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Phytochemical evaluation, spectral analysis and antiepileptic potential of *Euphorbia nivulia* in Swiss albino mice

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Euphorbia nivulia (EN)(Buch-Ham.), one of the members of Euphorbiaceae family, is known as Indian Spurge in English. Traditionally it is used in the treatment of various diseases like inflammation, fever, worm infection, asthma, cough, wounds and diabetes. In current study fresh as well as dried aerial parts of the plant were used for phytochemical evaluation, spectral analysis and determination of antiepileptic potential. The phytochemical analysis of EN shows that it contains flavonoids, tannins and alkaloids and also rich in saponins. Results of FTIR spectroscopic analysis confirms the presence of various chemical constituents including aromatics, diketones, alkenes, carbonyls, carboxylic acids and amide compounds. The study revealed some functional groups with different spectral peaks regarding chemical and structural nature of various compound(s) having anticonvulsant and antiepileptic activity. Its juice was found to be more toxic than its extracts and both juice and aqueous ethanolic extracts possess sedative effects in animals. The extract exhibited significant anticonvulsant and antiepileptogenic activity against PTZ-induced convulsions in a dose dependent manner as observed at doses 30 mg/kg, 50 mg/kg and 100 mg/kg, respectively in different groups of Swiss albino mice (n=6).

Keywords: *Euphorbia nivulia*, FT-IR spectroscopy, epileptogenesis.

INTRODUCTION

Epilepsy is one of the oldest neurological disorder known to mankind. The term ‘‘epilepsy’’ is derived from Greek word ‘‘epilambanein’’, which means ‘‘to seize upon’’ or ‘‘to attack’’. Epilepsy is the most prevalent neurodegenerative disorder of this modern aged world after stroke. More than 2 million Americans and 50 million people worldwide are suffering from this disease (Strine, T W et al. 2005) from which 80% population belongs to less economical and developing countries (De Boer, H M et al. 2008). According to the Commission on Classification and Terminology of the International League Against Epilepsy (ILAE), epilepsy is defined as the abnormal electrical activity surrounds both hemispheres of the brain (Engel, J 1998).

Data of WHO shows that for the primary health care need, about 80% of world population depends on natural medicine. 40% urban Chinese, 70 % of India is reported to use the herb for primary cure, 70 % German physician use the herbal medicine or patients are referred to traditional prescribers, and 90 % rural used herbs,49 % population of Japan use phyto-medicine for remedies. European use the herbal medicines of 6 billion$/year. According to a FDA survey 16 million Americans actively use the herbal medicines. This extensive use of herbal medicine is because of their general consideration being safe as they are natural, but the boot is on the other leg. The most plants reported with cardiac, anti-diabetic, diuretic, sedative, hypertensive, hypo-tensive or anti-coagulant effect have interactions (Haq, I 2004).

Euphorbia nivulia is traditionally used in folklore use belong to family Euphorbiaceae. Common names include; in English is Leafy milk hedge, Holy milk hedge, Dog’s tongue in Hindi. Worldwide 2000 species of Euphorbia are

existed. Only in india, its 195 species reported in widely diverse habitats the genus includes herbs, shrubs and trees having imperative biological activities, Euphorbia nivulia Buch.-Ham. attracts the researchers in all over the world (Mahajan, R T and Badgujar, S B 2011).

Leaf extract used in treatment of Jaundice (Tareen, 2010) Stem can be used to cure bone fractures and latex of plant is antiseptic (Kumar, 2010). It is used to kill Intestinal worms. (Nandagopalan; 2011) Leaf, latex and root are useful in Skin disorders, ear disorders, retention of urine, swelling, worm infection (Jothi, 2008).

In our current study, a primary focus of research to date has been in the areas of pharmacology and phytochemistry. Research in the pharmacology of medicinal plant (*Euphorbia nivulia*) has involved assays of bio-activity i.e; anti-epileptic activity for possible active phytomedicinal compound(s). In the area of phytochemistry, medicinal plant has also been characterized for their possible bioactive compounds by using FT-IR analysis and subjected to functional groups analysis.

MATERIALS AND METHODS

Collection of plant

The plant of *Euphorbia nivulia* was collected from rural areas of district Bahawalpur. The plant was authenticated from the botanist, department of Islamia University Bahawalpur under voucher number i.e., EN-AP-05-12-041.

Preparation of crude extract

Euphorbia nivulia Buch.-Ham was cut down into small pieces and spread under shade for drying. On complete drying, the plant was subjected for grinding, coarse powder was macerated in 70% ethanol at room temperature for fifteen days. Filter it and filtrate was evaporated in rotary evaporator under the reduced pressure. A thick and semisolid pasty mass was obtained.

Phytochemical analysis

Phytochemical Evaluation

Qualitative phytochemical screening

Different chemical tests were conducted, using reported methods, to check out the presence of various phytoconstituents from ethanol extract (Edeoga *et a*l., 2005; Evans, 2003; Kokate, 2007; United States Pharmacopoeia, 2009).

Quantitative phytochemical screening

The total phenolic contents present in the plant material were estimated by reported methods (Chaovanalikit and Wrolstad, 2004; Kokate, 2007).

Animal handling

Adult, male Swiss Albino mice weighing (20-30gm) were kept in polycarbonate cages (47×34×18 cm) and housed under standard conditions of temperature (24±1 C˚), humidity and dark light cycle (12h-12h) and were given standard daily animal feed.

Development of *in-vivo* epileptogenic model

Once screening in the acute seizure model was done, then developed epilepsy process (epileptogenesis) using the chemical kindling model. The induction of the kindling was accomplished according to De Sarro *et al.* method (De Sarro G et al. 2000). The animals were divided into four groups (n = 8). The normal control group and the drug control group were given daily dose of 0.5 ml of normal saline (0.9%NaCl) and diazepam (7.5 mg/kg) i.p., respectively. Similarly the test groups were given with test extract once daily dose of extract 100 mg/kg. Animals scored according to a pre-validated scoring scale for the severity of the seizure activity they showed. Seizure patterns during the gradual development of kindling are classified into five distinct behavioral stages which are given in the tabular form Table 2.

FTIR spectroscopic analysis

FTIR (Tensor, Bruker, Germany) was used to record FTIR spectra. The powdered sample was scanned at room temperature at 4000 to 400 cm–1 spectral range. Each Sample was run three times and all of them were undertaken within a day period (Mariswamy, Y et al. 2012).

Statistical analysis

Data collected was analyzed and expressed by using SPSS, as mean ± standard error of mean (S.E.M) with 95% confidence interval (CI). ANOVA test was applied for analysis of data. p values <0.05 were considered significant. All the data were compared with control group and statistical analysis by the use of Graph Pad Prism.

RESULTS

Phytochemical Evaluation

Qualitative phytochemical screening

The qualitative phytochemical analysis was used to evaluate the occurrence of different chemical constituents like alkaloids, glycosides, tannins, saponins, terpenoids, fats, phenols and flavonoids in methanol extract and its various fractions. The findings are shown in Table 1.

Table 1: Phytochemical analysis of *Euphorbia nivulia*

Group Tests	Identification tests	Methanol
Carbohydrates	Molisch test	+

	Benedict's test	+
	Fehling's test	+
Amino acids and Proteins	Ninhydrin test	+
	Biuret test	+
	Millons test	+
Alkaloids	Mayer's test	+
	Dragendorff's test	+
	Wagner's test	+
	Hager's test	+
Glycosides	Keller-Killiani test	+
	Modified Borntrager's Test	+
	Modified Borntrager's Test	+
	Salkowski's test	+
Tannins	Ferric chloride test	+
	Gelatin test	+
Flavonoids	Lead acetate test	+
	Alkaline reagent test	+
Saponins	Foam test	+

Quantitative phytochemical screening

The values of total phenolic contents (TPC) were found to be 16.20 mg/g as gallic acid equivalent of dry plant material. The percentages of Alkaloids and glycosides contents were 2.60 and 0.32 respectively. Results are presented in figure 1.

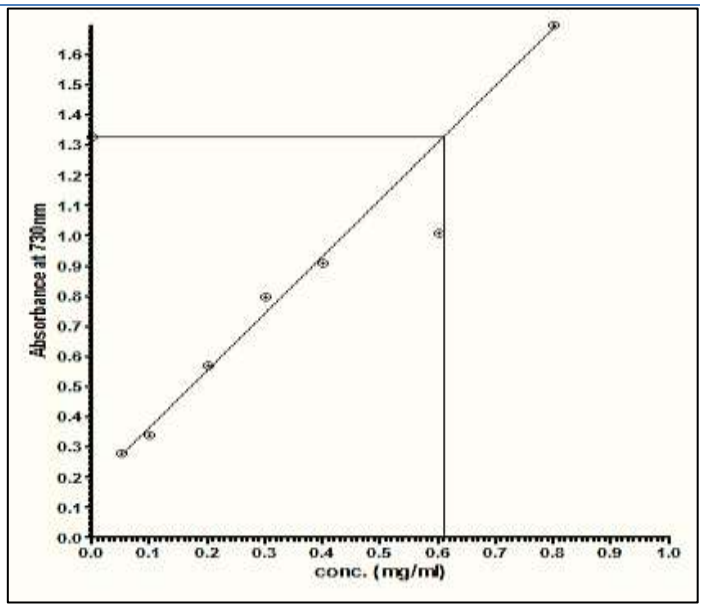

Figure 1: Graphical representation of Phenolic contents of *E.nivulia*.

Development of in-vivo antiepileptic potential

The Diazepam control group did not show any seizure pattern till the end of experiment. In the light of our previous results obtained from acute testing, we used only 100 mg/kg of crude extract for further kindling model of epileptogenesis. Diazepam treated group protected the animals from developing the seizures throughout the treatment protocol given in table 2.

Table 2: Duration (sec) of different Seizures induced in different groups

Groups	Treatment mg/kg	Animal Used	Onset of Jerks (sec)	Rear & Falling (sec)	HLTE (sec)	No. of animals (convulsed)	No. of dead animals	% Morality
PTZ	90	6	99.6 ±3.16	132.3 ±3.91	247.8 ±5.12	6/6	6	0
E.nivulia	30	6	173.8 ±7.23	203.8 ±2.50	304.0 ±2.82	6/6	5	83.3
E.nivulia	50	6	265.6 ±13.33	334.1 ±3.77	650.5 ±24.75	6/6	4	66.6
E.nivulia	100	6	544.0 ±18.03	0	0	6/6	2	16.6
Diazepam	7.5	6	563.1 ±21.38	0	0	6/6	0	100
Normal Saline	0.25ml	6	-	-	-	0/6	0	100

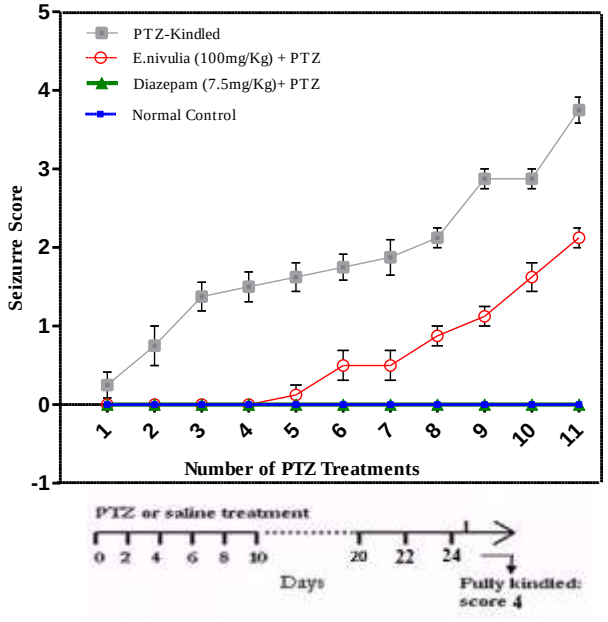

Figure 2: Graphical representation of development of *in vivo* epileptogenic model

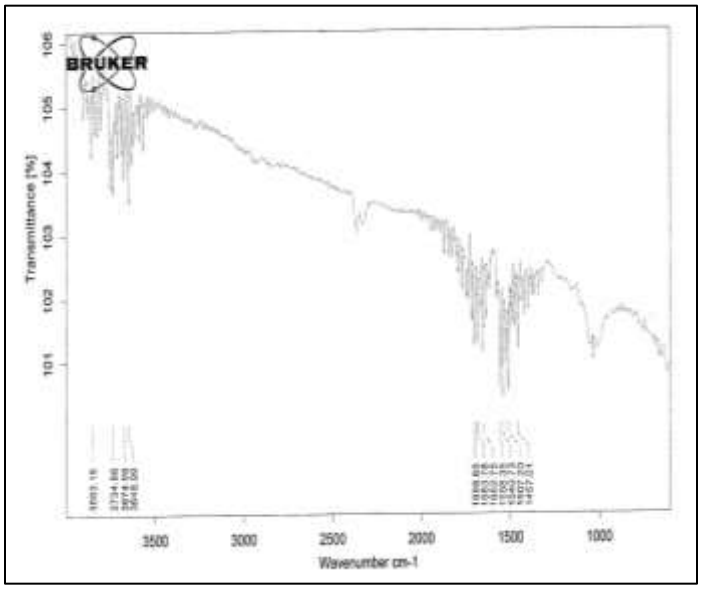

Figure 3: FT-IR Spectra of *Euphorbia nivulia* (crude powder)

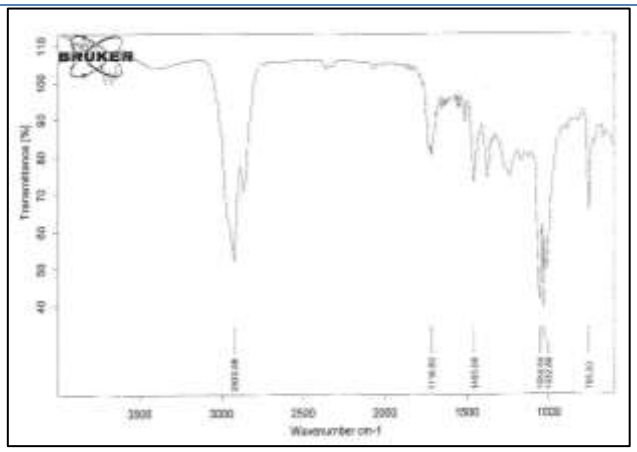

Figure 4: FT-IR Spectra of *Euphorbia nivulia* (Ethanolic extract)

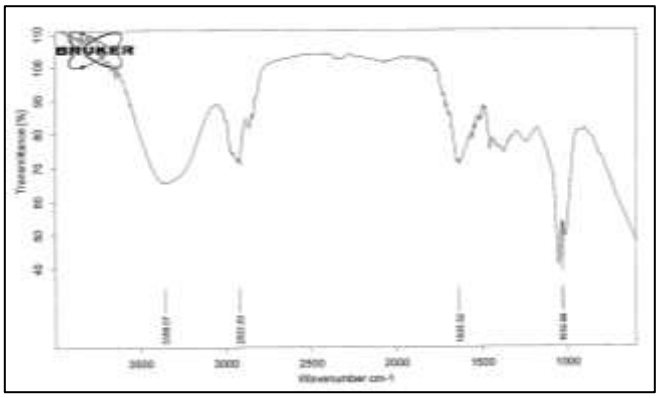

Figure 5: FT-IR Spectra of *Euphorbia nivulia* (Methanolic extract)

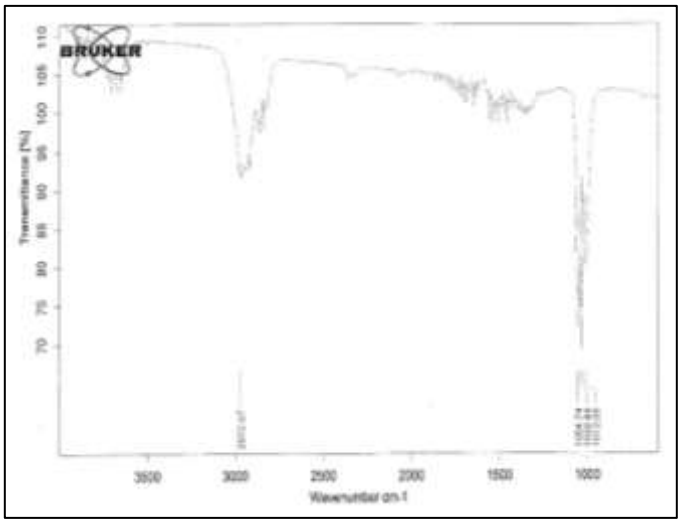

Figure 6: FT-IR Spectra of *Euphorbia nivulia* (n-Hexane extract)

FTIR analysis

The FT IR analyses were performed for different forms of the plant extract e.g. crude powder, ethanolic extract, methanolic extract and n-Hexane extract. FTIR (Tensor, Bruker, Germany) was used to record FTIR spectra.

DISCUSSION

The percentage yield of the crude extract was 23.2% after soaking the materiel three times over a period of 15 days in ethanol.

In our study, we have used diazepam (7.5 mg/kg) as a reference drug. The protection of mice observed in diazepam-treated kindled mice was expected. Since various authors have shown that diazepam exerts its anticonvulsant activity by enhancing GABA-mediated inhibition.

We noticed that *E. nivulia* exhibited anticonvulsant effects according to the Dessaro Method (De Sarro G et al. 2000) at the dose of 100 mg/kg with only 16.67 % mortality rate . Whereas, 67.77 % and 83.33 % mortality rate was exhibited by 30 mg/kg and 50 mg /kg dose, respectively. In the light of this acute anticonvulsant testing, we proceeded with chronic chemical Kindling model to evaluate the extract against the development of epileptogenesis. And for this purpose 100 mg/kg of plant extract was selected. At the end of experiment the average score of 2.0 was recorded in the Euphorbia nivulia administered mice group which means that it significantly slowed down the progression of epileptogenesis (Fig, 2). Once the anti-epileptogenic activities of *Euphorbia nivulia* was confirmed, next we were interested to analyze about the possible active constituents present in this medicinal plant.

FT-IR spectrum reflecting objectively the panorama of chemical components of plant extracts is a most credible method to validate and identify the mix-substance systems such as traditional medicine and herbal medicine. The results of the present study spectrum also revealed the functional groups present in the crude powder and different extracts (Ethanol, Methanol, n-Hexane) of *E.nivulia*. Many workers have applied the FTIR spectrum as a tool for discriminating, classifying and differentiating closely related plants. The results of the present study also provided the previous observations and revealed the similarity and variation in functional groups at extract (Ethanol, Methanol, n-Hexane) level also. Therefore, the present work on *E.nivulia* showed different phytochemical markers as useful analytical tool to check not only the quality of the powder and extracts but also to identify the medicinally important plant. Further advanced spectroscopic studies are needed for the structural elucidation and identification of various beneficial compounds present. The crude powder and extracts (Ethanol, Methanol, n-Hexane) were subjected to FTIR analysis for the detection of different functional groups present in *E.nivulia*. The FTIR study showed the similarity and variation among the extracts and crude powder of *E.nivulia* based on the absorption spectrum and functional group presence. Because of the similarity of the extraction method by Rotary Evaporator, the chemical components in the concentrated extracts are relatively consistent and therefore they show higher comparability and repeatability in FT-IR spectra.

Results of FTIR spectroscopic analysis in the different solvent extracts of *E.nivulia* have revealed the presence of various chemical constituents (Fig 3, 4 and 5). This study revealed some functional groups with different spectral peaks from which we can assess the chemical and structural nature of compound(s) having anticonvulsant and antiepileptic activity. The values at 1457.01, 1507.20, (1540.73, 1558.35), 1652.75, 1683.78, 1698.65, (3648.99, 3674.95), 3734.86 and 3853.15 attributed Aromatics, Amide, Diketones, Alkenes, Carbonyls, Carboxylic acids and Amide compounds, respectively in the crude powder. While the dried ethanolic extract of *E.nivulia* plant FTIR analysis revealed the presence of Alkenes, Aliphatic amines, Alcohols, Carboxylic acids, Esters, Aromatics, Esters and Alkenes which shows major peaks at 755.20, 1032.80, 1054.54, 1456.00, 1716.60, and 2922.89 respectively. The dried methanolic extract of *E.nivulia* plant FTIR analysis also revealed the presence of cyclohexane ring vibrations, Alkenes, Alkanes which shows major peaks at 1032.86, 1635.32, and 2922.83 respectively. While spectral lines at 3558.07 are still unknown suggesting further investigation into it for the possible discovery of new compounds in it with medicinal potentials. Similarly, the results of n-Hexane extract of *E.nivulia* plant FTIR analysis confirmed the presence of Aliphatic fluoro compounds, (Alcohols, Ethers, Carboxylic acids, Esters) and Alkanes which shows major peaks at (1012.65,1032.88), 1054.74 and 2972.67, respectively.

CONCLUSION

The current study showed that *Euphorbia nivulia* exhibited significant anticonvulsant and antiepileptogenic activity with the possibility of the presence of novel compounds. In future, the plant extract and its different fractions can be obtained for the isolation of the active constituents and ultimately elucidate their chemical structures. Furthermore, we can explore the mechanism of action of those active constituent(s). This initial study will paved the way for further scientific screening of this medicinal plant with the possibility of developing novel antiepileptic drug(s) with better efficacy and tolerability.

The plant extract contained 610mg of Gallic acid equivalent per gram of *E.nivulia* extract.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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AUTHOR CONTRIBUTIONS

HR, MYK and SA performed the experimental work. NA and MI wrote the manuscript. MSR, QAJ and RAR assisted throughout the allelopathy survey, MH and GR performed statistics. MAG and IA designed the experimental work and assisted throughout the experimental as well as theoretical work.

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