Journal of Applied Pharmaceutical Science
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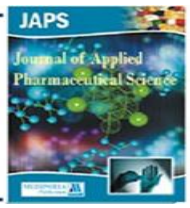

ISSN: 2231-3354
Received on: 05-07-2012
Revised on: 09-07-2012
Accepted on: 17-07-2012
DOI: 10.7324/JAPS.2012.2739

Phytochemical and Anticonvulsant Studies on the Aqueous Ethanol Extract of the Root-Back of *Ficus Abutilifolia* (Miq.) Miq. (Family: Moracea)

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ABSTRACT

Ficus abutilifolia, belonging to the family Moraceae is a small to medium sized tree that grow mostly in the African continent. It was reported to be used traditionally, in promoting fertility in humans and in the treatment of skin wart and management of epilepsy. Preliminary phytochemical investigation of the powdered root revealed the presences of flavonoids, saponins and tannins among others. The intraperitoneal LD_{50} of the 70% aqueous ethanol extract was found to be 2154.1 mg/Kg in mice. The anticonvulsant studies of the extract revealed that a single administration (at the dose of 100 – 400 mg/Kg) produced a dose-dependent protection against MEST; however, the extract did not offer significant protection against pentylenetetrazole- and 4-amino pyridine–induced seizures. These finding suggest some level of protection by the aqueous ethanol extract against MES induced seizure in chicks, thereby giving support to the traditional claim for the use of the plant in the treatment and/or management of convulsion and epilepsy.

Keywords: Anticonvulsant plants; *Ficus abutilifolia;* Ethanol extract; Phytochemical screening.

INTRODUCTION

Plants and their products have been in use in the treatment of chronic ailments, pains and infectious diseases before the modern civilization. And to date, they still remain the almost exclusive source of drugs for more than 60% of the world's population, especially in developing countries, and also serve as important source of new drugs, new drug leads, and new chemical entities. It is in record that the potential of plants in general and higher plants in particular as a source of new drugs has not been fully explored. Some individual plant extract may have been subjected to specific pharmacological test (e.g. for cardiac activity only) however, the same extract may be examined for other types of activities such as pain reliving, anti-inflammation, anti-diarrhea etc (Balunas and Kinghorn, 2005; Li and Vederas, 2009; Phillipson, 2001). *Ficus abutilifolia* (Miq.) Miq. is commonly called large-leaved rock fig or rock wild fig and belongs to the family Moraceae. It is a small to medium sized, deciduous to semi-deciduous tree that may grow up to 15 m high (though it seldom exceeds 5 m). The bark of the plant is yellowish-white, smooth and flaking. The trunk is usually twisted. Leaves are broadly ovate and cordate at base, they usually measured 7.5 – 20.0 X 6.5 – 18.0 cm. Fruits are 1.5 – 2.5 cm in diameter usually borne singly or in pairs at the leaf axils, smooth or slightly hairy.

A decoction of the leaf of the plant is reported to be used in promoting fertility in humans and the milky latex is used to remove skin warts. Bark decoction is taken by men as a strengthening tonic (Burring, 2006).

Information made available to us (personal communication, 2004) showed that the root of *F. abutilifolia* is also used as part of a preparation in the management of epilepsy. We therefore, investigate and report here the preliminary phytochemical screening and for the first time the anticonvulsant properties of the *F. abutilifolia* root-bark.

MATERIALS AND METHODS

Plant Material

The plant specimen was collected in October, 2006 around Basawa-Zaria, Kaduna State Nigeria. It was identified by the herbarium keepers of the Department of Biological Sciences, Ahmadu Bello University, Zaria-Nigeria, where a specimen (voucher specimen number 900, 742) was deposited for future reference. The root of the plant was cleaned, air dried and ground to powder using pestle and mortar.

Extraction of the Plant Material

Powdered plant (150 g) was macerated with 750 ml of 70% aqueous ethanol for four days after which, it was filtered and evaporated to dryness on water bath to give a brownish residue. The residue, subsequently referred to as the extract was stored in an air tight container until required for further use.

Animals

Swiss albino mice of both sexes, weighing 18 – 25 g were obtained from the Animal House facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. One day old Ranger cockerels were obtained from National Animal Production Research Institute (NAPRI) Shika-Zaria, Nigeria. The mice were kept under well–ventilated conditions, 12 hours light/dark cycle, temperature of 25 ± 2^0C and fed on standard laboratory animal Feeds and had access to water *ad-libitum.*

Drugs

4-aminopyridine (BDH, UK); Pentylenetatrazole (PTZ) (Sigma-Aldrich, Germany); Phenobarbitone (Pfizer, USA); Phenytoin (Sanofi synthelabo, USA). All drugs/chemicals were prepared (fresh each time) in distilled water, to the desired concentration.

Preliminary Phytochemical Screening

The powdered root of *F. abutilifolia* was subjected to preliminary phytochemical analysis to test for the presence of phytochemical constituents using the following methods:

Anthraquinones [Borntrager's test: 100 mg of powdered plant in 5 ml of chloroform, filtered. 2 ml filtrate + 2 ml 10% NH_4OH. A bright pink colour indicates the presence of anthraquinones; Modified Borntrager's test: 200 mg plant material boiled in 5ml 10% HCl, filtered. Filtrate extracted with 5ml benzene, and benzene layer shaken with 5 ml 10% NH_4OH. A rose pink or cherry red colour indicates the presence of anthraquinone derivatives (Evans, 1996).

Tannins [200 mg sample boiled in 20 ml distilled water, filtered; 2 ml filtrate + 1 ml $FeCl_3$, blue-black or greenish-black precipitate indicates tannins; 1 ml filtrate, + 3 drops of lead sub acetate, a colored precipitate indicates the presence of tannins; 1 ml filterate + 3 drops of bromine water, a buff colour indicate the presence of tannins]. Saponins [frothing test: 0.5 ml filtrate + 5 ml distilled water, shaken for 30 sec, persistence frothing indicates saponins (Evans, 1996)].

Flavonoids [Shinoda's Test: (200 mg sample in 5 ml ethanol, filtered) 1 ml filtrate, + magnesium ribbon + conc. HCl a pink or red color indicates the presence of flavonoids; 200 mg sample was de-tanned with acetone and extracted with water. Extract + $FeCl_3$, a greenish-black colour indicates phenolic nucleus; 2 ml extract + 2 ml 10% NaOH a yellow solution that becomes colorless with dil. HCl indicate flavonoids]. Terpenses/steroids [Liebermann – Burchard's Test: (200 mg plant material in 10 ml chloroform, filtered; 2 ml filtrate + 2 ml acetic anhydride + 1 ml of conc. H_2SO_4. A blue – green ring indicates the presence of terpenses/steroids (Parekh and Chanda, 2007)].

Alkaloids [200 mg plant material boiled in 20 ml of 1% H_2SO_4 in 50% ethanol, filtered; filtrate + 5 drops conc. NH_4OH + 20 ml chloroform and the two layers separated. Chloroform layer was extracted with 20 ml dilute H_2SO_4. Extract + 5 drops of Mayer's/ Wagner's/ Dragendorff's/ Hager's reagents, a creamy/brownish-red/orange-red/yellow precipitate indicates the presence of alkaloids (Evans, 1996).

Acute Toxicity Study

LD_{50} determination was conducted using the method of Lorke (1983). Nine mice were divided into 3 groups of 3 mice each. The first group received the extract (*i.p.*) at a dose of 1000mg/kg; group 2 received the extract at a dose of 100mg/kg (*i.p.*), while the last group received the extract at the dose of 10mg/kg body weight. Animals were observed for general signs and symptoms of toxicity including mortality over a period of 24 hours.

In the second phase, 4 mice were divided into 4 groups of one mouse each. The extract was administered at the dose of 600, 1000, 1600, and 2900 mg/Kg (*i.p.*) to animals respectively, based on the result of the first phase. LD_{50} was calculated as the square root of the geometrical mean of highest non lethal dose for which the animal survived and the lowest lethal dose for which the animal died.

Anticonvulsant Activity

Maximal Electroshock-Induced Seizures in Chicks (MEST)

The methods previously described by Swinyard and Kupferberg (1985) and Browning, (1992) were employed. Fifty one day old cockerels were randomly divided into 5 groups of 10 chicks per group. The first group received normal saline *i.p.*; groups 2 – 4 received the extracts (100, 200 and 400 mg/kg, *i.p.*

respectively). While the fifth group received phenytoin 20 mg/kg, (*i.p.).* Thirty minutes later, maximal electroshock was delivered to induce seizures in the chicks using Ugo basile electroconvulsive machine (model 1801) 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 90mA, 1.0 sec, 200 Hz and 1.0 ms^{-1} respectively. An episode of tonic extension of the hind limbs of the chicks was considered as full convulsions. Lack of tonic extension of the hind limbs was regarded as protection. The recovery time was taken for the unprotected animals.

Pentylenetetrazole–Induced Seizures in Mice (Sc-PTZ)

The method of Swinyard *et al.* (1952) was employed. Twenty-five mice were randomly divided into 5 groups of five mice per group. The first group which served as negative control was treated with normal saline (*i.p.*). Groups 2 – 4 received different doses of the aqueous ethanol extract reconstituted in water (100, 200 and 400 mg/kg, *i.p*. respectively). Group 5 which served as positive control was treated with 200 mg/kg *i.p.* valproic acid.

Thirty minutes later, 85mg/Kg of freshly prepared solution of pentylenetetrazole was administered subcutaneously to all the mice. The mice were observed for 30 minutes for the onset and incidence of seizures. An episode of clonic spasm of at least 5 seconds was considered as seizure. Lack of threshold convulsion during 30 minutes of observation was regarded as protection. The number of mice protected was noted and the anticonvulsant properties of the extract expressed as percentage protection.

4-Amino Pyridine-Induced Seizure in Mice

The method of Yagamuchi and Rogawski, (1992) was adopted. Twenty-five mice were randomly divided into 5 groups of five mice per group. The first group which served as negative control was treated with normal saline. Groups 2 – 4 received different doses of the aqueous ethanol extract reconstituted in water (100, 200 and 400 mg/Kg, *i.p.* respectively). Group 5 which served as positive control was treated with 20 mg/Kg (*i.p.*) phenobarbitone.

Thirty minutes later, 15mg/Kg of freshly prepared solution of 4-aminopyridine was administered subcutaneously to all the mice. The mice were observed for 30 minutes for characteristic behavioral signs such as hyperactivity, trembling, intermittent forelimb extension, tonic seizure and death. Lack of threshold convulsion during 30 minutes of observation was regarded as protection. The number of mice protected was noted and the anticonvulsant properties of the extract expressed as percentage protection.

Statistics Analysis

Data were expressed as Mean ± Standard Error of Mean. Statistical analysis was carried out using one-way ANOVA, followed by Dunnett's test and Chi-square for percentage protection and $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening tests for the powdered root of *F. abutilifolia* (table 1) revealed the presence of saponins, flavonoids, tannins, terpenoids and/or steroids. However, alkaloids were not detected in the root. The presence of the above chemical compounds may alone or in combination contribute to the observed anticonvulsant effect of the root extract.

Table .1: Preliminary Phytochemical Screening Result of Powdered root-bark of *F. abutilifolia.*

CONSTITUENT TESTED	RESULT
Anthraquinones:	
Borntrager's test	Absent
Modified Borntrager's test	Absent
Saponins:	
Frothing test	Present
Cardiac glycosides:	
Keller-Keliani test	Present
Kedde's test	Absent
Terpenes and/or steroids:	
Liebermann-Burchard test	Present
Salkowski's test	Present
Flavonoids:	
Shinoda's test	Present
Ferric chloride test	Present
Sodium hydroxide test	Present
Tannins:	
Ferric chloride test	Present
Bromine water test	Present
Lead sub-acetate test	Present
Alkaloids:	
Mayer's test	Absent
Wagner's test	Absent
Dragendorff's test	Absent
Hager's test	Absent

The median lethal dose of the aqueous ethanol extract of *F. abutifolia* was estimated to be 2, 154.1 mg/Kg body weight. The acute toxicity index (LD_{50}), serve as a means of giving an idea about the toxic effect of any potential drug substances. The result of the present study shows that the 70% aqueous ethanol extract of *F. abutilifolia* has an LD_{50} of over 2,000 mg/Kg body weight in mice when administered through intraperitoneous route. The extract can be regarded as slightly toxic but the risk of acute intoxication is minimal (Lorke, 1983).

The aqueous ethanol root extract of *F. abutifolia* dose dependently protected the animals against maximal electroshock seizure with the highest protection of 40% produced at the dose of 400 mg/Kg. However, the extract did not offer any significant protection against pentylenetetrazole and 4-aminopyridine induced seizures in mice. The hind limb tonic extension (HLTE) produced electrically as in the maximum electroshock test (MEST), is a common feature in many animal species including humans. And the response of the brains of the animals to anticonvulsant is similar to that of humans. Extracts of *F. abutilifolia* afforded dose depended protection to the laboratory animals against the HLTE showing the ability of the extract to inhibit or prevent seizure discharge within the brainstem seizure substrate (Browning, 1992). This suggests that the 70% aqueous extract of the plant under study contain some compounds that may be beneficial in the treatment of generalized tonic-clonic and partial seizure.

Table . 2: Effects of different doses of aqueous ethanol extract of *F. abutilifolia* root-bark on the convulsive activities of electroshock .

Treatment	Mean Recovery time ± SEM (min.)	Quantal Protection	Percentage Protection
Normal saline	7. 00 ± 1.36	0/10	0
100 mg/Kg	6.12 ± 0.92	1/10	10
200 mg/Kg	7.31 ± 1.22	2/10	20
400 mg/Kg	5.97 ± 1.52	4/10	40
Phenytoin 20 mg/kg	-	10/10	100

Table. 3: Effects of aqueous ethanol extract of *F. abutilifolia* root on pentylenetetrazole-induced seizure in mice.

Treatment	Mean onset of seizure ± SEM (min.)	Quantal Protection	Percentage Protection	Percentage Mortality
Normal saline	9.60 ± 2.84	0/5	0	80
100 mg/Kg	7.80 ± 3.35	0/5	0	40
200 mg/Kg	6.00 ± 2.24	0/5	0	60
400 mg/Kg	5.40 ± 1.67	0/5	0	60
Valproic acid 200 mg/kg	-	5/5	100	0

Table. 4: Effects of different doses of aqueous ethanol extract of *F. abutilifolia* root on the convulsive activities of 4-aminopyridine.

Treatment	Mean Onset of Convulsion ± SEM (min.)	Quantal Protection	Percentage Protection
Normal saline	9.40 ± 0.40	0/5	0
100 mg/Kg	9.60 ± 1.03	0/5	0
200 mg/Kg	9.20 ± 0.49	0/5	0
400 mg/Kg	9.80 ± 0.66	0/5	0
Phenytoin 20mg/kg	19.60 ± 3.33	0/5	0

The chemically-induced seizure using PTZ test usually identifies drugs that raise seizure threshold in the brain (White *et al*, 1998). The standard convulsant PTZ has also been shown to interact with γ-amino butyric acid (GABA) – a neurotransmmiter – and the GABA receptor complex (Bum *et al*, 2001). Drugs that inhibit the PTZ activity such as diazepam and valproic acid exert their effect by enhancing GABA mediated inhibition in the brain. The inability of the aqueous ethanol extract of *F. abutilifolia* to protect the animals against PTZ-induced seizure suggests that it may not be effective in the treatment of absence and/or myoclonic seizures. The potassium channel blocker - 4-aminopyridine - is a convulsant that penetrates the blood-brain barrier (Yagamuchi and Rogawski, 1992), and causes convulsion by enhancing spontaneous and evoked neurotransmitters. The inability of the extract to afford any protection to the laboratory animals against the chemically-induced seizure by 4-aminopyridine indicates that the extract does not produce its activity via potassium channel.

CONCLUSION

F. abutilifolia root extracts afforded protection to laboratory animals against maximum electroshock, indicating that it may be useful in the management of grand mal epilepsy. However, the absence of any significant protection to the animals against the chemically induced seizures of PTZ- and 4AP suggests that, at this dose the extracts may not be beneficial in the management of petit mal epilepsy.

ACKNOWLEDGMENT

The authours wish to thank: Malam Usman Mai-almajirai Hayin-mallam, Jama,a village Zaria-Kaduna State Nigeria, for an interview, and Mal Ibrahim Adamu, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria for technical assistance.

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