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PHARMACOGNOSTICAL SCREENING AND *IN-VIVO* ANTIEPILEPTIC ACTIVITY OF LEAVES EXTRACTS OF *FICUS SARMENTOSA* USING ALBINO WISTAR RATS

Arvind Negi*[1] and Anju Rawat[2]

*[1,2]Department of Pharmacy, GRD (PG) Institute of Management and Technology, Rajpur Road, Dehradun.

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***Corresponding Author**
Dr. Arvind Negi
Department of Pharmacy, GRD (PG) Institute of Management and Technology, Rajpur Road, Dehradun.

ABSTRACT

Ficus sarmentosa belonging to the family Moraceae is evergreen shurb, leaves alternate, ovate-elliptic to oblong, smaller on creeping branches, larger in fruting branches. Even though this plant has gained scientific importance recently, there is a need for pharmacognostic standardization. In the present study, pharmacognostic screening and In-vivo antiepileptic activity of leaves extracts of *Ficus sarmentosa* was evaluated on PTZ and MES induced epileptic albino wistar rats. In the microscopical studies, the different cell structures and arrangements were studied and in physical evaluation the ash values and extractive values were studied. In antiepileptic activity, maximal electroshock seizures were elicited by a 60 Hz alternating current of 150 mA intensity for 0.2 sec. A drop of electrolyte solution (0.9% NaCl) with lignocaine was applied to the corneal electrodes prior to application to rats. The duration of various phases of epilepsy were observed. In PTZ model, PTZ (110mg/kg) i.p. was injected. Diazepam 4mg/kg i.p. is used as standard drug. Behavioral responses of all animals were determined; antiepileptic response was evaluated by using criteria like onset of convulsion, duration of convulsion, protection against GTCS and mortality. The result of the study showed that *F. sarmentosa*, test doses do protection against mortality following PTZ administration, as well as MES model respectively. The present work was taken up with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant extract, shows significant antiepileptic potential, it could be a potential candidate for further drug development.

KEYWORDS: *Ficus sarmentosa*, fluorescence analysis, Physical parameter. Pentylenetetrazole, MES, antiepileptic.

INTRODUCTION

Diseases have been known for thousands of years and many ingenious methods have been followed for the relief of mankind. Almost every source of life and matter surrounding human being has been used in some form or other to treat diseases. Since the beginning of the history of mankind plants have been used for the treatment of various diseases.[1] Plants are an indispensible source of therapeutic preparations, both preventive and curative. With the resurgence in the consumption and demand for medicinal plants.[2]

Animal seizure models have played a significant role in epilepsy research including identification and characterization of new antiepileptic drugs (AEDs).[3] Recent years have witnessed a significant progress towards uncovering the cellular and molecular mechanisms of convulsion. This has provided impetus to the target-based drug design and mechanism-oriented approach for the identification of new AEDs. However, the current understanding of molecular biology of epilepsy is still too limited to employ *in-vitro* screening as the only tool in the search for new AEDs. Consequently, animal models continue to form useful component of a panel of screening tests for AED identification.[4] There remains a need for reliable animal models which are easy to use and which allow assessment of drug's ability not only to suppress the seizures but also to prevent development of epilepsy (epileptogenesis). Epilepsy is a brain disorder including unpredictable and recurrent tendency of seizures that interrupt normal brain functioning.[5] Epilepsy is one of the most common neurological clinical- pathological disorders that affect about 10-15% of the general population of India. Which have abnormal electrical discharge of neuronal circuit due to abnormal sensory, motor and psychomotor experiences which manifest and emphasis to criticize in center nervous system, on the basis of pathological justification reveal that seizures occurred because a group of excessive cortical neurons discharge, abnormal in ascending as well as descending tract of corticospinal pathways.[6] Seizure can be controlled with current therapies however the treatment option which are available now a days for treating seizure is only preventive not curable. Anti-seizure drugs have low neuroprotective activity or their side effects which are the outcomes of long therapy overcome their therapeutic benefits. Therefore considerations now focus on neuroprotective effects of various components. Considerable numbers of clinical and epidemiological studies reveal that about one third of adult patients (20-30%) are

suffering from epilepsy but they do not respond to drug therapy or surgical treatment hence come under resistant type epilepsy. Anything that disturbs the normal homeostasis or stability of neurons can trigger hyper excitability and seizures.[7]

Experimental animal models are beings used in order to explore basic mechanisms underlying epilepsy and to invent new anticonvulsant drugs, since intracellular recording of intact human brain. There are many etiological factors like liver disease[8] chronic injury at central nervous system due to severe glucose deficiency, major electrolyte disturbance, severe hypokalamia and other medical condition may involve in the epilepsy like genetic mutations, chromatic brain injury,[9] intracerebral artery pressure elevation due to high blood pressure, genetic predisposition to seizures has been seen in many ways of primary generalized epilepsy. Mental retardation of patient, cerebral trauma, necrotic plaques in the CNS, or strokes are at an enhance risk tool for seizures. Mental retardation frequently has been determined by the intelligence quotient.[10] In the elderly, the cause of focal neuronal injury is typically depending on stroke, neurodegenerative disorders. For example Alzheimer's disease and other conditions like excitotoxity in neuronal circuit many neurotransmitters are involve in abnormal function of the brain. With over 10 billion of neurons and an estimated connection of synapse and axonal transportation in the human brain basis for uncontrolled automaticity complexity unlike myocardial tissue where electrical signal spared through a syncytium of cells proper functioning of the brain requires distinct isolation electrical signal and thus demand of a fighter level of regulation.[10] A seizure can present with a variety of symptoms and result from of a various cause.

Pentylenetetrazole come under the categories as CNS stimulant epileptogenic properties have been used to find out antiseizure phenomenon of herbal drugs with high efficacy and to identify pharmaceuticals entities that may control seizure susceptibility. basic mechanism of PTZ is that a non-competitive antagonize by GABA, the antagonize action of PTZ on inhibitory neurotransmitter as GABA can lead excitotxicity of neuronal cells and spontaneously depolarization of after administration of PTZ rats are observed for seizure episode ranking and determine of latencies and onset of convulsion and duration of convulsion as well as general tonic clonic seizure was determined with the help of PTZ.[11]

F. sarmentosa is common, straggling on rocks or higher trees, nearly shady moist ravines, to 2500m. Leaves provide good fodder fruits edibles bark powder is taken to cure boils and secrete more milk during delivery. Root extract is used in malaria.[12] Bark is also used for

bone fracture.[13] however leaf extract is used as antiepilepsy on the basis of traditional justification. In spite of the numerous medicinal uses attributed to this plant; however, there is no Pharmacognostical report on the leaf or petiol of the plant to determine the anatomical and other physicochemical standards required for quality control of the crude drug. Hence, the present investigation includes morphological and anatomical evaluation, determination of physicochemical constants and the preliminary phytochemical screening of the different extracts of *F. sarmentosa* on the basis of Pharmacognostical evaluation may help to determine pharmacological justification.

MATERIALS AND METHODS

Plant material

The plant was collected in the month of November and December 2014 from the Forest of Nagdhar Chamoli Dist. Uttrakhand India. Plant was identified and authenticated from Botanical Survey of India, Dehradun. The specimen no is 1127805.

Drugs, Chemicals and reagent

Phenytoin**,** Diazepam and PTZ was purchased from Yarrow Chem product Mumbai, India. Solvent and other chemical and reagent was used of analytical grade.

Physicochemical Examination

Physicochemical values such as percentage of ash values and extractive values and loss on drying were performed according to the official methods.[7-8] Fluorescence characteristics of the powder of drug was examined under ultra-violet light according to the methods suggested by Kokoski et al.[14] Behavior of the powder of drug with different reagents observed under ordinary light and UV-radiation according to the methods suggested by Chase, C.R. et al.[15]

Determination of macroscopic characteristics

Soon after authentication, the plant leaves was dried at room temperature, until they were free from moisture and subjected to physical evaluation with different parameters. The parameters used for screening were nature, colour, odour, taste, size, shape, width and length. Hence finally all the parts were subjected to reducing size to get coarse powder and then passed through sieve no.40 to get powder uniformity. Powder was subjected to standardization with different parameters as per Indian Pharmacopoeias/literatures.[16]

Determination of microscopic characteristics

The microscopy of the plant was studied according to the method of Brain and Turner.[17] In the microscopical study, cross sections were prepared and stained as per the procedure of Johansen.[18] powder analysis and leaf constant was done according to the official method.

Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out by using standard procedures described by Kokate[19] and Harborne.[20]

pH Determination

pH of 1% and 10% solution of powdered aerial parts of *Ficus sarmentosa* was determined using a standardized glass electrode pH meter and adjust pH accordingly.

Foreign Matter Analysis

A sample of plant material was weighed. It was spread in a thin layer and foreign matter was sorted into groups either by visual inspection, using a magnifying lens or with the help of a suitable sieve according to the requirements. The remainder of the sample was sifted through a sieve no. 250; dust was regarded as mineral admixture. The portion of this sorted foreign matter was weighed. The content of each group was calculated in grams per 100g of air dried sample.[21]

Swelling Index and Foaming Index

Swelling Index and Foaming index were determined as per the methods prescribed in WHO guidelines.[22]

Preparation of ethanolic extract

The leaves of plant shade dried at room temperature and extracted with ethanol 95% v/v for 48 hrs using hot soxhlation method and extract was dried at 50 ^{0}C on water bath the percentage yield formula was adopted from (Gakunga *et al*. 2014).[23] The formula is Percentage yield = (M2/M1) X 100; where M2 is the mass of the semi-solid portion of ethanol extract and M1 is the mass of dried leaves prior to extraction. M2 = 40.79 g and M1 = 620.13 g. Substituting the given values: Percentage yield = (81.23 g /720.12 g) X 100 = 11.28%.

Animal

Wistar albino rats (120-180) of either sex obtained from, GRD (PG) IMT Rajpur Road Dehradun. Rats are acclimatize for 10 days, under a room temperature of 24±2^{0}c relative humitidity 45-55^{0}c with 12:12 hrs light and dark cycle. The animals had free accesses to food (Ashirivad food industry Mohali, Chandigarh) and water ad lebitum. The animals had habituated to laboratory condition for 48 hrs prior to the experimental protocol to minimize the non specific stress. The institutional animal ethics committee of GRD (PG) IMT Rajpur Road Dehradun, Uttarakhand, India, approved the experimental protocol in accordance with the guideline provided by committee for purpose of control and supervision of experimental on animals (CPCSEA) with the registration no 1145/a/07/CPCSEA/2011/6.

LD_{50} Determination

Acute toxicity studies, Healthy Wistar albino rats of either sex weighing 120-180 g maintained under standard laboratory conditions were used for acute oral toxicity test according to Organization for Economic Cooperation and Development guidelines 423. Animals were observed individually at least once during first 30 min after dosing, periodically during first 24 h (with special attention during the first 4 h) and daily thereafter for period of 3 days (OECD, 423). Observations were done daily for changes in skin and fur, eyes, mucus membrane (nasal), respiratory rate, circulatory signs (heart rate), autonomic effect (salivation, lacrimation, perspiration, urinary incontinence and defecation) and central nervous system (drowsiness, gait, tremors and convulsion) changes. The leaf extract of plant *F. sarmentosa* at a dose of 2 gm/kg body weight was given to 6 animals and was continuously observed for 14 days for mortality and general behavior. No deaths were observed till the end of this study. The plant extract was considered to be safe up to a dose of 2 gm/kg body weight. From these results, test drug dose of 100, 200 & 300 mg/kg body weight was chosen for the efficacious studies.[24]

Experimental design

Effect on Maximal electroshocks (MES) induced seizures

Albino wistar rats of either sex weighing 120 to 180 gm were divided into five groups of six animals each. The first group received vehicle control (normal saline 1ml/100 g) whereas Group-II received standard drug Phenytoin, 25mg/kg ip, Group-III, IV and V received ethanolic extract of *F. sarmentosa (EEFS)* 100, 200 and 300 mg/kg body weight p.o. respectively for 7 days. On the 7th day, Seizures are induced to all the groups by using an

Electro convulsiometer. Maximal electroshock seizures were elicited by a 60 Hz alternating current of 150 mA intensity for 0.2 sec. A drop of electrolyte solution (0.9% NaCl) with lignocaine was applied to the corneal electrodes prior to application to the rats. This increases the contact and reduces the incidence of fatalities. The duration of various phases of epilepsy were observed. The percentage protection was estimated by observing the number of animals showing abolition of Hindleg Tonic Extension (or) extension not greater than 90°.[25]

Effect on Pentylenetetrazole (PTZ) induced seizures

Albino wistar rats of either sex weighing 160 to 220 gm were divided into five groups of six animals each. The first group received vehicle control (normal saline 1ml/100 g) whereas Group-II received standard drug Diazepam, 4mg/kg intraperitoneally, Group-III and IV, and test doses of ethanolic extract of *F. sarmentosa (EEFS)* 100, 200 and 300 mg/kg body weight P/O respectively for 7 days. On the 7th day, Pentylenetetrazole (PTZ) 110 mg/kg body weight IP was administered to all the groups to induce general tonic-clonic convulsions as well as myoclonic seizures. Animals were observed for a period of 30 min post PTZ administration. These animals were also observed for the next 72 hours for mortality. The parameters noted were mean onset time of convulsions, duration of convulsion and recovery/Death (% recovery or % of survival) due to PTZ (Hosseinzadeh and Sadeghnia 2007).[26]

Statistical Analysis

In animal study, the data are expressed as mean ± SD. For statistical analysis data was subjected to analysis of variance (ANOVA) by using Graph Pad Instat. Values are considered statistically significant **P<0.01 (n=6).

RESULTS

In the present study *Ficus sarmentosa* was selected on the basis of literature survey and traditional uses from the local area of Chamoli (Garhwal) it was observed that all the plants were extensively used in the treatment of variety of diseases.

Swelling Index and Foaming Index

Swelling Index and Foaming index were determined as per the methods prescribed in WHO guidelines the swelling index and foaming index shown in Table no.1.

Table 1: Observation of Morphological Characters of Ficus sarmentosa leaves.

S.NO.	Parameters	Ficus sarmentosa
1	Shape/Texture	Elliptic-ovate/ glabrous
2	Colour	Dark green
3	Odour	Characteristic
4	Taste	Characteristic
5	Length	6-12cm
6	Width	3-5cm

Leaf Macroscopic characteristics

The macroscopy of leaf revealed that leaf is compound 6-12 cm long 3-5 cm broad, colour dark green, divergent venationand characteristic odour and taste (figure 1 (A)) results of organoleptic observations are presented in Table 2.

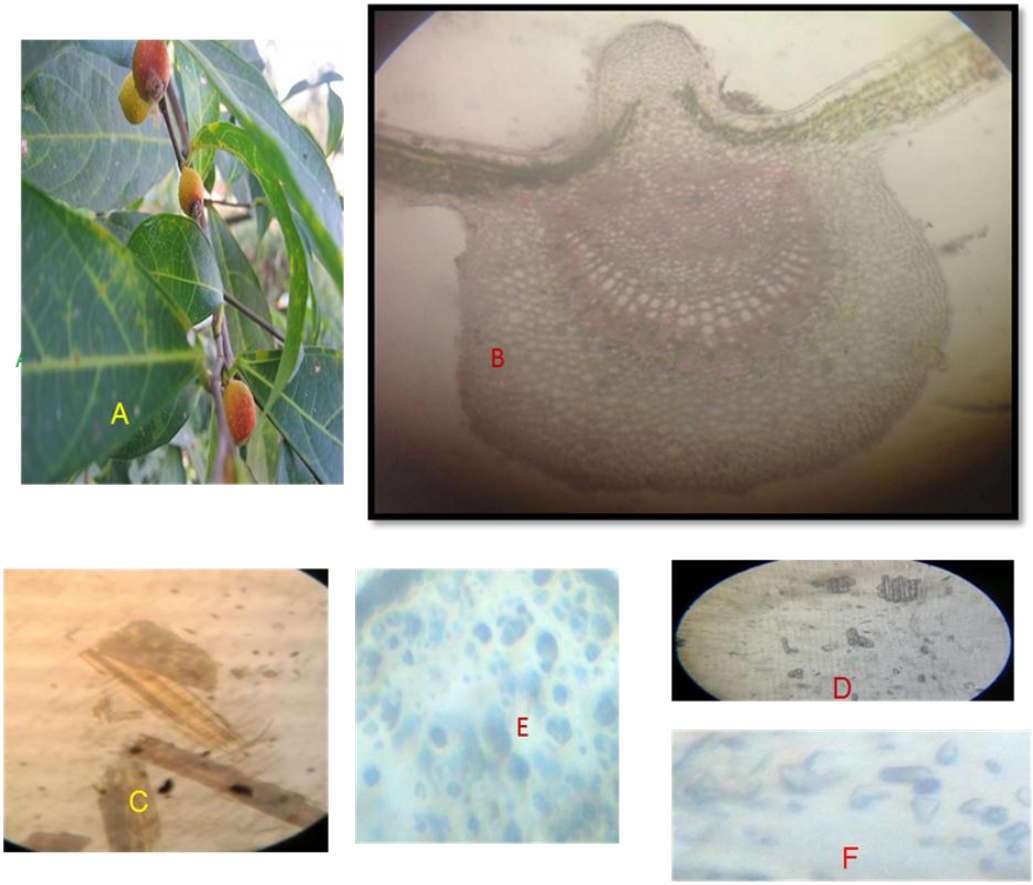

Figure 1: (A)- A young plant of Ficus sarmentosa. (B)- T.S. of *Ficus sarmentosa* leaf. (C)- Lignified fibers. (D)- Stomata. (E)- Starch grains. (F)- Calcium oxalate crystals.

Physical Parameters

The study of various physicochemical parameters of the aerial parts of *Ficus sarmentosa* Total ash, acid insoluble ash, extractive values was carried out and the result are as shown in

Table 2. The loss on drying at 105°C was found to be 4.715%w/w which gives an idea about the likely deterioration time for the crude drug. The amount of earthy materials and minerals accompanying the crude drug as indicated by the total ash was 4.214%w/w while the siliceous matter i.e. acid insoluble ash content was 1.138%w/w.

Preliminary phytochemical study

The preliminary phytochemical tests have confirmed the presence of a wide range of chemical constituents including secondary metabolites as alkaloids, glycosides, and phenolic compounds likely to be responsible for the therapeutic effects thus justifying its traditional use. In order to identify the chemical composition of various extracts preliminary qualitative analysis was carried out. These results are summarized in Table 3. It was observed that extracts of *F. sarmentosa* leaves showed the presence of sterol, steroidal glycosides, triterpenoids, carbohydrates and free amino acids.

Behavior of powder with chemical reagents

Behavior of leaf powder with different chemical reagents was studied to detect the presence of phytoconstituents with color changes under daylight by reported method[22] and the results were shown in Table 4. The powder was subject to fluorescence analysis as per the standard procedure and shown in Table 5.

Table 2: Physical Parameters.

S.NO.	Parameters	Values		
1	Foreign matter analysis	0.80%w/w		
2	Loss on drying	4.715%w/w		
3	Ash values A. Total ash B. Acid insoluble ash C. Water soluble ash	4.214 %w/w 1.138%w/w 0.80%w/w		
4	Extractive values	Cold maceration(%w/w)	Hot extraction(%w/w)	Successive extraction (%w/w)
	A. Petroleum ether	2.56	4.90	5.21
	B. Ethyl acetate	4.66	8.97	15.25
	C. Methanol	8.25	16.12	18.90
	D. Aqueous	45.20	28.25	34.59
5	Crude fibre content	4.29%w/w		
6	Swelling iindex	Absent		
7	Foaming index	Less than 100		
8	pH determination A. 1% solution	8.20		
	B. 10% solution	11.5		

Table 3: An observation of phytochemical screening of Ficus sarmentosa leaves extracts.

Extract constituents	TEST	PETROLIUM ETHER	ETHYL ACETATE	METHANOL	AQUEUOS
Test for sterols	Salkowski'stest	+	-	+	-
	Libermann-Buchardt's test	+	-	+	-
Test for steroidal glycosides		+	-	-	+
Test for triterpenoids		-	+	-	-
Test for saponins	Foam test	-	-	+	-
	Haemolytic test	-	-	+	-
Test for flavonoids	Alkaline reagent Test	-	-	+	-
	Shinoda test	-	-	+	-
Test for carbohydrates	Molisch's test	-	+	+	+
	Fehling's test	-	+	+	+
Test for alkaloids	Mayer's test	-	-	-	+
	Dragendorff's test	-	-	-	+
	Hager's test	-	-	+	+
	Wagner's test	-	-	-	+
Test for tannins and phenols	Lead acetate Test	-	+	+	+
	Ferric chloride test	-	+	+	+
Test for proteins	Xanthoproteic Test	-	-	-	-
	Millon's Test	-	-	-	-
	Biuret test	-	-	-	-
Test for free amino acids	Ninhydrin test	-	-	-	-

(+) Present; (-) Absent

Extractive values

The extracts were prepared according to the polarity and they were concentrated and their values were calculated with reference to air dried drug. The percentage yield obtained for each solvent is tabulated in Table 6. The highest yield was obtained from aqueous solvent and lowest yield was obtained from petroleum ether.

Quantitative Analysis

The fresh leaf samples were subjected to quantitative analysis for various leaf constants like stomatal number, stomatal index, vein islet number, vein termination number, palisade ratio. The results are shown in Table 7.

Microscopical characteristics of *Ficus sarmentosa* leaf

The transverse section of leaf was prepared by standard method. Slides of powdered leaf material were also prepared and studied. The slides of T.S. of leaf of the plant were prepared and subjected to microscopical examination. The section view of the leaf show following part:-

The T.S. of (figure 1 (B)) midrib shows dorsiventral structure and a distinct biconvex out line in the basal and middle regions where as in the apical region become Plano convex. The T.S. show single layered epidermis covered with distinct charterstic cuticle. Epidermal cells of the ventral side and dorsal side are more or less round to oval in shape and distinct thinking on radial walls. Beneath the epidermal cells on both the sides the layers of collenchymatous cells is wider towards the ventral side and more or less double in width that of dorsal side. However, the collenchymatous cells on the dorsal side are bigger in size. All the collenchyamtous cells show distinct angular thinking on their walls which becomes lesser towards the central region. The collenchyma is followed by parenchyma both on dorsal as well as ventral side. The parenchymatous cells are mostly round or oval in shape but the parenchymatous cells on dorsal side have distinctly bigger space as compared to ventral side. On the dorsal side beneath the parenchyma there is a discontinuous layer of fibers with intervening parenchymatous cells. The T.S. of lamina shows a single layered epidermis composed of either tangentially or radial elongated cells. The epidermal cells of the ventral sides are distinctly bigger than the epidermal cells of ventral side. Large numbers of epidermal cells get modified into stomata on both dorsal and ventral side which is usually paracytic. Epidermis on dorsal and ventral side is covered with distinct striated cuticle. The epidermal trichomes extend and forms covering trichomes which are similar to the tirchomes described in mid-rib and the distribution pattern is also similar as described in case of mid-rib. Epidermis on the ventral side is followed by a single layer of palisade cells which are long and rod shaped but their size gradually reduces towards the margin of leaf where they appear like the parenchymatous cells in both shape and size. The palisade cells are followed by 3-5 layers of spongy parenchyma which are usually round to oval in shape showing large spaces.

Table 4: Behavior of leaf powder Ficus sarmentosa with different reagents observed under ordinary light and UV-radiation.

Interaction of powder drug with different reagent	Colour produced under ordinary light	Colour under UV-radiation	
		254nm	366nm
Drug (P)as such	Green	Green	Black
P +Fecl3	Black	Black	Black
P+1N HCL	Brown	Green	Black
P+conc. H2SO4	Dark brown	Green	Dark brown
P+iodine solution	Brown	Green	Black
P+picric acid	Yellowish	Light green	Dark green
P+alcohol (50%)	Green	Green	Black
P+acetic acid	Light green	Light green	Dark green
P+lead acetate (5%)	Light brown	Green	Dark green
P+HNO3	Reddish brown	Light green	Black

Table 5: Fluorescence characteristics of leaf powder of Ficus sarmentosa leaf powder.

Treatment	Fluorescence observed
Powder as such	Greenish
Powder treated with methanol	Greenish
Powder treated with nitrocellulose in amyl acetate	Green
Powder treated with1N Hcl in methanol	Green
Powder treated with1N sodium hydroxide in methanol	Dark brown
Powder treated with 50% sulphuric acid	Dark brown
Powder treated with 50% nitric acid	Reddish brown

Characteristics of Leaf Powder

The powered characteristics of leaf revealed (figure1(C, D, E & F)) the presence of starch grains, calcium oxalate crystal, stomata and lignified fibers shown when treated with Phloroglucinol.

Table 6: The percentage yield of plants extracts.

S.NO	Plant	Solvent	% yield
1	Ficus sarmentosa	Petroleum ether	2.00
2		Ehtyl acetate	3.70
3		Mthanol	7.28
4		Etanol	14.27

Table 7: Leaf constants of F.sarmentosa (in average).

Parameters	Ficus sarmentosa		
Stomatal number	Apex	Middle	Base
Upper epidermis	7-10	34-45	10-19
Lower epidermis	35-55	60-90	13-25
Stomatal index			
Upper epidermis	34.5-54.3	60.43-78.34	45.35-70.50
Lower epidermis	40.0-67.6	60.56-65.0	50.0-58.9
Vein- islet number	12.14	21.50	13.20
Vein- termination number	8-11	10-13	9-13
Palisade ratio	1:3	1:5	1:7

Antiepileptic Potential

Effect of EEFS on Maximal electroshocks (MES)

Effects of EEFS on MES Induced Epilepsy at doses of 300, 400 and 500 mg/kg were protect animals from seizures and significantly ($p<0.01$) reduced the duration of tonic hindleg extension. With dose and concentration dependent manner whereas the standard drug phenytoin treated animals' exhibits abolished tonic hindleg extension. Phenytoin treated animals have shown 100% protection against MES induced seizures whereas EEFS 100 mg/kg, 200 mg/kg and 300 mg/kg have shown 49.8%, 66.4% and 83% protection respectively shown in table no 8.

Table 8: Effect of ethanolic extract of *F. sarmentosa* (EEFS) On MES induced seizers in rats.

Treatment	Flexion	Extensor	Clonus	Stupor	Recovery	%Protection
Vehicle 1ml/100gm	9.2±0.15	17.42±0.51	42.79±0.32	42.52±0.11	202.36	0%
Phenytoin 25mg/kg	4.5±0.22	1.2±0.32	16.69±0.21**	16.12±0.22**	89.28	100%
EEFS 100mg/kg	8.5±0.36	7.2±0.13	38.61±0.34**	34.26±0.25*	165.59	49.8%
EEFS 200mg/kg	7.8±0.36	5.21±0.32	28.56±0.22	28.52±0.14	139.85	66.4%
EEFS 300mg/kg	5.6±0.20	3.11±0.39	19.62±0.15**	19.72±0.18**	118.36	83%

Statistical significance was determined by one way ANOVA followed by dunnett test values are expressed as mean ± SD, n=6 $p<0.05$** as compared with normal control group: $p<0.01$ ** as compared with group II, III, IV and V respectively.

Effect of EEFS on Pentylenetetrazole (PTZ) Induced epilepsy

Intraperitoneal administration of lower dose of ethanolic extract of *F. sarmentosa* (100 mg/kg) had no potent effects on generalized tonic-clonic seizures (GTCS), while injection of 200 & 300 mg/kg caused significant increase in GTCS latencies ($p<0.01$). In this study diazepam, (4 mg/kg) 1 hrs prior to PTZ, significantly increased GTCS latency. Diazepam

treated animals have shown 100% protection against PTZ induced seizures whereas EEFS 100 mg/kg 76.6%, 200 and 300 mg/kg have shown 100% protection respectively shown in table no 9.

Table No. 9: Effect of ethanolic extract of EEFS in Pentylenetetrazole induced convulsions in rats.

Treatment	Dose mg/kg	No of Animal Exhibiting seizure	Onset of Convulsion (GTCS)	Duration of Convulsion	Animal Mortality	Protection against seizure
Vehicle +PTZ	1 ml/10g	6	39.5±7.61	139±40.3	5	16.6%
Diazepam +PTZ	4mg/kg	6	513.5±48.72	32.3±7.9*	0	100%
EEFS +PTZ	100mg/kg	6	269.16±24.71**	83.3±17.04	2	66.4%
EEFS +PTZ	200mg/kg	6	297.16±60.82**	78.66±16.51**	0	100%
EEFS +PTZ	300mg/kg	6	428.53±56.88**	48.5±11.041*	0	100%

Statistical significance was determined by one way ANOVA followed by Dennett test values are expressed as mean ± SD, n=6 **p<0.05 as compared with normal control group**p<0.01 as compared with PTZ group.

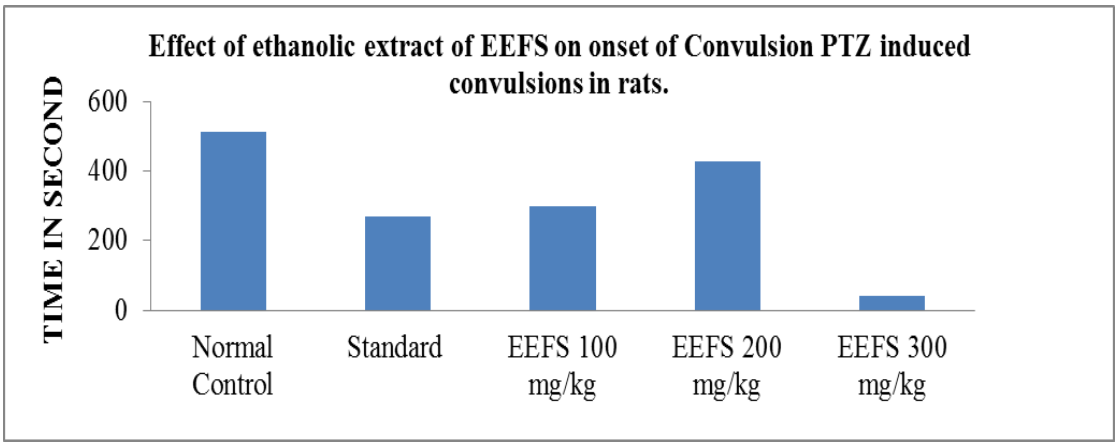

Figure no. 2: Effect of Ethanolic extract of EEFS on onset of convulsion PTZ induced convulsions in rats.

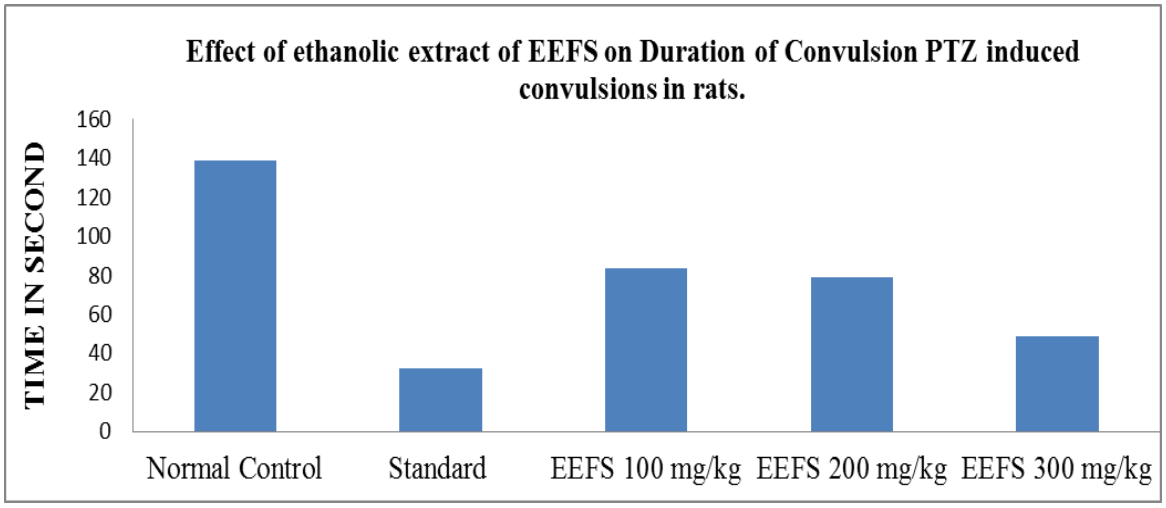

Figure no. 3: Effect of Ethanolic extract of EEFS on onset of convulsion PTZ induced convulsions in rats.

DISCUSSION

From the study, important diagnostic characters that might be useful in determining authenticity and identifying adulteration of the crude drug are observed. ***Ficus sarmentosa*** belonging to the family Moraceae is evergreen shurb, leaves alternate, ovate-elliptic to oblong, smaller on creeping branches, larger in fruting branches. The moisture content of 0.8%, which is within the recommended range of 8–14% for vegetable drug was an indication that the plant can be stored for a long period of time with less probability of microbial attack. The water extractive value of 34.59% showed that water permeates the cells of the leaves and thus, a better extracting compared to alcohol with extractive value of 18.90%. Total ash of 4.214% was low implying that the crude plant has low inorganic components. The aim of ashing was to remove all traces of organic matter.[27] The total ash value can be used to detect foreign organic matter and adulteration with sand and earth, therefore, reflecting the kind of care that must be taken in preparing the plant for drug. The T. S. of midrib shows dorsoventral structure and a distinct biconvex out line in the basal and middle regions where as in the apical region becomes Plano convex. All the parts were subjected to size reduction to get coarse powder and then passed through sieve no. 40 to get uniform powder. Then the uniform powder was subjected to standardization with different parameters as per Indian Pharmacopoeias. Phytochemical investigation revealed the presence of different chemical constituents like steroids, saponins, alkaloids, proteins, sugars, flavonoids, tannins, phenols, amino acids and steroidal glycosides in different plant extracts. Different types of Antiepileptic drugs are available so far treat any kind of epilepsy like general Tonic-Clonic seizure Atonic[28] etc. but there are many side effect and contraindication, so there is always a

strong need for minimization of ADR and maximization of therapeutic benefit. Some time we are fail to manage epilepsy so we can use herbal medicine to manage any type of convulsion without any side effect. The MES model is the well definite screening method to find out or identified anticonvulsant activity of drugs for the generalized tonic-clonic seizures as well as myoclonic seizure This method is based on observation of the excitation by recurrent and spontaneously electrical impulses induced by different neuronal circuit one characteristics standard of convulsion activity In our recent study, it is found that rats are treated with EEFS significantly reduces in tonic hindleg extensor stage in MES induced epilepsy. Since, EEFS significantly prohibited generalized tonic-clonic seizures in MES method our result demonstrate that *Ficus sarmentosa* have shows dose dependently affect on epilepsy using PTZ. The phytochemical investigated that different constituent found in the EEFS which shows antiepileptic activity and another neuroprotective.

CONCLUSION

As there is no pharmacognostic / anatomical work record of this much valued traditional drug, the present work was taken up with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. In other words, the pharmacognostic features examined in the present study may serve as tool for identification of the plant for validation of the raw material and for standardization of its formulations So it can be concluded that the ethanolic extract of whole plant of *Ficus sarmentosa* have shown significant antiepileptic potential with minimal toxicity However further study will need in human beings to explore best results.

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