *Candida albicans* (Abubakar et al., 2015), while its leaves possess antidiabetic, antioxidant (70% methanol extract), antitumor and antibacterial activities (Mousa, 1994). Also, aqueous extract of stem bark exhibits sedative and muscular activities (Sandabe et al., 2003; 2006).

The root extract of *F. abutilifolia* was reported to protect the laboratory animals against maximum electroshock induced-seizure; this indicates that the plant may be useful in the management of *grand mal* epilepsy. However, the plant could not protect the laboratory animals against pentylene-tetrazole (PTZ) or 4-aminopyridine induced seizures, and as such may not be beneficial in the management of *petit mal* epilepsy (Danmalam et al., 2012).

To the best of our knowledge, scientific evidence for the use of *F. sycomorus* in the treatment of epilepsy is lacking, and therefore this study was conducted to evaluate this claim. Three models of epilepsy were used, which include the maximal electroshock test (MEST), pentylenetetrazole (PTZ) and 4-amino pyridine induced seizures.

MATERIAL AND METHODS

Animals

Swiss albino mice of both sexes, weighing 18-25 g were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. In addition, day old white ranger cockerels obtained from National Animal Production Research Institute (NAPRI) Shika, Zaria were used. They were housed under standard conditions of temperature ($25 \pm 2^\circ\text{C}$), 12/12 hour light/dark cycle, fed with standard diet (Feeds Masters Plc. Ilorin, Nigeria), and given water

ad libitum. All experiments performed in this work followed the principles of laboratory animal care outlined by the ethical committee of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. An approval number, ABUCAUC/15/0873 was given by the ethical committee.

Plant collection and identification

The plant was collected in Zaria City, Kaduna State, Nigeria. It was identified and authenticated by a Taxonomist, Mr. Umar Gallah of the Herbarium of the Biological Sciences Department, Ahmadu Bello University, Zaria. A voucher specimen (Number, 1466) had been deposited in the same herbarium.

Preparation of the extract

The root bark was carefully removed, washed and cut into pieces. It was then air-dried and ground into powder using mortar and pestle. The powdered plant material (250 g) was macerated with 900 mL of 70% methanol in a separating funnel plugged with cotton wool (the cotton wool serves as a filter and separating funnel as percolator) for 48 hours. The filtrate was concentrated using rotary evaporator and was dried over a water bath 40°C.

Phytochemical screening

The phytochemical analysis of the methanol root bark extract of *F. sycomorus* was conducted using standard qualitative methods as described by Harbone (1998) and Evans (1996).

Test for carbohydrates

The extract (5 g) was boiled in 50 mL of distilled water for 5 minutes. The mixture was filtered while hot and allowed to cool to room temperature. The filtrate was divided into two portions and used as follows:

Molisch's test: To the first portion, 1 mL of Molisch's reagent was added, this followed by 1 mL of concentrated sulphuric acid down the side of the test tube. A reddish colored interfacial ring indicates the presence of carbohydrate.

Fehling's test: To the second portion, 5 mL of equal mixture of Fehling's solution A and B was added and the mixture was boiled for minutes. A brick red colored precipitate indicates the presence of reducing sugar.

Test for anthraquinones

Borntrager's test: The extract (200 mg) was boiled in 5 mL of 10% HCl and then filtered. The filtrate was extracted with 5 mL of benzene, and the benzene layer was shaken with 5 mL of 10 % NH₄OH. A rose pink or cherry red color indicates the presence of anthraquinone derivatives.

Test for saponins

Frothing test: The extract (0.5 g) was shaken with water in a test tube. Frothing which persisted for 15 minutes indicates the presence of saponins.

Test for cardiac glycosides

The extract (2 g) was boiled in 10 mL of 95 % alcohol for five minutes; it was then cooled and filtered. Lead sub acetate solution (3 mL) was added to the filtrate and then filtered again. The filtrate was divided into two portions (first filtrate). To the first portion of the first filtrate, 10 mL of chloroform was added and the mixture was shaken for 5 minutes. The lower chloroform layer was run off into a beaker (second filtrate).

Keller-Killiani test: The second filtrate was transferred into an evaporating dish and evaporated to dryness on a water bath. The residue was dissolved in 1 mL of glacial acetic acid containing traces of FeCl₃ solution and then transferred into a dry test tube; 2 mL of concentrated sulphuric acid was run down the side of the test tube to form a lower layer. A purple-brown ring at the interface indicated the presence of deoxysugars, while a pale green color in the upper acetic acid layer is due to the presence of steroid, which indicates the presence of cardiac glycosides.

Kedde test: To the second portion of the first filtrate, 1 mL of 2% solution of 3,5-dinitrobenzoic acid in 95% alcohol was added. The solution was made alkaline by the addition of 5% sodium hydroxide. The appearance of a purple-blue color indicated the presence of cardinolides.

Test for triterpenoids/steroids

Liebermann-Buchard test: Anhydrous acetic acid (1 mL) was added to 1 mL of chloroform and cooled to 0°C, and then a drop of concentrated sulphuric acid and the extract (0.5 g) were added. A blue-green ring was taken as an indication for the presence of terpenoids.

Salkowski test: The extract (0.5 mg) was dissolved in 2 mL of chloroform, thereafter; 1 mL of concentrated sulfuric acid was added down the test tube to form two phases. Formation of red or yellow coloration was taken as an indication for the presence of sterols.

Test for flavonoids

Shinoda test: About 0.5 g of the extract was dissolved in 2 mL of 50% methanol and filtered. Magnesium filings and 3 drops of hydrochloric acid were added to the filtrate. A pink or red color was considered as an indication for the presence of flavonoids.

Sodium hydroxide test: The extract (0.5 g) was dissolved in 2 mL of 10% aqueous sodium hydroxide solution and filtered to give yellow color, a change in color from yellow to colorless on addition of dilute hydrochloric acid indicated the presence of flavonoids.

Test for tannins

Ferric chloride test: The extract (200 mg) was boiled in 20 mL of distilled water and filtered. Ferric chloride (1 mL) was then added to the filtrate. The formation of green precipitate indicated the presence of tannins.

Test for alkaloids

The extract (0.5 g) was stirred with 5 mL of 1% aqueous hydrochloric acid on a water bath and filtered. The filtrate was divided into three portions. To the first portion, 1 mL of freshly prepared Dragendoff's reagent was added and observed for the formation of orange to brownish precipitate. To the second portion, 1 mL of Mayer's reagent was added and observed for the formation of white to yellowish colored precipitate. To the third portion, 1 mL of Wagner's reagent was added to give a reddish-brown precipitate.

Acute toxicity studies

The intraperitoneal median lethal dose (LD₅₀) was conducted using the method of Lorke (1983). It was carried out in two phases. In the first phase, nine mice were divided into 3 groups of 3 mice each. The first group received the extract (i.p) at a dose of 1000 mg/kg body weight; followed by 100 mg/kg and 10 mg/kg to the second and third group respectively. Animals were observed for general signs of toxicity and death over a period of 24 hours. In the second phase, four mice were divided into four groups of one mouse each. The extract was administered to group 1, 2, 3 and 4 at the dose of 200, 400, 800 and 1600 mg/kg body weight, and final LD₅₀ was calculated as the square root of the geometrical mean of the highest non-lethal dose and the lowest lethal dose.

Maximal electroshock test in chicks

The methods of Swinyard and Kupferberg (1985) and Browning (1992) were adopted. Fifty (50) one day old chicks were randomly divided into 5 groups of 10 chicks per group. The first group received normal saline 10 mL/kg body weight (i.p), groups 2-4 received the extract (i.p) at the doses of 37.5, 75, and 150 mg/kg body weight respectively. The fifth group received phenytoin 20 mg/kg (i.p), 30 min later, maximal electroshock was delivered to induce a seizure in the chicks using an electroconvulsive machine (model 1801, Ugo Basile, Italy) with corneal electrodes placed on the upper eyelid of the chick after dipping them in normal saline. The current, shock duration, frequency and pulse width were set and maintained at 90 mA, 1.0 s, 200 Hz and 1.0 m/s, respectively. An episode of tonic extension of the hind limbs of the chicks was considered as full convulsion while the lack of tonic extension of the hind limbs was considered as protection. The time of recovery from seizures was also recorded.

Pentylenetetrazole induced-seizure test in mice

The method of Swinyard et al. (1952) was employed. Twenty-five mice were divided into five groups of five mice per group. The first group received normal saline 10 mL/kg body weight (i.p). Groups 2-4 received the extract (i.p) at the doses of

37.5, 75, and 150 mg/kg body weight respectively, while the fifth group received valproic acid at a dose of 90 mg/kg (i.p). Thirty minutes later, mice in all the groups were administered with freshly prepared solution of pentylenetetrazole (PTZ) at a dose 90 mg/kg body weight (s.c). The mice were observed for 30 min for the onset and incidence of seizures. An episode of tonic extension of the hind limbs or clonic spasm, which persisted for a minimum of 30 s, was considered as a threshold convulsion. Lack of threshold convulsion during 30 min of observation was considered as protection.

4-Aminopyridine-induced seizure in mice

The method described by Yagamuchi and Rogawski (1992) was adopted. Twenty-five albino mice were randomly divided into 5 groups of five mice per group. The first group received normal saline 10 mL/kg body weight (i.p). Groups 2-4 received the extract at the doses of 37.5, 75, and 150 mg/kg body weight respectively, while the fifth group received phenobarbitone at a dose of 30 mg/kg body weight (i.p). Fifteen minutes post treatment; 4-aminopyridine was administered at a dose of 15 mg/kg body weight to each group (s.c). The mice were observed for 30 min for characteristic behavioral signs, such as hyperactivity, trembling, intermittent forelimb extension, tonic seizures and death. The ability of the extract to protect the mice from lethality within 30 min observation period was considered as an indication for anticonvulsant activity.

Statistical analysis

Results were presented in tables and expressed as mean \pm SEM. The level of significance was tested using One-way ANOVA followed by Duncan Multiple Range Test (DMRT). Results were regarded as significant when $p < 0.05$. All statistical analyses were performed using SPSS software, version 21.0 (Released August 14, 2012), United States of America.

RESULTS

Phytochemical screening

Phytochemical constituents identified in the methanol root bark extract of *F. sycomorus* include: carbohydrates, saponins, cardiac glycosides, steroids, terpenoids, flavonoids, tannins and alkaloids (Table 1).

Acute toxicity studies

The signs of toxicity observed in both phases include loss of appetite, restlessness and general weakness. Death was recorded in the third group (1000 mg/kg) of the first phase, and also in the third (800 mg/kg) and fourth (1600 mg/kg) groups of the second phase of the toxicity studies. Based on the above results, the median lethal dose (LD_{50}) of the methanol root bark extract of *F. sycomorus* was estimated to be 565.69 mg/kg.

Maximal electroshock test in chicks

The methanol root bark extract of *F. sycomorus* afforded a slight protection, 30% (highest dose used, 150 mg/kg body weight) to the animals against the maximal electroshock induced convulsion. The duration of convulsion decreases slightly at all the three doses but it was not statistically significant ($p > 0.05$) when compared to the untreated group (Table 2). The standard anticonvulsant drug, phenytoin (at a dose of 20 mg/kg body weight) protected all the animals in the positive control group by giving 100 % protection.

Pentylenetetrazole-induced seizure test in mice

The methanol root bark extract of *F. sycomorus* root did not protect the animals against the chemically induced convulsion of PTZ, but it afforded a slight reduction in the duration and onset of convulsion, which was statistically significant ($p < 0.05$) when compared to the untreated group (Table 3). The standard antiepileptic drug, valproic acid protected all the animals in the positive control group.

Table 1. Phytochemical constituents of the methanol root bark extract of *F. sycomorus*.

Metabolites	Identification test	Inference
Carbohydrates	Molisch's test	Present
	Fehling's test	Present
Anthraquinones	Borntrager's test	Absent
Saponins	Frothing test	Present
Cardiac glycosides	Keller-Keliani test	Present
	Kedde's test	Present
Triterpenoids /steroids	Liebermann-Burchard test	Present
	Salkowski's test	Present
Flavonoids	Shinoda test	Present
	Sodium hydroxide	Present
Tannins	Ferric chloride test	Present
Alkaloids	Mayer's reagent	Present
	Dragendorff's reagent	Present
	Wagner's reagent	Present

Table 2. Effects of different doses of methanol root bark extract of *F. sycomorus* on the convulsive activities of electroshock in chick.

Treatment	Dose (mg/kg)	Mean onset convulsion (min)	Quantal protection	Protection (%)	Mortality (%)
Normal saline	10 mL/kg	7.70 ± 0.83 ^a	0/10	0	0
FSE	37.5	7.60 ± 1.35 ^a	1/10	10	0
	75	7.40 ± 1.38 ^a	2/10	20	0
	150	7.30 ± 1.69 ^a	3/10	30	0
Phenytoin	20	-	10/10	100	0

Values represent mean ± SEM of n=10. Values in the same column with different superscript differs significantly (p<0.05). FSE: Methanol root bark extract of *Ficus sycomorus*.

Table 3. Effects of different doses of methanol root bark extract of *F. sycomorus* on the convulsive activities of pentylenetetrazole in mice.

Treatment	Dose (mg/kg)	Mean onset convulsion (min)	Mean duration convulsion (min)	Quantal protection	Protection (%)	Mortality (%)
Normal saline	10 mL/kg	6.40 ± 0.22 ^d	7.40 ± 1.78 ^d	0/5	0	60
FSE	37.5	7.25 ± 0.27 ^c	6.20 ± 1.66 ^b	0/5	20	20
	75	8.50 ± 0.28 ^b	5.80 ± 1.39 ^b	0/5	20	20
	150	9.67 ± 0.07 ^a	5.20 ± 1.74 ^a	0/5	40	0
Valproic acid	90	-	-	5/5	100	0

Values represent Mean ± SEM of N=5. Values in the same column with different superscript differs significantly (p<0.05). FSE: Methanol root bark extract of *Ficus sycomorus*.

Table 4. Effects of different doses of methanol root bark extract of *F. sycomorus* on the convulsive activities of 4-aminopyridine in mice.

Treatment	Dose (mg/kg)	Mean onset convulsion (min)	Quantal protection	Protection (%)	Mortality (%)
Normal saline	10 mL/kg	9.60 ± 0.22 ^a	0/5	0	100
FSE	37.5	9.40 ± 0.27 ^a	0/5	0	100
	75	9.70 ± 0.28 ^a	0/5	0	100
	150	9.97 ± 0.07 ^a	0/5	0	100
Phenobarbitone	30	-	2/5	20	20

Values represent mean ± SEM of N=5. Values in the same column with different superscript differs significantly (p<0.05). FSE: Methanol root bark extract of *Ficus sycomorus*.

4-Aminopyridine-induced seizure in mice

The methanol root bark extract of *F. sycomorus* root did not protect the animals against the chemically induced convulsion of 4-aminopyridine solution as shown in Table 4.

DISCUSSION

The different phytochemical constituents identified in the methanol root bark extract of *F. sycomorus* are considered as significantly important biologically active compounds of plant origin. Phytoconstituents such as tannins, flavonoids and saponins have been shown to modulate central nervous system activities. For example, a saponin present in *Bacopa monnieri* was reported to be responsible for the observed anticonvulsant activity of the plant (Darpan et al., 2009). Similarly, tannins identified from

the aqueous fraction of the stem bark extract of *Xeromphis nilotica* was believed to be responsible for the effects observed in mice (Danjuma et al., 2009).

The acute toxicity test gives a clue on the range of doses that could be toxic to the animals, and can also be used to estimate the acute toxicity index (LD₅₀) of drugs and xenobiotics (Rang et al., 2001). The result of this study showed that the methanol root bark extract of *F. sycomorus* has an LD₅₀ that can be considered as slightly or moderately toxic (Matsumura, 1975), but the risk of acute intoxication is minimal (Lorke, 1983).

The electroshock assay is used primarily as an indication for compounds, which are effective in *grand mal* epilepsy. Protection against hind limb tonic extension in maximal electroshock test predicts the anticonvulsant effect that prevents the spread of epileptic seizure discharge from an epi-

leptic focus during seizure activity. The extract was only able to give a slight protection and reduction in the duration of convulsion, which was not significant and thus has no effect on *grand mal* epilepsy (Browning, 1992).

PTZ is a well-known convulsant, and the chemically induced seizure using PTZ test usually identifies compounds that raise seizure threshold in the brain. PTZ has been shown to interact with GABA neurotransmitter and GABA receptor complex (De Deyn et al., 1992). The methanol root bark extract of *F. sycomorus* root did not protect the animals against the chemically induced convulsion of PTZ. This finding indicates that the extract may not contain compounds that can raise seizure threshold in the brain (White et al., 1998). On the other hand, the stem bark extract of *F. sycomorus* conferred 100% protection to the animals treated with a convulsive dose of PTZ (Sandabe et al., 2003). The ability of the extract to increase the latency time to onset of a seizure in the PTZ test suggested a possible interaction of the extract with GABA-ergic neurotransmission and anticonvulsant activity against *petit mal* epilepsy (Vida, 1995). This finding is in contrast to a similar test conducted on *F. abutilifolia*, which failed to show anticonvulsant activity against PTZ (Danmalam et al., 2012), and this may be due to differences in chemical constituents between the two *Ficus* species.

CONCLUSIONS

The findings in this study showed that the root extract of *F. sycomorus*, at the doses tested and under the experimental conditions reported, may not contain psychoactive principles that are relevant to the management of *grand mal* epilepsy as claimed by the traditional medicine practitioners, but may be useful in the treatment of *petit mal* epilepsy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Contribution	Abubakar US	Danmalam UH	Musa KY	Ahmed A	Jajere UM	Abdullahi S
Concepts or Ideas	X	X	X		X	
Design	X	X	X	X	X	X
Definition of intellectual content	X	X	X	X	X	X
Literature search	X	X	X			
Experimental studies	X	X	X	X	X	X
Data acquisition	X	X	X	X	X	X
Data analysis	X	X	X	X	X	X
Statistical analysis	X	X	X	X	X	X
Manuscript preparation	X	X				
Manuscript editing			X			X
Manuscript review				X	X	

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