International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 158-170 www.ijprajournal.com ISSN: 2249-7781

Anti-Convulsant Activity of Ethanolic Leaf Extract of Euphorbia milii Des moul using rodent models

N. Vedika *, Dr. M. Sandhya Rani**.
**Student, Department of pharmacognosy, Centre of pharmaceutical sciences, University College of Engineering, Science and Technology, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad.*
*** Centre of pharmaceutical sciences, University College of Engineering, Science and Technology, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad.*

Submitted: 25-12-2023 Accepted: 05-01-2024

ABSTRACT
The existence of natural chemicals in medicinal plants makes them a key source of molecules with therapeutic characteristics. Medicinal plants are beneficial in treating human ailments and have a significant impact on healing because they contain phytochemical elements. The use of complementary and traditional medicine is widespread worldwide. In several Asian and African nations, traditional medicine provides primary treatment to about 80% of the population. The oldest continuously used traditional medicine is still Ayurveda. Nowadays, nutraceuticals are regarded as cutting-edge medications with established health advantages.People with epilepsy and those who care for them may experience psychological and emotional effects from this prevalent medical condition. Around 80 percent of individuals with epilepsy (PWE) reside in developing nations, where there are 40 to 70 new instances of epilepsy for every 100,000 persons in the general population each year. Approximately 65 million people worldwide suffer from epilepsy. Thus, the current study's research focused on the ethanolic extract of Euphorbia milii des moul leaves' anti-convulsive activity (EEEM). Pentylenetetrazole-induced convulsions and in-vivo maximal electroshock models were investigated for EEEM at varying dosages (200 mg/kg, 400 mg/kg), and the results were compared with the efficacy of standard medication phenytoin (25 mg/kg). At a 400 mg/kg dose, EEEM reduced convulsions. This study may be helpful as a baseline for future research on the anti-convulsive properties of Euphorbia milii.

Keywords: Nutraceuticals, Anti-convulsion, Euphorbia milii des moul, Phenytoin.

I. INTRODUCTION

In order to treat, diagnose, and prevent illnesses or maintain well-being, traditional medicine refers to health practices, approaches, knowledge, and beliefs that include manual techniques and exercises, spiritual therapies, and medicines derived from plants, animals, and minerals (WHO 2003). These practices can be used alone or in combination. Up to 80% of people in Africa receive their main medical care from traditional practitioners, and the worldwide market for herbal medicines is currently valued at more than US $60 billion yearly and is expanding significantly (WHO 2003). Ethnopharmacological investigations remain the most successful way for finding medicinal plants nowadays (1,2).

There seems to be a limit to the amount of progress that can be made in the development of novel pharmaceuticals using only contemporary technologies. Since the 1980s, the pharmaceutical industry has tended to use combinatorial chemistry and high-throughput synthesis in the creation of novel medications; nevertheless, despite significant efforts in this direction, the anticipated level of drug productivity has not been achieved. It is extremely difficult for certain big pharmaceutical businesses to create new medications. In light of this, natural products have received more attention over the past twelve years while looking for innovative medications in conjunction with cutting-edge technologies like high-throughput selection (4,5). Due to their distinct chemical variety, which has developed over millions of years, natural products have a wide range of biological activity and drug-like qualities. These goods are now among the most crucial tools for creating novel scaffolds and lead compounds. Natural goods will be used continuously to address the pressing need to create efficient medications, and they will take the lead in the development of medications for treating human illnesses, particularly serious illnesses (6).

Herbal medications are made up of active substances, plant parts, or plant materials in their raw or processed form along with some excipients, such as solvents, preservatives, or dilutions (7,8).

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 158-170 www.ijprajournal.com ISSN: 2249-7781

These active components give plants their scent, flavour, and colour while shielding them from harm and disease. Scientifically speaking, they are referred to as phytochemicals and fall into a number of groups, including alkaloids, terpenoids, glycosides, saponins, and flavonoids (2009). The study of identifying the therapeutic or medical properties of herbs or herbal products is known as herbal medicine, or herbalism.Although they can be made from any part of the plant, the most common ones are roots, leaves, flowers, bark, and seeds. They can be administered directly to the skin, swallowed, breathed, or taken orally. A variety of naturally occurring phytochemicals are commonly found in herbal products, many of which support the medicinal qualities of the plant(11). Ayurvedic herbal medicine (derived from the Sanskrit phrase Ayurveda, which means "the science of life") started about 5000 years ago in India and spread to nearby nations like Sri Lanka. Herbal medicine is often divided into a few basic categories.

- In China, herbal medicine is a branch of traditional oriental medicine.
- African herbal remedies.
- The Western World's use of herbs, which began in classical Greece and Rome and subsequently spread to South America, Europe, and North America .

The hallmark of epilepsy, a chronic neurological condition or cluster of disorders, is the recurrent (two or more) epileptic seizures that typically occur randomly in the absence of triggers. A clinical manifestation associated with an aberrant and excessive discharge from a group of neurons in a particular brain region is known as an epileptic seizure. This clinical manifestation is characterized by abrupt and transient aberrant occurrences that can include changes in motor, sensory, autonomic, mental, or altered states of consciousness [1]. Seizures, which are defined by an excessive, aberrant release of neurotransmitters from cortical neurons, are not the same as epilepsy. Concerns regarding epilepsy frequently include loss of consciousness, disruption of the sensory motor system, personal goals, and health.

Herbal medicine is defined by the World Health Organization (WHO) as the use of herbs, herbal materials, herbal preparations, and completed herbal products that have plant parts, other plant materials, or combinations of plant materials as active ingredients [9]. Plant parts like leaves, stems, blossoms, roots, and seeds are the source of these herbs [10].

Euphorbia milii Des Moul is an evergreen shrub that grows to a height of 60 to 90 cm. It is scrambling and has many branches. It grows best in full sun on well-drained, dry to slightly damp soil.

In the winter, it is susceptible to temperatures below 350F. Enjoys some midday shade in hot summer climates. Even though Euphorbia milii Des Moul can withstand drought and poor soil conditions, particularly rocky-sandy soils, regular treatments of mild hydration may promote bloom and reduce leaf drop. Particularly in the winter, wet soils can be fatal. It functions best in areas with sufficient airflow. Indoor plants grow best in potting mixes made of coarse dirt and require a lot of light. Proliferate from tip cuts. The common name Euphorbia milii Des moul refers to the belief of some that the stems of this plant were used to form the crown of thorns that Jesus Christ wore during his crucifixion.(15)

GEOGRAPHICAL DISTRIBUTION

A blooming plant native to the Inselberg region of Madagascar's Central Plateau, Africa is called Euphorbia milii Des Moul. It is grown and naturalised in Europe, Africa, Asia, South America, North America, and the Caribbean. It has become widely popular as an ornamental in a range of tropical and subtropical locations. Euphorbia milii Des Moul is a decorative plant that is imported and widely distributed over the globe (16). India's Assam, Gujarat, Kerala, Maharashtra, Manipur, and Tamil Nadu are the main growing regions for it (17). In traditional medicine, Euphorbia milii Des Moul is commonly used to treat trichiasis, hepatitis, cancer, and warts (in southern Brazil) (20). The seeds are used as a kid laxative, the whole plant paste is applied on displaced animal bones, and the leaves are used to treat ringworm and snake bites. Oral administration of Euphorbia milii Des Moul flower powder and whole plant ash, at dosages of 500 mg three times day and 250–500 mg twice daily, respectively, is useful to treat asthma (18). Numerous other medical conditions can be treated using Euphorbia species, such as blood disorders, genitourinary syndromes, microbiological infections, scorpion stings, pregnancy and puerperium, and sensory impairments. The formulations are utilised as skin remedies for their emollient, antiseptic, disinfecting, and wart-relieving properties, as well as for dermatitis, acne, sunburn, boils, rashes, and irritation (21). It has been shown that the undiluted latex of Euphorbia milii Des Moul irritates mammals' eyes and skin. Compared to other closely related ingenol and

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 158-170 www.ijprajournal.com ISSN: 2249-7781

phorbol derivatives, many diterpene esters of ingenol have less propensity to cause tumours, despite being potent skin irritants. It was discovered that milli amines extracted from Euphorbia milii Des Moul latex were extremely molluscicidal (22).

II. MATERIALS AND METHODS

Collection and identification of plant materials

In Hyderabad, Telangana, India, on the campus of JNTUH university, Euphorbia milii Des moul were collected. The plant, which has voucher number OUAS-97 and is a member of the Euphorbiaceae family, was morphologically identified and authenticated by Dr. A. Vijaya Bhasker Reddy, assistant professor, Department of Botany. Since this sample contains volatile oils, it was shade dried for two to three weeks. After that, the material was ground into a fine powder and kept for later use in an airtight container.

Chemicals and kits used

Ethanol, Water, Ethyl acetate, Methanol, Phenytoin, Pentylenetetrazole, Sodium chloride

Extraction Procedure

For the soxhlation process, 30g of powdered Euphorbia milii Des Moul leaves were added to a 250ml conical flask holding 200ml of ethanol. After heating the extraction solvent in the flask, the vapours condensed. The filter paper bag with the powdered leaves was filled with the condensed extract by drips. The liquid contents of the chamber syphon were collected into a flask when the liquid level reached the top of the syphon tube. This procedure was kept up until the syphon tube was completely empty. The extract is further filtered, and the filtrate is stored in a rota evaporator to be evaporated.

Experimental animals

Adult Wistar albino rats (180-200 g) and adult Swiss albino mice (20-25 g) were used to test the pharmacological action. They were housed in polypropylene boxes with 12 hours of light and darkness per day, a temperature of 25± 2 °C, and a relative humidity of 45–55%. All of the animals were allowed a week to acclimatise to the lab environment prior to use. They have unrestricted access to water and frequent animal feed. All of the experimental protocols have the permission of the institutional animal ethics committee (IAEC).

Evaluation of Anti-convulsant activity

Maximal Electroshock Induced Convulsions (MES): We bought Wistar albino rats weighing between 150 and 250 grammes. After a week of acclimation, every rat was housed at normal temperature. Animals should be divided into 4 groups, each with 6 animals. Group 1, the control group, received an equivalent volume of normal saline (0.9% Nacl). Oral administration of a low dose (200 mg/kg bd wt) and a high dose (400 mg/kg bd wt) of ethanolic leaf extract of Euphorbia milii was done in Groups 2 and 3. Group 4's animals received the regular medication phenytoin (25 mg/kg bd.wt). All of the rats will receive electroshock using an electroconvulsiometer via ear electrodes at an intensity of 150 mA, 60Hz for 0.2 seconds following the administration of the aforementioned medications for 30 minutes. After then, a number of parameters were noted.(23).

Table 1: Experimental study design for Maximal Electroshock Induced Convulsions (MES)

Groups	Treatment	No.of animals
Group 1	Control(Normal saline)+MES	6
Group 2	EEEM(200 mg/kg bd wt.,p.o)+MES	6

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 158-170 www.ijprajournal.com ISSN: 2249-7781

Group 3	EEEM(400 mg/kg bd wt.,p.o)+MES	6
Group 4	Standard drug phenytoin (25 mg/kg bd wt.,i.p)+MES	6

Pentylenetetrazole Induced Convulsions (PTZ): We bought three-month-old Swiss albino mice weighing 25–30 g. Every mouse was housed at room temperature and given a week to become used to their new surroundings. Group 1 animals were given an equivalent volume of normal saline (0.9% Nacl) as the control group. Ethanolic leaf extract of Euphorbia milii Des moul was given orally to Groups 2 and 3 at low doses (200 mg/kg bd.wt) and high doses (400 mg/kg bd.wt).Group 4 animals received Phenytoin (25 mg/kg bd.wt), a conventional medication. Following the following medications' 60-minute administration, Pentylenetetrazol (PTZ) was given to each animal, and a number of parameters were noted.(23).

Table 2: Experimental study design for Maximal Electroshock Induced Convulsions (MES)

Group	**Treatment**	**No.of animals**
Group 1	Control (Normal saline)+PTZ	6
Group 2	EEEM(200 mg/kg bd wt.,p.o)+PTZ	6
Group 3	EEEM(400 mg/kg bd wt.,p.o)+PTZ	6
Group 4	Standard drug phenytoin (25 mg/kg bd wt.,i.p)+PTZ	6

Statistical Analysis

The standard error of the mean (SEM) was represented as Mean ±. The unpaired student's "t" test was used to determine whether the differences between the treatment and control groups in the experiments were significant. $P<0.05$ values were regarded as statistically significant.

III. RESULTS AND DISCUSSION

Extractive Values

The extraction values of Euphorbia milii Des moul leaves in various solvents are shown in table 3. Using a straightforward maceration method over the course of seven days and three distinct solvents, the extract was made from powdered Euphorbia milii Des moul leaves. Ethanol, methanol, and ethyl acetate were produced in crude extract proportions of 13.2%w/w, 12%w/w, and

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 158-170 www.ijprajournal.com ISSN: 2249-7781

6%w/w, respectively. Consequently, it was discovered that there were greater amounts of ethanol and methanol solvent—13.2g and 12g, respectively. It was suggested that ethanol extract be used in the future.

Table 3: The values of Euphorbia milii Des moul leaves extracted using various solvents.

Nature of extract	**Colour**	**Consistency**	**Extractive values (gm)**
Ethanol soluble	Reddish brown 	Semisolid	13.2%w/w
Methanol soluble	Greenish yellow 	Semisolid	12%w/w

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 158-170 www.ijprajournal.com ISSN: 2249-7781

Ethyl acetate soluble	Yellowish brown 	Semisolid	6%w/w

Preliminary Phytochemical Analysis

Standard analytical methods were used to evaluate the results of the preliminary phytochemical examination. Alkaloids, flavonoids, terpenoids, steroids, and tannins were detected in the leaves of Euphorbia milii Des Moul in ethanolic, methanolic, and ethyl acetate extracts. The presence of different compounds in the solvents ethanol, methanol, and ethyl acetate is determined in Table 4 below.

Table 4: Phytoconstituents in various extracts of Euphorbia milii Des moul.

S.No.	**Tests**		**Ethanol**	**Methanol**	**Ethyl acetate**
1.	Alkaloids				
		a)Dragendroff's test	+	-	-
		b)Hager's test	+	-	-
		c)Wagner's test	+	-	-
		d)Mayer's test	-	-	-
2.	Amino acids	a)Millon's test	-	-	-
		b)Ninhydrin test	-	-	-
3.	Flavonoids	a)Shinoda test	+	+	+
		b)Alkaline reagent test	+	+	+

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 158-170 www.ijprajournal.com ISSN: 2249-7781

4.	Terpenoids and steroids	a)Liebermanns Buchards test b)Salwoski test	+ +	+ +	+ +
5.	Tannins	a) Fecl3 test b) Gelatin test	+ +	+ +	+ -
6.	Carbohydrates	Molisch test	-	-	-

Note :- (+) Indicates presence ; (-) Indicates absence in Leaves of Euphorbia milii Des moul. Tannins, alkaloids, flavonoids, terpenoids, and steroids were all detected in the ethanolic extract. The presence of flavonoids, terpenoids, steroids, and tannins was demonstrated by the methanolic extract. Flavonoids, terpenoids, steroids, and tannins were detected in ethyl acetate extract (Fecl3 test).

GC-MS of Ethanolic leaves extract of Euphorbia milii Des moul.

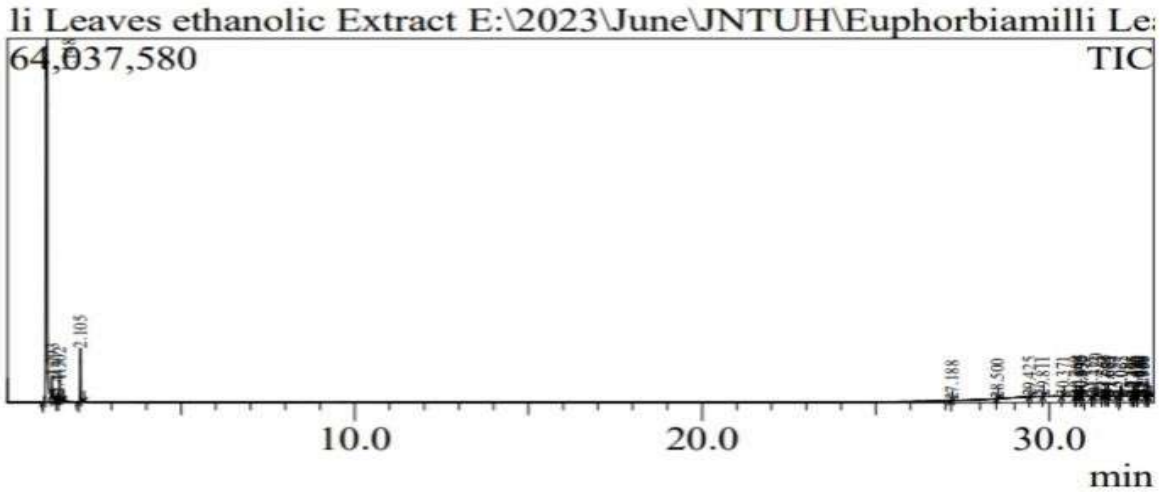
The extract's GC-MS analysis revealed the following (Table 5) bioactive compounds.

CENTRAL ANALYTICAL FACILITY

UNIVERSITY COLLEGE OF TECHNOLOGY
OSMANIA UNIVERSITY

Sample Information

Analyzed by	: B. Sammayya
Analyzed	: 6/22/2023 3:53:43 PM
Sample Name	: Euphoriamilli Leaves ethanolic Extract
Injection Volume	: 1.00
Data File	: E:\2023\June\JNTUH\Euphorbiamilli Leaves ethanolic Extract.QGD
Method File	: C:\GCMSsolution\Data\Project1\GC_MS Fatty acid.qgm
Instrument Model	:GCMSQP2010, SHIMADZU



International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 158-170 www.ijprajournal.com ISSN: 2249-7781

Table 5: Chemical components of EEEM

Peak Report TIC

Peak	R.Time	Area	Area%	Height	A/H	Base m/z	Name
1	1.148	231736892	89.16	63893040	3.63	46.80	Methylsulfidtiole
2	1.293	4411919	1.70	3705707	1.19	59.10	1-Propene, 2-fluoro-
3	1.447	1380022	0.53	1088747	1.27	43.10	Ethyl Acetate
4	1.502	5691310	2.19	3603446	1.58	43.10	1-Propanol, 2-methyl-
5	2.105	11465281	4.41	9386297	1.22	45.10	Ethane, 1,1-diethoxy-
6	27.188	79836	0.03	59495	1.34	57.10	2-Methyldocosane
7	28.500	208730	0.08	104444	2.00	57.10	Docosane
8	29.425	74866	0.03	42575	1.76	281.10	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
9	29.811	59999	0.02	63489	0.95	207.05	Demecolcine
10	30.371	199928	0.08	86478	2.31	207.05	4,4,6a,6b,8a,11,12,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b
11	30.760	108317	0.04	70972	1.53	207.05	1,3-Dioxolane, 2-(2,4-dimethylphenyl)-2,4,5-trimethyl-, (2.alpha.,4.alpha.,5.beta.)-
12	30.845	866581	0.33	252796	3.43	207.00	D:B-Friedo-B':A'-neogammacer-5-en-3-ol, (3.beta.)-
13	30.895	44349	0.02	74947	0.59	281.10	1-Pentene, 1,3-diphenyl-1-(trimethylsilyloxy)-
14	30.936	11219	0.00	30000	0.37	281.10	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
15	31.215	154384	0.06	37588	4.11	281.10	Benzoic acid, 4-[(trimethylsilyl)amino]-, trimethylsilyl ester
16	31.320	1041033	0.40	341466	3.05	123.20	Squalene
17	31.553	792892	0.31	253998	3.12	95.10	1-Cyclohexene-1-butanal, alpha.,2,6,6-tetramethyl-
18	31.664	236347	0.09	104167	2.27	209.00	Heptanedioic acid, 4-(ethoxycarbonylmethylene)-, diethyl ester
19	31.675	39291	0.02	97070	0.40	207.05	Pyridine, 1,2,3,6-tetrahydro-1-methyl-4-[4-chlorophenyl]-
20	31.685	46889	0.02	81610	0.57	207.05	Cyclotetrasiloxane, octamethyl-
21	32.027	268286	0.10	74685	3.59	207.05	1,2-Dihydro-2,4-diphenyl-quinazoline
22	32.065	81203	0.03	47898	1.70	253.05	2-Propenoic acid, 3-(4-phenylphenyl)-, ethyl ester
23	32.395	134684	0.05	108031	1.25	209.05	Acetic acid, 4,4a,6b,8a,11,11,12b,14a-octamethyl-3-oxodocosahydropicen-2-yl ester
24	32.405	87266	0.03	85590	1.02	207.05	Benzoic acid, 4-methyl-2-trimethylsilyloxy-, trimethylsilyl ester
25	32.470	78010	0.03	58628	1.33	281.10	1,3,5,7-Tetraethyl-1-butoxycyclotetrasiloxane
26	32.530	35856	0.01	46141	0.78	207.05	1,2-Dihydro-2,4-diphenyl-quinazoline
27	32.617	195276	0.08	32163	6.07	207.05	Arsenous acid, tris(trimethylsilyl) ester
28	32.760	196921	0.08	69812	2.82	207.05	Silicic acid, diethyl bis(trimethylsilyl) ester
29	32.838	47853	0.02	53624	0.89	207.05	Silicic acid, diethyl bis(trimethylsilyl) ester
30	32.909	135489	0.05	57070	2.37	207.05	Silicic acid, diethyl bis(trimethylsilyl) ester
		259910929	100.00	84011974			

In-Vivo Anti-convulsant Activity

Maximal Electroshock Induced Convulsions (MES):

Rats in the control group experienced 5.5 seconds, 18 seconds, 16 seconds, 43.5 seconds, while rats in the EEEM (200 mg/kg bd.wt) group experienced 4.8 seconds, 13 seconds, 3.5 seconds, and 12 seconds, while rats in the EEEM (400 mg/kg bd.wt) group experienced 3 seconds, 11.6 seconds, 0.8 seconds, 7.6 seconds, and rats in the phenytoin (25 mg/kg bd.wt) group experienced 2.3 seconds, 11.6s, 0 seconds, 6.6 seconds, and all of the groups were recovered. The ethanolic extract of Euphorbia milii Des moul leaf, known as "EEEM (400 mg/kg bd.wt)", shown notable anticonvulsant effect in the current investigation as compared to the EEEM (200 mg/kg bd.wt). The highest anticonvulsant activity of EEEM (400 mg/kg bd.wt) was equivalent to that of phenytoin (25 mg/kg bd.wt), the standard medication. The entire set of findings from this investigation were displayed in the table 6.

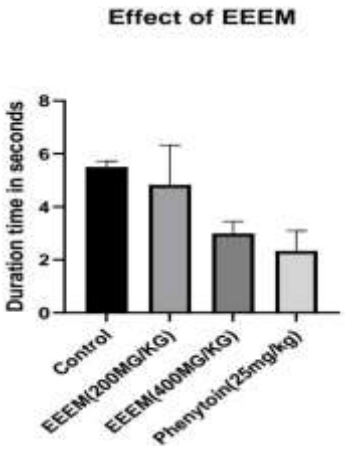
International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 158-170 www.ijprajournal.com ISSN: 2249-7781

Table 6: Effects of EEEM on Maximal Electroshock Induced Convulsions (MES)

S.No.	Treatment	Flexion (sec)	Extension (sec)	Clonus (sec)	Stupor (sec)	Death or Recovery
1.	Control (Normal saline).	5.5±0.204	18±0.408	16±0.408	43.5±0.612	Recovery
2.	EEEM(200 mg/kg bd wt.,p.o)	**,**4.8±1.36 2	**,**13±1.333	**,**3.5±1.64 5	**,**12±5.049	Recovery
3.	EEEM(400 mg/kg bd wt.,p.o)	***,***3±0.408	**,***11.6±2.45 7	***,**0.8±0.7 6 0	***,***7.6±2.54 5	Recovery
4.	Phenytoin (25 mg/kg bd wt.,i.p)	2.3±0.693	11.6±1.953	0±0	6.6±3.849	Recovery

Values are represented as Mean ± SEM (n=6). Statistical analysis performed using one way ANOVA followed by Turkey's multiple comparison test, compared with control *p<0.05, ****p<0.0001 and compared with standard **p<0.0186, ****p<0.0001.

FLEXION **EXTENSION**



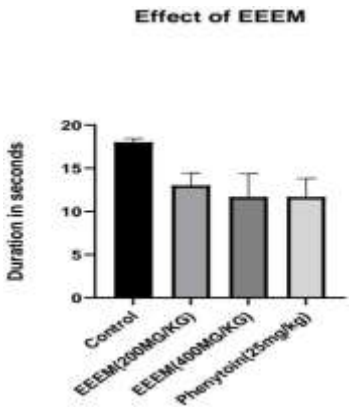
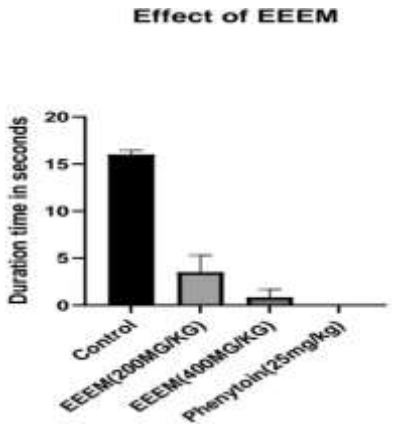


Figure 1: Effect of EEEM showing flexion **Figure 2:** Effect of EEEM showing Extension

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 158-170 www.ijprajournal.com ISSN: 2249-7781

CLONUS **STUPOR**



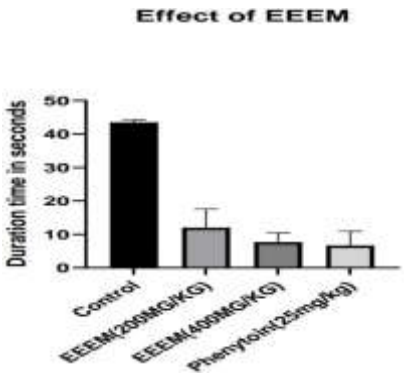


Figure 3: Effect of EEEM showing Clonus. **Figure 4:** Effect of EEEM showing Stupor.

Pentylenetetrazole Induced Convulsions (PTZ)

Using PTZ-induced convulsions in mice, the anticonvulsant activity of ethanolic leaf extract of Euphorbia milii was tested. The duration of the convulsions was 1.95 seconds, 14.04 seconds in the control group, 4.43 seconds, 10.11 seconds in the EEEM (200 mg/kg bd.wt) group, 5.33 seconds, 8.42 seconds in the EEEM (400 mg/kg bd.wt) group, and 5.4 seconds, 8.31 seconds in the phenytoin (25 mg/kg bd.wt) group. All mice were recovered. The ethanolic extract of Euphorbia milii leaf, known as "EEEM (400 mg/kg bd.wt)", shown notable anticonvulsant effect in the current study as compared to the EEEM (200 mg/kg bd.wt). The highest anticonvulsant activity of EEEM (400 mg/kg bd.wt) was equivalent to that of phenytoin (25 mg/kg bd.wt), the standard medication. Table 7 shows all of the findings from this investigation.

Table 7: Effects of EEEM on Pentylenetetrazole Induced Convulsions (PTZ)

S.No.	Treatment	Latency of Convulsions (sec)	Duration of Convulsions (sec)	Death or Recovery
1.	Control (Normal saline).	94.5±0.020	864±0.289	Recovery
2.	EEEM(200 mg/kg bd wt.,p.o)	**,**261±0.164	*,**611±0.063	Recovery
3.	EEEM(400 mg/kg bd wt.,p.o)	***,**303.3±0.138	**,***535.3±0.445	Recovery
4.	Phenytoin (25 mg/kg bd wt.,i.p)	320.6±0.176	524.6±0.409	Recovery

Values are represented as Mean ± SEM (n=6). Statistical analysis performed using one way ANOVA followed by Turkey's multiple comparison test, compared with control *p<0.05, ****p<0.0001 and compared with standard **p<0.0186, ****p<0.0001.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 158-170 www.ijprajournal.com ISSN: 2249-7781

LATENCY OF CONVULSIONS.DURATION OF CONVULSIONS

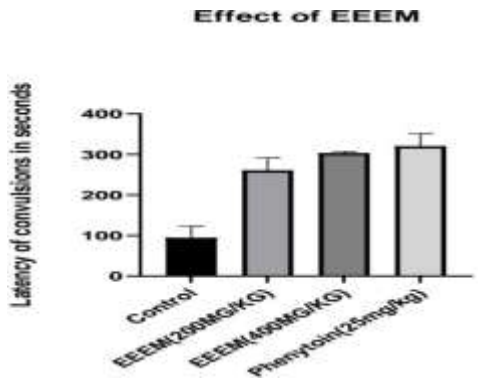


Figure 5: Effect of EEEM showing .

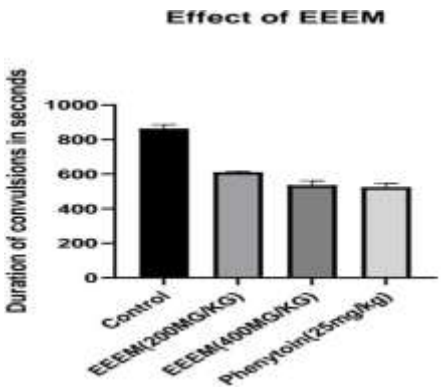


Figure 6: Effect of EEEM showing Latency of Convulsions Duration of Convulsions

IV. DISCUSSION:

The purpose of this study was to assess the ethanol extract of Euphorbia milii Des moul leaf's anticonvulsant properties. This study used acute seizure models, namely PTZ- and MES-induced seizure testing. The medications that counteract the seizures brought on by PTZ have a reputation for working well for mild epilepsy. It is well known that PTZ exhibits GABA antagonistic properties. The chemicals that are useful in treating grand mal epilepsy are mostly identified by use of the MES-induced seizure test in mice. Antiepileptic drugs inhibit the tonic extension of the hind limb elicited by electrical stimulation. It is well established that antiepileptic medications that prevent MES-induced seizures also prevent the spread of seizures. The models were created more than 60 years ago, and many AED screening programmes used them as standards when they first started. This is employed due to its ease of use, economy of time and money, high repeatability across laboratories, thorough validation with many AEDs, and clinical activity prediction. In Significant anticonvulsant effectiveness against PTZ-induced clonic seizure was demonstrated by an EEEM (400 mg/kg) dose of crude extract in the PTZ-induced seizure mouse investigation. This could be caused by the plant's presence of bioactive secondary metabolites and the potential localization of the active components. Agents that decrease the T-type calcium channel and/or increase GABAergic neurotransmission eliminate PTZ-induced seizures. It has been discovered that medications that block PTZ-induced seizures work well against absence and myoclonic seizures in general. Thus, it is plausible to suggest that either T-type calcium channel inhibition or increased GABAergic neurotransmission is responsible for the study plant's anticonvulsant effects in the PTZ-induced seizure test. Nonetheless, more research is required. Crude extract, primarily EEEM (400 mg/kg) dosage, dramatically lowered the mean duration of THLE in the MES-induced seizure paradigm. This suggests that, in comparison to the EEEM dose of 200 mg/kg, the larger dose of 400 mg/kg may be used as the highest efficacious dose. The presence of high concentrations of active chemicals may be the cause of this. AEDs that are effective against this seizure model include phenytoin, carbamazepine, lamotrigine, and valproate. These medications work by blocking sodium channels, stabilising their inactive state, and lowering high frequency firing of action potentials. Actually, these medications work well in both animal models and have a variety of modes of action. Consequently, the crude extract at larger doses has anticonvulsant efficacy; phytoconstituents may be the cause of this. broad-spectrum anticonvulsant properties found in the extract, such as inhibiting the Na+ channel and/or enhancing GABAergic neurotransmission, can prevent generalised tonic-clonic seizures. An initial phytochemical examination of Euphorbia milii Des moul showed the presence of terpenoids, alkaloids, flavonoids, steroids, and tannins. It is impossible to definitively link the observed active principle or principles to the anticonvulsant activity of the substance based on the current understanding of its chemical elements. Nonetheless, in animal models of anxiety, sedation, and convulsion, a number of flavonoids may function as benzodiazepine-like compounds in the central nervous system and alter GABA-mediated chloride channels. It has been shown that in MES and PTZ experimental seizure

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 1 Jan-Feb 2024, pp: 158-170 www.ijprajournal.com ISSN: 2249-7781

models, certain terpenoids and steroids exhibit anticonvulsant properties.

V. CONCLUSION:

The anticonvulsant activity of an ethanolic leaf extract of Euphorbia milii Des moul was assessed in the current investigation, and the following conclusions were made:

1. The yield percentage of Euphorbia milii des moul leaf was determined to be 16.66% w/w after the leaf was identified, harvested, and extracted.
2. During the initial phytochemical screening, the EEEM was found to include alkaloids, flavonoids, proteins, phenols, triterpenoids, carbohydrates, and steroids.
3. The presence of luteolin, naringenin, cloartenol, cyclotetrasiloxane, squalene, ethyl lansiolate, 2-methyl docosane, docosane, and demecolcine was determined by GC-MS analysis.
4. Quercetin, Naringenin, and Luteolin are the recognised flavonoids.
5. An acute toxicity investigation showed that Euphorbia milii Des moul was non-toxic at doses up to 2000 mg/kg, bd. wt., meaning the extract was safe.
6. MES conducted an anti-epileptic activity screen on EEEM. Convulsions caused by pentylenetetrazole were observed in Swiss albino mice and Wistar albino rats. Compared to the control group, EEEM reduced the duration of flexion, extension, clonus, and stupor in convulsions caused by MES. Comparing EEEM to the disease control group during PTZ- induced convulsions, the latter showed a dose-dependent decrease in convulsion duration but an increase in latency period.

Additionally, in order to identify the active principle causing the anti-convulsant effect and investigate its mechanism, the active components must be isolated. The work's future focus would be on identifying the active ingredient and isolating it so that a preclinical study formulation may be created.

REFERENCE:

1. J Muazucorresponding author1 and A H Kaita2; A Review of Traditional Plants Used in the Treatment of Epilepsy Amongst the Hausa/Fulani Tribes of Northern Nigeria; Afr J Tradit Complement Altern Med. 2008; 5(4): 387–390.
2. Igoli JO, Ogaji OG, Tor-Anyiin TA, Igoli NP. Traditional Medicine Practice Amongst the Igede People of Nigeria. Part II. Afr J Trad CAM. 2005;2(2):134–152. [Google Scholar] .
3. Haidan Yuan,1,2 Qianqian Ma,1 Li Ye,1 and Guangchun Piao1,2,*; The Traditional Medicine and Modern Medicine from Natural Products; doi: 10.3390/molecules21050559 PMCID: PMC6273146PMID: 27136524; 2016 May; 21(5): 559.
4. Ngo L.T., Okogun J.I., Folk W.R. 21st Century natural product research and drug development and traditional medicines; doi: 10.1039/c3np20120a. [PMC free article] [PubMed] [CrossRef] [Google Scholar]; Nat. Prod. Rep. 2013;30:584–592.
5. Zhu F., Ma X.H., Qin C., Tao L., Liu X., Shi Z., Zhang C.L., Tan C.Y., Chen Y.Z., Jiang Y.Y. Drug discovery prospect from untapped species: Indications from approved natural product drugs. PLoS ONE. 2012;7:e39782. doi: 10.1371/journal.pone.0039782. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
6. Galm U., Shen B. Natural product drug discovery: The times have never been better. Chem. Biol. 2007;14:1098–1104. doi: 10.1016/j.chembiol.2007.10.004. [PubMed] [CrossRef] [Google Scholar]
7. Bent S. Herbal medicine in the United States: Review of efficacy, safety, and regulation. Journal of General Internal Medicine. 2008;23(6):854-859. DOI: 10.1007/s11606-008-0632-y
8. Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Brazilian Journal of Medical and Biological Research. 2000;33:179-189. ISSN: 0100-879X
9. Saxena M, Saxena J, Nema R, Singh D, Gupta A. Phytochemistry of medicinal plants. Journal of Pharmacognosy and Phytochemistry. 2013;1(6):168-182. ISSN: 2278-4136
10. Shivali Sagar & Monika Bisht. A REVIEW ON PHYTOPHARMACOLOGY OF MEDICINAL PLANT: EUPHORBIA

MILII DES MOUL; INTERNATIONAL RESEARCH JOURNAL OF PHARMACY; Int. Res. J. Pharm. 2021, 12 (6) 67; ISSN 2230 – 8407

11. Kunle, Folashade O, Egharevba, Omoregie H and Ahmadu, Ochogu P. Standardization of herbal medicines - A review. International Journal of Biodiversity and Conservation 2012; 4(3):101-112.
12. Bandaranayake WM. Quality Control, Screening, Toxicity, and Regulation of Herbal Drugs: Ahmad I, Aqil F, Owais M. Modern phytomedicine; 2006:25-57.
13. Eucare., European white paper on epilepsy-call to action. Epilepsia, 2003. 44(6): p. 4-8
14. Organization WH. General guidelines for methodologies on research and evaluation of traditional medicine 2000. Geneva: World Health Organization.
15. Euphorbia milii. Missouri Botanical Garden. 2021 [cited 22 June 2021]. Available from:http://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?kemperco de=b633.
16. Fonseca KC, Morais N, Queiroz M, Silva M, Gomes M, Costa J, Mamede C, Torres F, Silva N, Beletti M, Canabrave H, Oliveria F. Purification and biochemical characterization of Eumiliin from Euphorbia milii var. hislopii latex. Phytochemistry 2010; 71(7):708-715.
17. Euphorbia milii (crown-of thorns) [Internet]. Cabi.org. 2021 [cited 22 June 2021]. Available from: https://www.cabi.org/isc/datasheetreport/21368.
18. M. Flory, Vinusha C. Phytochemical screening and antimicrobial studies in leaf extract of Euphorbia Milii. International Journal for Multidisciplinary Research 2015; 1(1):13-19.
19. Pascal OA, Bertrand AE, Esaie T, Sylvie HA, Eloi A. A review of the ethnomedical uses, phytochemistry and pharmacology of the Euphorbia genus. The Pharma Innovation Journal 2017; 6(1):34-39.
20. Aleksandrov M, Maksimova V, Gudeva L. Review of the Anticancer and Cytotoxic Activity of some species from Genus Euphorbia. ACS 2019; 84(1):1-5.
21. Kemboi D, Peter X, Langat M, Tembu J. A Review of the Ethnomedicinal Uses, Biological Activities, and Triterpenoids of Euphorbia Species. Molecules 2020; 25(17):1-29.
22. Islam N, Khan I, Rauf A, Muhammad N, Shahid M, ShahMR. Antinociceptive, muscle relaxant and sedative activities of gold nanoparticles generated by methanolic extract of Euphorbia milii. BMC Complementary and Alternative Medicine 2015; 15(1):1-11.
23. K. D. Tripathi, Essentials of medical pharmacology, Jaypee brothers medical publishers(p) ltd, 6th edition, 401-402.