doi.org/10.36721/PJPS.2026.39.3.REG.14501.1

GC-MS correlated-antiepileptic screening for probable GABA-modulator by PTZ-induced acute seizure model in Swiss albino mice and phytochemical profiling of ethanol extract of *Ficus benghalensis*

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Abstract: **Background**: Plants, such as *Ficus benghalensis* (family: Moraceae), contain diverse phytoconstituents against diseases, including epilepsy, affecting more than 50 million people worldwide and is characterized by high neuronal activity in the brain that results in seizures and convulsions. **Objectives**: This study was designed to investigate the antiepileptic potential and to correlate possible phytochemicals responsible for it. **Methods**: The ethanol extract was subjected to GC-MS analysis, spectroscopic & phytochemical profiling and antiepileptic evaluation by *in-vivo* screening in PTZ-induced acute seizure model in mice. **Results**: The plant is found to be rich in polyphenols, flavonoids, sterols and terpenes, identified by spectroscopic techniques. The ethanol extract of the plant retarded the onset of seizures (p<0.0001), seizure intensity by delaying tonic-clonic seizures (p=0.0006) and seizure-duration (p=0.0001). Moreover, the extract also improved the recovery-time of mice after tonic-clonic seizures (p<0.0001) with a significant decline in overall seizure scores (p=0.0054). **Conclusion**: The findings suggest that the phytoconstituents of *Ficus benghalensis*, such as polyphenols, flavonoids, sterols and previously unidentified alkaloids, play a key role in mitigating oxidative stress, neuroinflammation and GABAergic dysfunction associated with epilepsy, which demonstrates its potential as a source of lead compound for new antiepileptic drugs. Detailed investigations on isolated phytochemicals can be done to explore their antiepileptic potential.

Keywords: Alkaloids; Antiepileptic activity; *Ficus benghalensis*; GC-MS; Phytochemistry; UV & IR spectroscopy

Submitted on 22-04-2025 – Revised on 26-08-2025 – Accepted on 02-09-2025

INTRODUCTION

Epilepsy is a serious chronic disease, characterized by abnormally high neuronal activity in the brain, affecting more than 50 million people of all ages across the globe. The majority of epilepsy patients (about 80%) belong to low and middle-income countries (LMIC) like Pakistan, where epilepsy is the most common disorder (80 to 100 per 100,000 people per year) due to a lack of health professional training, unavailability of medications, a poor healthcare system, basic sanitation & hygiene and lofty speculation of brain infections and traumatic brain injury (Thijs *et al*., 2019, WHO, 2022). Epilepsy is characterized by recurrent unprovoked seizures and may arise due to congenital abnormalities, hippocampal sclerosis, trauma leading to penetration in dura mater, tumors resulting in structural lesions involving frontal, parietal, or temporal regions of cerebral hemispheres and vascular and other neurodegenerative diseases in later age (Maxine A. Papadakis *et al*., 2024). The symptoms of epilepsy are according to the site of origin of neuronal firing in the brain (Harvey *et al*., 2012). All the presently used antiepileptic drugs manifest dose-dependent idiosyncratic toxicity and side effects, with an inability to control seizures in about 30% of the epileptic patients. Therefore, to develop a safer, effective and more selective antiepileptic drug with the least side effects is the need of the hour (Wei *et al*., 2015). Medicinal plants are considered the richest sources of therapeutic agents against morbidities globally. Due to increased efficacy of pharmacological actions, non-toxicity & least side-effects of medicinal plants and recent swing in international trends for natural and organic products, there is an increased interest in herbal medicines, claimed as "Return to Nature" (Khan and Ahmad, 2019). Over 10% of the known plant species are used in pharmaceutical and cosmetic formulations and the demand has increased by 8% to 15% every year in eastern and western continents of the world over the last decade. According to an estimation, more than 50% of the currently available drugs are related to medicinal plants (Jamshidi-Kia *et al*., 2018). However, it is not safe to consume them as such, as some of these plants are toxic and might show adverse side effects if taken as such. Therefore, active compounds should be standardized from bioactive extracts and undergo safety studies (Khan and Ahmad, 2019). To utilize them effectively and cognitively, plant materials are standardized by proximate analysis and phytochemical investigations. Proximate analysis of herbal powder includes determining moisture, ash contents, extractable values, physical properties like foaming & swelling indices and nutritional contents such as carbohydrates, lipids, proteins and caloric values of the plant. Proximate analysis is significant for legitimate comparison for the purpose of validation of different plants of the same species and those found in different areas of the world (Thangaraj, 2016). Bioactive compounds (i.e., secondary metabolites) are isolated from plants by suitable extraction methods. Gas

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chromatography coupled with mass spectrometry (GC-MS) is a good tool for identifying important phytochemicals in plants, while ultraviolet-visible (UV-visible) and Fourier Transform Infrared (FTIR) spectroscopies are utilized for the qualitative and quantitative determination of individual compounds in plant materials (Lozada-Ramírez *et al*., 2021).

Ficus benghalensis is an evergreen perennial plant of the family Moraceae, indigenous to South Asia. The Indian fig tree is known for treating various ailments for centuries in the traditional medical system. A number of biological studies have been done on its different parts to understand its pharmacological potential, which also include neuropharmacological propensities comprising antiseizure, anxiolytic and anti-amnesic activities along with other natural pharmacological properties (Ahmad *et al*., 2011, Gopukumar and Praseetha, 2015, Murugesu *et al*., 2021) but a lack of phytochemical data i.e., the specific phytoconstituent responsible for antiseizure activity, limits its scope (Panday and Rauniar, 2016). The wide distribution and plentiful pharmacological potentials make it a very cheap source of prospective lead compounds in finding novel, economical and better therapeutically active drugs.

Pentylenetetrazole (PTZ) inhibits the function of the $GABA_A$-receptor in central neurons by acting as a noncompetitive antagonist, thereby reducing inhibitory neurotransmission and inducing epileptic seizures. The PTZ-model is a frequently applied model in rats and mice to induce myoclonic and tonic-clonic seizures to spot out the potential of novel drugs against seizures and epilepsy that function by antagonizing $GABA_A$ receptors and categorize seizures on an intensity scale correlating electroencephalographic parameters with behavior during seizures (Lüttjohann *et al*., 2009). Despite the prevalence of commercially available anti-epileptic drugs, due to multiple mechanisms involved in the pathophysiology of epilepsy, such as disruption in ion channels, genetic factors, neuroinflammation and oxidative damage, these drugs are not clinically suitable for all types of epileptic patients (Duan *et al*., 2024). Thus, the present study was proposed to identify novel and effective anticonvulsant agents that can be explored for improved clinical treatment of epilepsy. Several currently used antiepileptic drugs have been developed by utilizing simple models such as Pentylenetetrazole (PTZ)-model, Maximal Electroshock Seizure (MES)-model, etc. (Löscher, 2017). Considering all these aspects, the current study has focused on the phytochemical profiling, spectroscopic analysis and acute antiepileptic screening of the *Ficus benghalensis* ethanolic extract, followed by its correlated interpretation with GC-MS analysis.

MATERIALS AND METHODS

Plant material

Almost fifteen kilograms of different parts of the Indian banyan tree, such as aerial roots (adventitious roots), leaves, branches, stem and bark were collected locally from Chah Miran, Lahore, Pakistan. Google Maps coordinates of the collection site are 31.586161, 74.348234 (31°35'10.2"N 74°20'53.6" E). The plant parts were botanically identified and authenticated from the Botany Department of Government College University (GCU), Lahore and a sample of the plant materials as a specimen voucher was submitted in the herbarium of GCU, Lahore with the authentication number GC.Herb.Bot.3998 dated 10-07-2023. The issued number of the specimen voucher is used as a reference.

Chemicals and apparatuses

Analytical grade solvents and chemicals were procured from Merck, Germany. Diazepam (DZP) and normal saline (NS) solutions were purchased from the local pharmacy in Lahore. The chemical solutions used were freshly prepared right before the experiment. The glassware was bought from Pyrex glass, which complied with American Society for Testing and Materials (ASTM) guidelines for class A for apparatuses and glassware. An acrylic box with suitable-sized compartments was constructed from the local market at Lahore, while a two inch long stainless steel curved oral gavage tube of gauge 18 fitted on a 3cc syringe was provided from the pharmacology laboratory, Punjab University College of Pharmacy.

Instruments

ARE heating magnetic stirrer, model F-20520162 of VELP Scientifica (Italy), ATR-FTIR spectrophotometer (ECO-ATR) of Bruker (Germany), distillation apparatus (WSB/4) of Hamilton laboratories Glass Ltd. (China), electric carbolite furnace of Sheffield (Germany), electronic weighing balance (model BL-410S) of Setra (Boxborough USA), heating oven universal (digital PID control) model UNB-500 of Memmert (Germany), rotary vacuum evaporator of Heidolph (Germany), UV-Visible double beam spectrophotometer model UV-1700 of Shimadzu Scientific Instruments (Japan) with operating system UV-Probe 2.21, flash 2000 organic elemental analyzer (CHNS-O) of Thermo Fisher Scientific (UK) equipped with thermocouple detector, vortex mixer (SLV-6) of Seoulin Bioscience (Korea) and ultrasonic mixer (DSA 50 CKI-1.8L) of Brandson Ultrasonics (Germany) were used for the analysis. GC-MS (model QP-2010) with the Wiley spectral library search program of Shimadzu, Scientific Instruments (Japan). A high definition 1080P manual focus webcam from the RAPOO company (model C260) connected to a Dell Inspiron, Intel (R), Core (TM) i3-2350M laptop with operating system Windows 8.1 Pro.

Cleaning, drying and pulverization of the plant

The plant material was cleaned by rinsing with water to remove any type of dirt or filth, etc. and then dried in open air under shade. Dried parts were pulverized to coarse powder and stored at room temperature in an air-tight container.

Proximate analysis

Proximate analysis was done to determine moisture & ash contents, extractable values and swelling and foaming index of powder of the plant using the quality control guidelines of the World Health Organization for herbal materials and the United States Pharmacopoeia (WHO, 2011, USP, 2018).

Preparation of extract

The plant extract was prepared using the maceration technique by soaking 4 Kg of dried & pulverized plant material in 10 L of ethanol for 7 days. The resulting ethanol solution was filtered and subsequently concentrated to dryness using a rotary evaporator operated at 60°C under reduced pressure (200 to 300 mmHg) and a rotation speed of 25rpm. Ethanol obtained via rotary evaporator is again used to macerate the same powdered plant material for the next 7 days and the process is repeated until the plant material is exhausted. The dried extract was collected in a previously weighed, clean glass vial and kept at a cool, dry and clean place to protect it from any decay by moisture or microbes.

The percentage yield of the extract was calculated by applying the following formula:

$$\text{Percentage yield} = \frac{\text{weight of extract}}{\text{weight of dried powder taken}} \times 100$$

Determination of primary metabolites and nutritional composition

The powdered parts of *Ficus benghalensis* were tested to estimate different nutritional components as primary metabolites. Total proteins, lipids and carbohydrate contents were determined by using previously established procedures described in the literature. The method of Lowry *et al*., (1951) was used to estimate the total proteins in the dried powder and ethanolic extracts using bovine serum albumin (BSA) solutions ranging from 12.5-100µg/mL as reference (Lowry *et al*., 1951). Lipid contents were determined by using the methods reported by Sara Kethleen in the literature with slight modification (de Loiola *et al*., 2023). The formula of Al-Hooti *et al*., as described by Latif *et al*., was used to estimate total carbohydrates in the plant sample (Al-Hooti *et al*., 1998, Latif *et al*., 2023).

Total carbohydrates (%) = 100 - (Total moisture+ total ash+ total proteins+ total lipids)

Caloric value

The total calories per 100 grams were determined by using the "Atwater factor" system. The method calculates potential energy value as 4 kcal/g for protein and carbohydrates and 9 kcal/g for lipids. Total caloric value of *Ficus benghalensis* was calculated by multiplying crude proteins, lipids and carbohydrates percentages by 4, 9 and 4, respectively and taking the sum of the multiplication products (Souratié *et al*., 2025).

Caloric value (kcal/100g)= (4 × proteins %) + (9 × lipids %) + (4 × carbohydrates %)

Determination of secondary metabolites

The amount of secondary phytoconstituents in the ethanolic extract of *Ficus benghalensis* was determined by using the methods described previously (Khan *et al*., 2020). The total contents of flavonoids, glycosaponins, polyphenols, polysaccharides and tannins were determined by Chang *et al.*, (2002), Hussain *et al.*, (2008), Slinkard and Singleton (1977) and Deshpande *et al.,* (1986) (Slinkard and Singleton, 1977, Deshpande *et al*., 1986, Chang *et al*., 2002, Hussain *et al*., 2008).

Molecular spectroscopic analysis of the extract

The spectroscopic survey of the plant was done using UV-visible & FTIR spectroscopies, followed by GC-MS analysis on the ethanolic extract.

UV & IR analysis

The ultraviolet-visible spectrum of the ethanolic extract was taken by diluting to 1:10 with methanol and scanning over the UV-visible range of the electromagnetic spectrum (200-800nm) using a UV-visible spectrophotometer. The scan data was retrieved as an Excel document and the spectrum was developed using GraphPad Prism software (Hussain *et al*., 2023). The IR spectra of the ethanol extract were recorded with the help of an ATR-FTIR instrument from 4000 to 400 cm^{-1} at a spectral resolution of 2cm^{-1} by using the method reported by Azminah *et al*., with minute modifications (Azminah *et al*., 2023).

GC-MS analysis

The phytochemical composition of *Ficus benghalensis* was established by GC-MS analysis. The bioactive components were separated using a 30 m long, 0.25 mm silica film-coated capillary column (0.25 µm diameter) at a beginning temperature of 50°C, which was cautiously increased to 260°C at a rate of 10°C per minute. The sample was prepared by dissolving the ethanolic extract in ethanol and 2 µL of it was introduced into the preheated column, accompanied by helium as mobile phase carrier gas at 1 mL/min. The gas chromatograph was coupled to a mass spectrometer equipped with an electron impact ionizer (70 eV), and the sample was analyzed over 30 minute with a 0.5s scan interval. The outcome was obtained as a mass spectrum, which was matched with databases of the National Institute of Standards and Technology (NIST20), Agilent Retention Time Locked Pesticide and Endocrine Disruptor Mass Spectral library (RTLPEST3) and identified by their agreement with databases, taking 80 as a minimum value of quality factor (Hussain *et al*., 2023).

Elemental analysis

Elemental analysis was performed to quantify Carbon (C), Hydrogen (H), Nitrogen (N), Sulphur (S) and Oxygen (O)

by the method of Asmat Ullah *et al*. using Carbon, Hydrogen, Nitrogen, Sulfur and Oxygen (CHNS-O) Flash 2000 organic elemental analyzer (Ullah *et al*., 2023).

Acute antiepileptic activity

Experimental animals

Swiss albino mice (*Mus musculus*) of both sexes, weighing between 30g to 36g were kept in plexiglass cages for 3 days at 24 ± 2 °C and 60% relative humidity with a periodic 12-hour exposure to light and dark and unlimited access to food pellets and water. All the techniques and procedures used in the experiment were in accordance with the Guide for the Care and Use of Laboratory Animals (Council, 2011) and were approved by the Punjab University Institutional Ethics Review Board, University of the Punjab (ethical approval number: No.D/13/FIMS, dated 28-02-2024).

Preparation of extract solution for in-vivo study

400mg of the extract was taken in a Falcon tube and mixed with 0.5mL Tween-80 and 10mL NS. The contents were sonicated in an ultrasonic mixer until uniform.

Study groups

The rodents were divided into 4 different groups, each containing six mice (three mice of both genders (n=6)) (Table 1). The first group was given NS (0.9%w/v sodium chloride) solution orally and later on intraperitoneally (normal control group). The disease control group received only PTZ in NS via the intraperitoneal route to induce seizures 75mg/Kg (Ilhan *et al*., 2005, Kediso *et al*., 2021). The positive control group was administered with 5mg/Kg diazepam (DZP) solution in NS, 30 minutes before seizure induction by PTZ intraperitoneally (Duan *et al*., 2024). The treatment group received 400mg/kg plant extract, calculated from acute toxicity studies reported in the literature (Thakare *et al*., 2010, Bhardwaj *et al*., 2016).

Procedure

The rodents of each group were administered with their respective mixture orally, 30 minutes before the administration of PTZ. The mice were placed in separate plexiglass cages and each mouse was observed attentively for a duration of 30 minutes to observe any signs of convulsion. The revised Racine scale was used to score the extent and duration of seizure (Van Erum *et al*., 2019). The video of each mouse was recorded using a high-definition webcam to observe them more properly.

Study parameters

The epileptic parameters observed from the videos of mice under study include:

i. Onset of seizure
ii. Latency to first hind limb extension
iii. Latency to first tonic-clonic seizure
iv. Duration of seizure
v. Seizure frequency
vi. Intensity of seizure
vii. Total seizure score
viii. Duration of post-ictal phase

Onset of seizure was determined by the appearance of the first myoclonic twitch that occurred as a slight to a hard jerk. Tonic-clonic seizures and hindlimb extension were indicative of the severity of the epileptic attack and were ascertained by the time from the administration of PTZ to full body stiffening and jerking accompanied by wild jumping of mice. The number of seizures and their cumulative duration were recorded in seconds. The severity and intensity of seizures were established by the stages of seizures in mice that were categorized in seven different behavioral stages: stage 0) no apparent epileptic seizures revealed normal behavior, stage 1) sudden behavioral apprehension appeared as motionless staring that ends with a jerk, stage 2) facial jerking indicated with the nose often linked with head nodding, stage 3) forelimb clonus with lordotic posture, stage 4) forelimb clonus with rearing, often including hindlimb extension, stage 5) generalized tonic-clonic seizures with loss of postural tone, accompanied by wild jumping and tonic extension of hindlimbs (sometimes of forelimbs as well) and stage 6) death. Total seizure score was calculated by summing the stage scores of all seizures incorporating both seizure frequency and severity, where each seizure was given a score equal to the stage of seizure. The post-ictal phase was established by the time after the seizure, when mice start to behave normally again.

Statistical analysis

The original results are expressed as Mean ± SD (standard deviation). GraphPad Prism 8.0.1 was used for statistical analysis. For normally distributed data column analysis as one-way analysis of variance (ANOVA) (and nonparametric or mixed), followed by Tukey's multiple comparison test was applied. The p-value<0.05 was set as the statistical level of significance (Panday and Rauniar, 2016).

RESULTS

Proximate analysis

The standardization criteria, like moisture content, ash value, extractable matter, foaming & swelling indices, were carried out to establish the purity and identity validation of the plant. The results of the proximate analysis are summarized in table 2.

Percentage yield of ethanol extract

The cold extraction process gives 228.18g of ethanol extract with a percentage yield of 5.63%.

Determination of primary metabolites & nutritional composition

Total proteins were calculated from the bovine serum albumin standard curve using the regression equation ($y= 0.0017x+0.0173$). The linearity of the standard curve was established by the regression coefficient ($R^2 = 0.9979$) as shown in fig. 1.

Primary metabolites, including total proteins, total lipids and total carbohydrates, identified from the powdered parts of *Ficus benghalensis*, are given in table 3.

Determination of secondary metabolites

The amount of total polyphenols, flavonoid, tannins, polysaccharides, proteins and glycosaponins were determined in ethanolic extract of *Ficus benghalensis* by using regression equation of standard curves of gallic acid (*y = 0.0023x+0.0553*), quercetin (*y = 0.00695x+0.0868*), tannic acid (*y = 0.0145x+0.0993*), glucose (*y = 0.0018x-0.0022*) and bovine serum albumin (BSA) (*y= 0.0017x+0.0173*) respectively (Fig. 2).

The plant is specifically rich in proteins, polysaccharides and polyphenols. Other phytochemicals found in the extract and its fraction include flavonoids, tannins and saponins (Fig. 3). The results of the determined phytochemicals are given in table 4.

Elemental analysis

Elemental analysis of the ethanol extract declared the presence of carbon (65.56%), hydrogen (10.26%), oxygen (23.74%), nitrogen (0.19%) and sulfur (0.25%), indicating the diverse primary and secondary metabolites.

Molecular spectroscopic analysis

UV-Visible profile

The ethanol extract of *Ficus benghalensis* showed significant absorbance peaks of 1.658, 0.330 and 0.109 at 269nm, 402nm and 664nm, respectively, in the UV and visible regions of the electromagnetic radiation spectrum. The scan of the ethanol extract is shown in fig. 4 (a).

FTIR profiling

ATR-FTIR analysis of the ethanol extract of *Ficus benghalensis* identified several functional groups present in various compounds within the crude extract. The FTIR spectrum was characterized by distinct peaks corresponding to specific bond vibrations at different wavenumbers. The range of 400 to 1300 cm^{-1} is crowded with numerous fingerprints of functional groups. Important peaks identified in the functional group region are C-N stretching (1329 cm^{-1}), C-O stretching (1381 cm^{-1}), C=C stretching (1654 cm^{-1}), sp^3 C-H stretching (2887 cm^{-1}), sp^2 C-H stretching (2974 cm^{-1}) and O-H stretching (3328 cm^{-1}). FTIR spectrum of ethanol extract of the plant is shown in fig. 4 (b).

GC-MS profiling

GC-MS chromatogram of ethanol extract of *Ficus benghalensis* is shown in fig. 5. The GC-MS exploration revealed different phytochemical classes, including alkaloids such as piperine and its isomeric form piperine, (Z)- reported for the very first time in this plant. Piperine has also been reported in other Ficus species such as *F. religiosa* (Salehi *et al*., 2021). Other phytochemical classes include polyphenols, terpenoids, sterols and fatty acid esters. The names of compounds identified in GC-MS, their molecular formulae, molar weights, retention time, % area under curve (AUC) and the qualitative percentage of matching are concluded in table 5 and their structures are shown in fig. 6.

Antiepileptic activity

Onset of seizure

The time (seconds) for the onset of epileptic seizure is recorded as the appearance of the first twitch after seizure induction with PTZ (Fig. 7(a)). The initiation of seizure was rapid in the disease control group, with the mean onset time of 20.33±7.26 seconds, which was significantly delayed by diazepam in the positive control group and the ethanol extract treatment group by 96.33±25.38 seconds and 106.17±28.51 seconds, respectively (*p<0.0001*).

Latency to first hindlimb extension

PTZ-induced epileptic mice showed prominent hindlimb extension in 74.17±13.41 seconds as an important marker for the severity of the epilepsy. Hindlimb extension was significantly retarded in the ethanol extract group (*p=0.0003*) of the plant by 193.17±67.67 seconds compared to the positive control group, which exhibited complete inhibition of the hindlimb extension throughout the duration of observation of mice (30 minutes) (Fig. 7(b)).

Latency to first tonic-clonic seizure

Time (seconds) taken from the dose of administration of PTZ via the intraperitoneal route to the onset of tonic-clonic seizure is indicative of the ferocity of the epileptic attack. The ethanol extract significantly reduced tonic-clonic seizures compared to the negative control (p = 0.0006). The positive control group revealed that DZP thoroughly reversed the onset of tonic-clonic seizures (Fig. 7(c)).

Duration of seizures

The total duration of all the seizures in each mouse during the whole time of the study was noted and compared. The data of Tukey's multiple comparison test revealed that the ethanol extract and positive control significantly decreased the duration of seizures to 43.83±7.33 seconds and 23.83±10.28 seconds (*p=0.0001* & *p<0.0001*) compared to the negative control group with 120.17±46.07 seconds. (Fig. 7(d)).

Seizure frequency

The number of seizures per mouse after PTZ-triggering was recorded and analyzed statistically (Fig. 7(e)). The seizure frequency significantly dropped from 16.33±2.028 seizures in the disease control group to 8.83±1.222 seizures in mice treated with ethanol extract (*p=0.0016*) and 6.5±0.563 seizures in the treatment control group (*p<0.0001*).

Table 1: Study groups involved in experiment

Group title	Abbreviation used for group title	Drug administered
Normal control	NC	Normal Saline
Diseased control (Negative control)	PTZ	PTZ only
Treatment control (Positive control)	DZP	DZP & PTZ
Treatment group	Eth	Ethanol extract & PTZ

Table 2: Proximate composition of *Ficus benghalensis* air-dried powder

Parameter	contents (%w/w) ± SD
Total ash	3.85 ±0.076
Acid-insoluble ash	2.60 ±0.034
Water soluble ash	0.77±0.029
Moisture content	12.46±0.301
Water extractives	4.55±0.023
Ethanol extractives	5.51±0.083
Petroleum ether extractives	2.75±0.064
Chloroform extractives	3.05±0.070
Foaming index	0.72±0.076
Swelling index	3.45±0.206

(Values are presented as mean ± SD (n=3)

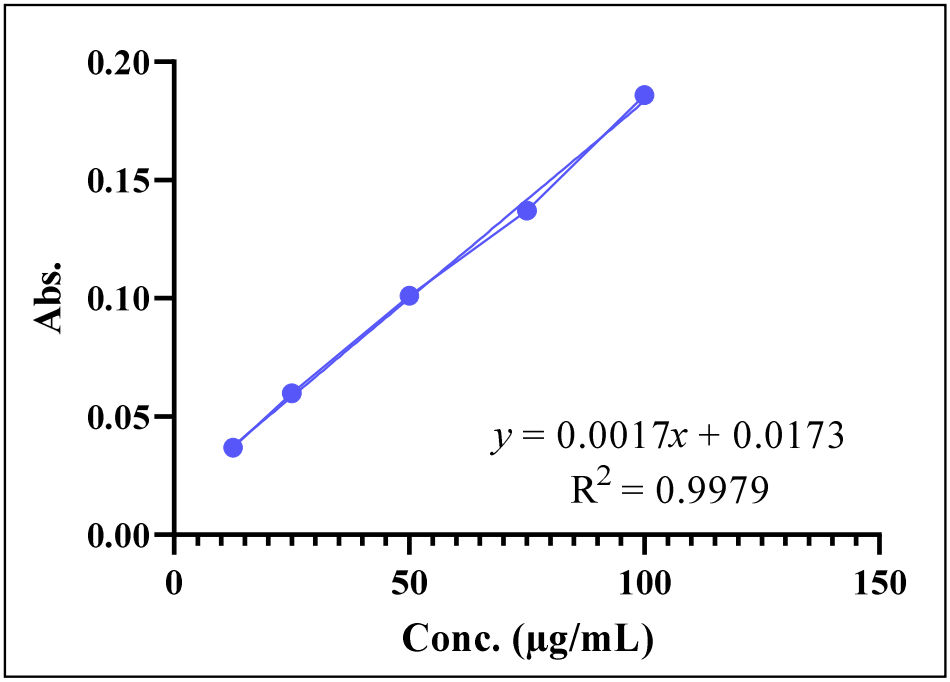

Fig. 1: Calibration curve of bovine Serum albumin (BSA) used for the determination of total protein contents

Table 3: Primary metabolites & nutritional composition

Primary metabolites	contents ((%w/w) ± SD
Total carbohydrates	20.74±0.582
Total lipids	2.07±0.011
Total proteins	60.87±0.385
Caloric value (calories per 100g)	345.06±1.280

(Values are presented as mean ± SD (n=3)

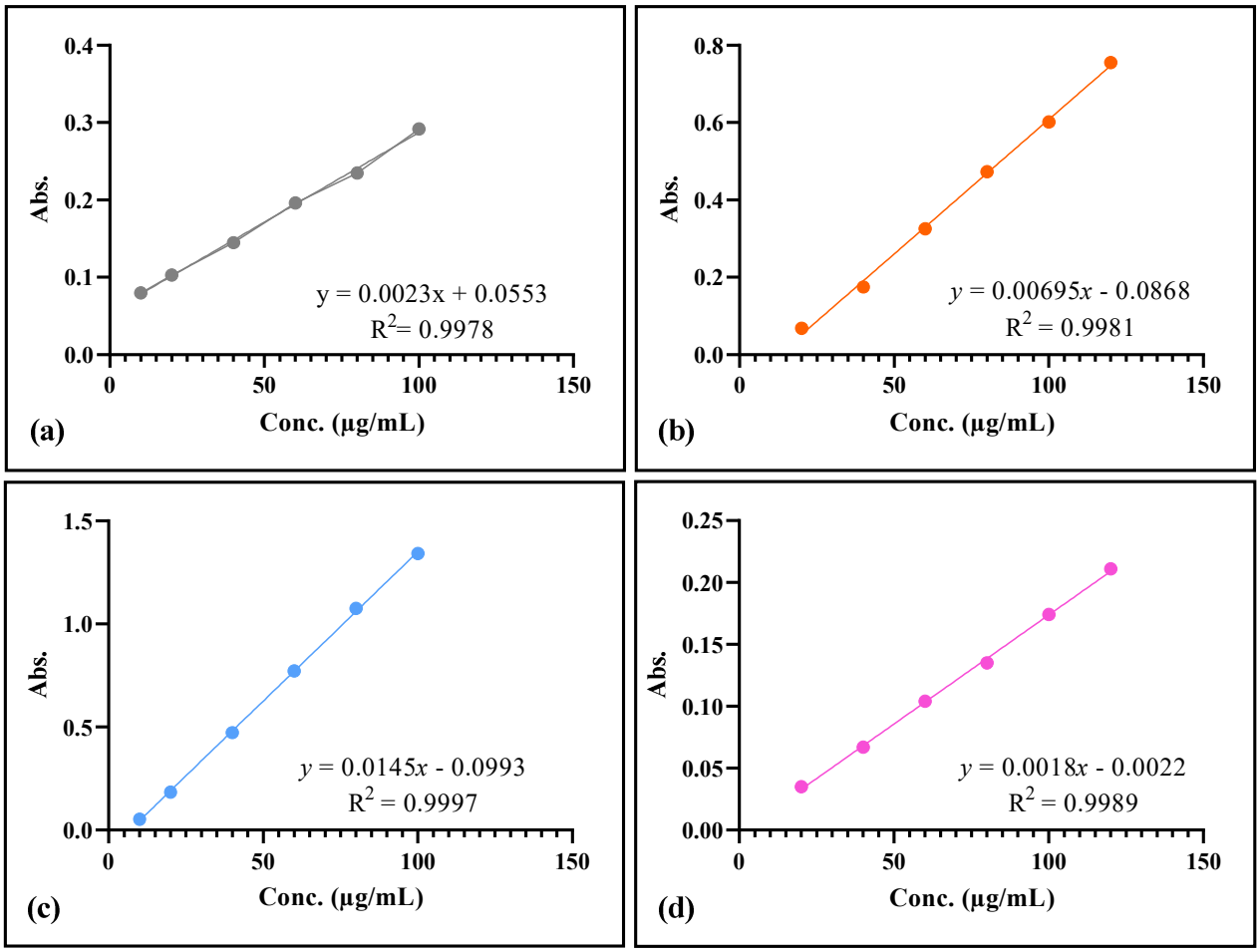

Fig. 1: Calibration curves of (a) gallic acid, (b) quercetin, (c) tannic acid and (d) glucose for the determination of phytochemicals

Table 4: Crude phytoconstituents in the air-dried powder and ethanol extract of *Ficus benghalensis*

Crude phytoconstituents	Air-dried Powder (mcg/g)	Ethanolic extract (mcg/g)
Total proteins	60.87±0.385	22.53±0.339
Total polysaccharides	116.04±0.361	34.07±0.848
Total polyphenols	119.60±1.697	55.31±0.251
Total flavonoids	22.94±0.059	152.01±0.082
Total tannins	10.97±0.077	43.16±0.159
Total glycosaponins	2.97±0.0028	2.88±0.006

(Values are presented as mean ± SD (n=3)

However, the intensity of the seizures varies for each group.

Intensity of seizure

The severity of epileptic seizures as the stage of seizure is unveiled in Fig. 7(f). The treatment of mice with ethanol extract decreased the severity of PTZ-induced seizures from stage 6 to stage 5 ($p=0.0012$). The diazepam treatment in the positive control group, on the other hand, strictly restricted the seizures to stage 1, which mostly appeared as minor jerks only ($p<0.00001$).

Total seizure score

The total score of each mouse for the number and extent of seizures is an indication of overall seizure conditions, as shown in Fig. 7(g). The ethanol extract group unveiled significant improvement in seizure intensity and frequency in contrast with the negative control group ($p=0.0054$).

Duration of post-ictal phase

The time taken for a mouse to recover to normal after a seizure is sum-up in Fig. 7(h). Mice treated with ethanol extract before the intraperitoneal administration of PTZ exhibited significantly faster recovery from seizures, in contrast to the disease control group ($p<0.0001$).

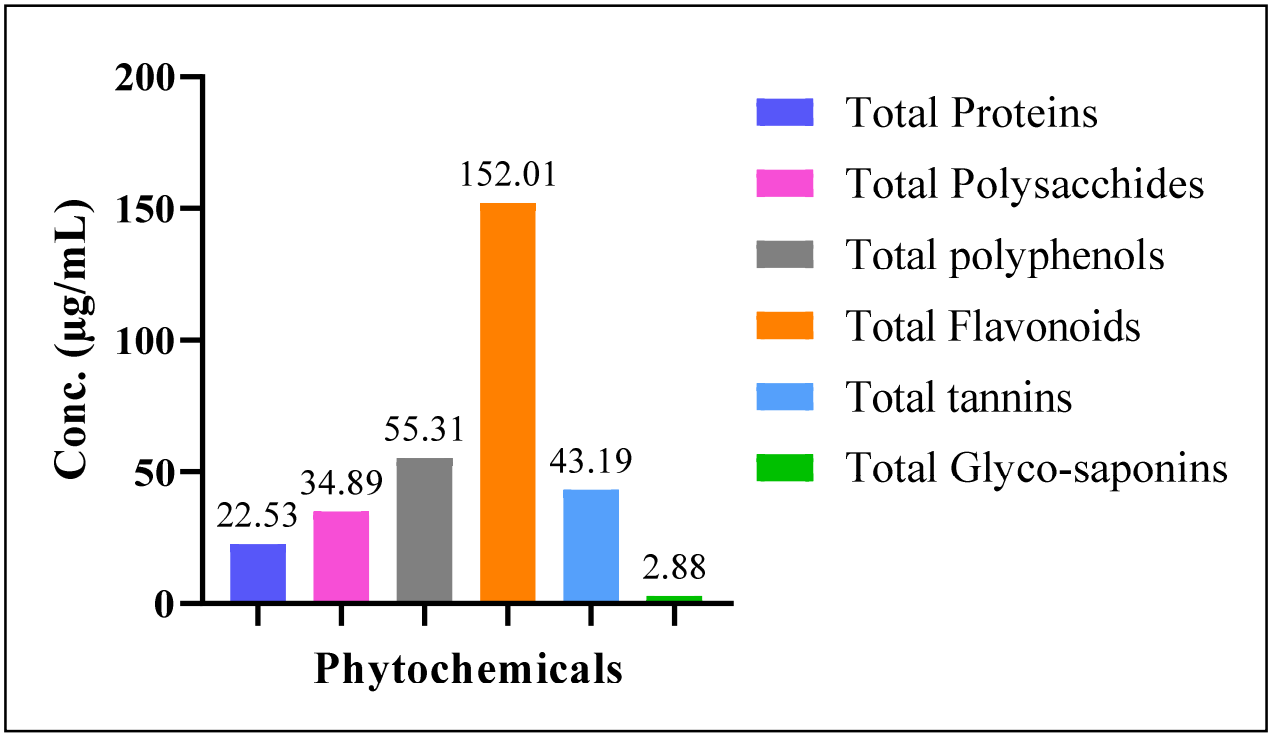

Fig. 3: Comparison of primary & secondary metabolites of *Ficus benghalensis* in the ethanol extract

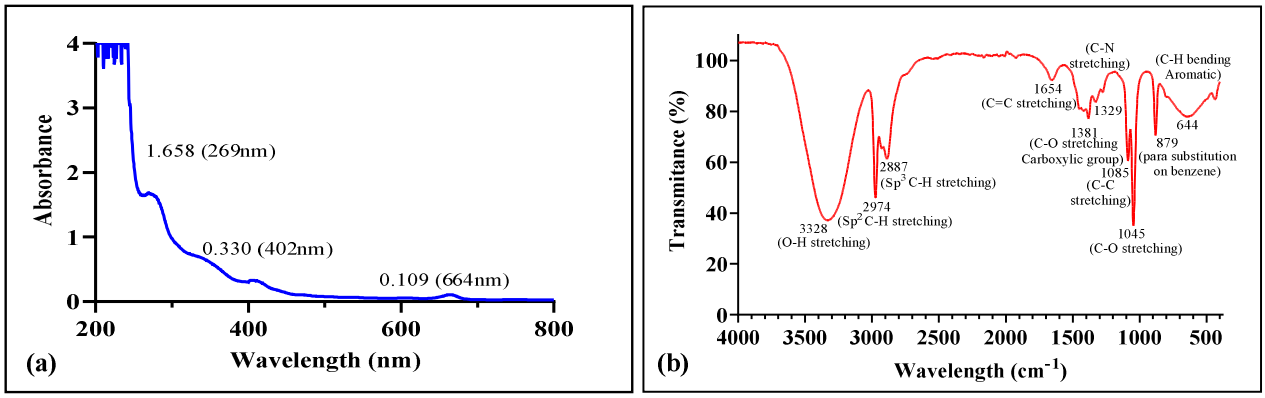

Fig. 4: (a) UV-Visible & (b) FTIR spectra of ethanol extract of *Ficus benghalensis*

An ordinary one-way ANOVA of all study parameters for each group is summarized in Supplementary Table 1.

DISCUSSION

Phytochemicals in plants exhibit a wide range of biological effects and can be used in the prevention and management of emerging diseases such as epilepsy. *Ficus benghalensis* holds several pharmacological properties that can be ascribed to its copious and diverse phytochemicals (Yaqub *et al*., 2025). The plant was standardized for its nutritional constituents and validation for regulatory compliance by proximate analysis of the powder which revealed the air-dried plant material contained a very small amount of water that could not encourage microbial growth or deterioration through hydrolysis. Similarly, ash values of crude products estimate the quality and purity of the herbal material as it contains a reasonable amount of minerals and lesser siliceous contents (WHO, 2011). The values of extractable matter are very crucial in the choice of solvent for extraction and show that slightly polar solvents are more effective for the extraction of *Ficus benghalensis*. Thus, by comparing different solvents, ethanol was chosen for cold extraction as it yielded the maximum amount of extractable matter as compared to chloroform, petroleum ether and water. The primary metabolites in *Ficus benghalensis* exhibit its high nutritional composition.

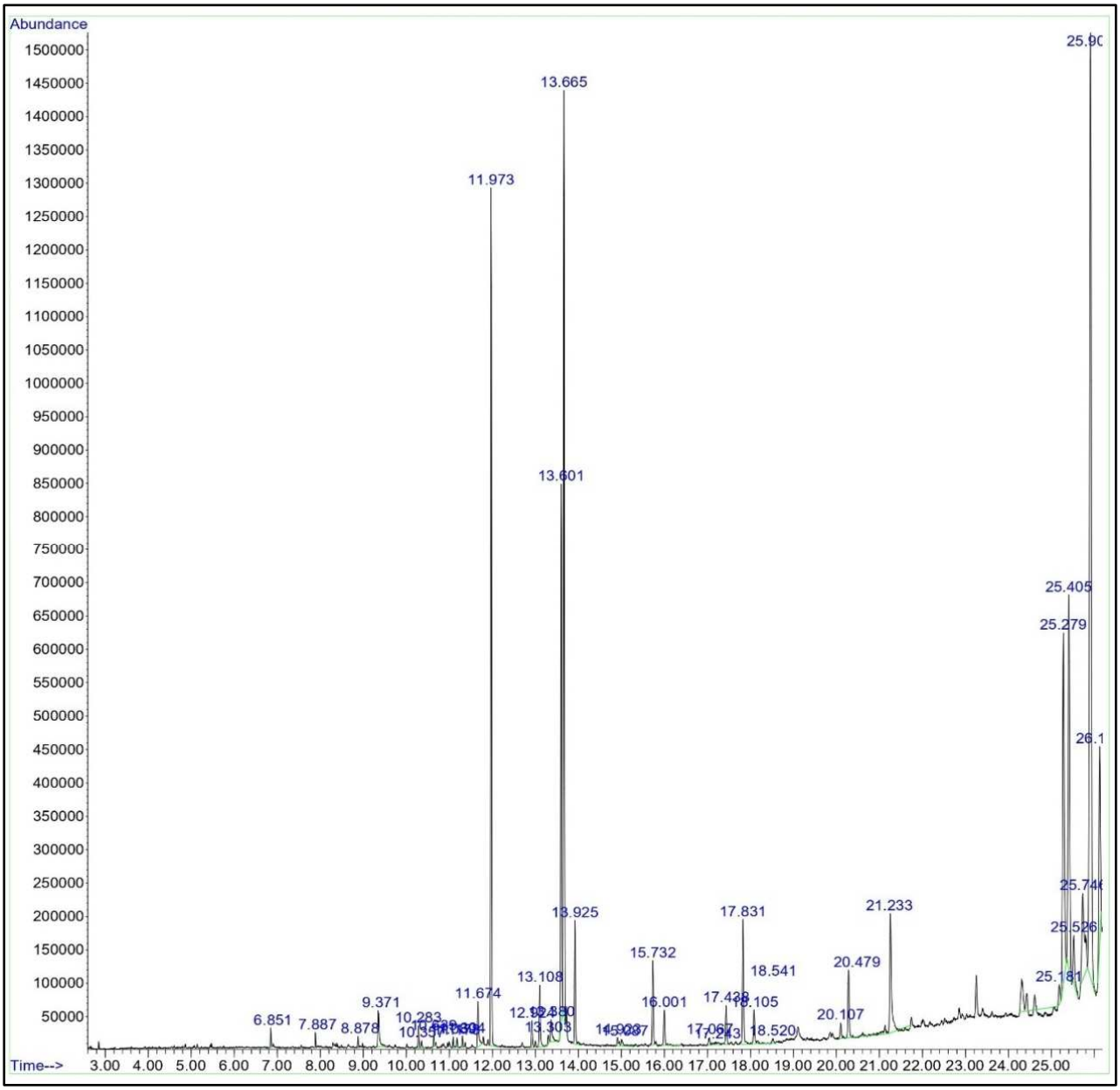

Fig. 5: GC-MS spectrum of ethanolic extract of *Ficus benghalensis*

Table 5: Interpretation of GC-MS data of ethanol extract of *Ficus benghalensis*

Sr. No.	Peak No.	Compound Name	Molecular Formula	Molecular weight (g/mol)	Area (%)	Retention time (min)	Qualitative percentage of matching
1	1	3-methyl-4-isopropyl phenol	$C_{10}H_{14}O$	150.2176	0.19	6.848	94
2	4	2-chlorodiphenyl methane	$C_{13}H_{11}Cl$	202.679	0.82	9.369	97
3	5	Tetra decanoic acid, ethyl ester	$C_{16}H_{32}O_2$	256.4241	0.27	10.285	95
4	7	Neo-phytadiene	$C_{20}H_{38}$	278.5157	0.15	10.639	83
5	8	Pentadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	270.4507	0.11	11.091	97
6	9	Lidocaine	$C_{14}H_{22}N_2O$	234.3373	0.12	11.178	80
7	10	Benzene, (1-methyl dodecyl)-	$C_{19}H_{32}$	260.4574	0.13	11.306	87
8	11	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	0.81	11.677	98

Table 5 is countinue……..

Sr. No.	Peak No.	Compound Name	Molecular Formula	Molecular weight (g/mol)	Area (%)	Retention time (min)	Qualitative percentage of matching
9	12	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.4772	10.99	11.972	99
10	13	Heptadecanoic acid, ethyl ester	$C_{19}H_{38}O_2$	298.5083	0.39	12.923	98
11	14	Phytol	$C_{20}H_{40}O$	296.5310	1.0	13.108	91
12	15	9,12-Octadecadienoic acid (z, z)-	$C_{18}H_{32}O_2$	280.4455	0.14	13.305	98
13	16	9,17-Octadecadienal, (z)-	$C_{18}H_{32}O$	264.4461	0.48	13.381	91
14	17	9(E), 11(E)-Conjugated linoleic acid, ethyl ester	$C_{20}H_{36}O_2$	308.4986	7.77	13.601	99
15	17	Linoleic acid, ethyl ester	$C_{20}H_{36}O_2$	308.4986	7.77	13.601	99
16	18	Ethyl Oleate	$C_{20}H_{38}O_2$	310.5145	13.11	13.665	99
17	19	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312.5304	1.8	13.926	99
18	22	11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	324.5411	1.36	15.734	70
19	23	Eicosanoic acid, ethyl ester	$C_{22}H_{44}O_2$	340.5836	0.67	16.001	99
20	26	Bis (2-ethylhexyl) Phthalate	$C_{24}H_{38}O_4$	390.5561	0.67	17.438	90
21	27	Ethyl-13-docosenoate (ethyl erucate)	$C_{24}H_{46}O_2$	366.6	2.23	17.833	99
22	32	Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (z, z)- [Piperine,(z)-]	$C_{17}H_{19}NO_3$	285.3377	2.67	21.235	99
23	32	Piperine	$C_{17}H_{19}NO_3$	285.3377	2.67	21.235	99
24	35	Gamma. -Sitosterol (γ-Sitosterol)	$C_{29}H_{50}O$	414.7067	8.15	25.281	99
25	36	Beta-Amyrone (β-Amyrin)	$C_{30}H_{48}O$	424.7015	8.37	25.403	99
26	36	Alpha-Amyrone (α-Amyrin)	$C_{30}H_{48}O$	424.7015	8.37	25.403	94
27	39	Lup-20 (29) –en-3-one [Lupenone]	$C_{30}H_{48}O$	424.7015	24.46	25.907	99
28	40	9,19 Cyclolanost-24-en-3-ol, (3.beta.)- [cycloartenol]	$C_{30}H_{50}O$	426.7174	4.22	26.122	99

The plant components are rich in proteins that constitute the major part of the nutritional content. Later phytochemical screening portrays the presence of numerous classes of metabolites in *Ficus benghalensis*, among which polyphenols and flavonoids are most abundant. Phenolic compounds such as polyphenols and flavonoids possess antioxidant, neuroprotective, antidiabetic, cytotoxic and antimutagenic properties and have also been found to impart their roles to angina pectoris, lymphocytic leukemia, rhinitis, cervical lesions, menopause, dermatopathy and cerebral infarctions (Ahirwar *et al*., 2018, Singh *et al*., 2023a, Wang *et al*., 2023). The high levels of polyphenols and flavonoids in *Ficus benghalensis* suggested that the reduction in overall PTZ-induced seizures might be due to the antioxidant and neuroprotective effects of these phytochemicals (Rabidas *et al*., 2023). Tannins have little therapeutic value because they are easily oxidized and polymerized in the solution and thus lose their astringent action (WHO, 2011). A high amount of polysaccharides has also been identified in ethanol extract, which acts as a storehouse of energy or as indigestible dietary fibers (Gidley and Yakubov, 2019). Regular intake of dietary bulk prevents hyperlipidemia, hypercholesterolemia, inflammation, malignancy, obesity, cardiovascular disorders and improves insulin sensitivity as well as the friendly flora of the gut (Kumar *et al*., 2023). Glycosaponins have also been detected in the air-dried powder of *Ficus benghalensis* and its ethanolic extract. Saponins are glycosides and are famous for having antimicrobial (both antibacterial & antifungal), hypoglycemic, hypolipidemic and virucidal effects. Moreover, they are also helpful in acute injuries, edema in chronic deep vein incompetence, benign prostatic hyperplasia, erectile dysfunction, as well as systemic lupus erythematosus (Wang *et al*., 2023).

Elemental composition of the extract was unveiled by a CHNS-O Flash 2000 elemental analyzer, which indicates high carbon and oxygen contents (Logesh *et al*., 2023). The high carbon and hydrogen contents in elemental analysis suggest that the extract is rich in organic compounds. The presence of oxygen is indicative of hydroxyl, carboxyl, ether and/or ester groups commonly present in polyphenols, flavonoids and fatty acids. The small percentage of nitrogen and sulfur suggested a minute presence of alkaloids &/or amines and organosulfur compounds such as thiol or sulfur-containing proteins (Singha *et al*., 2007, Sharma *et al*., 2009, Karthikeyan *et al*., 2019). The ratio of the element suggests terpenoids, polyphenols and flavonoids as major secondary metabolites that may contribute to the decline in epileptic seizures.

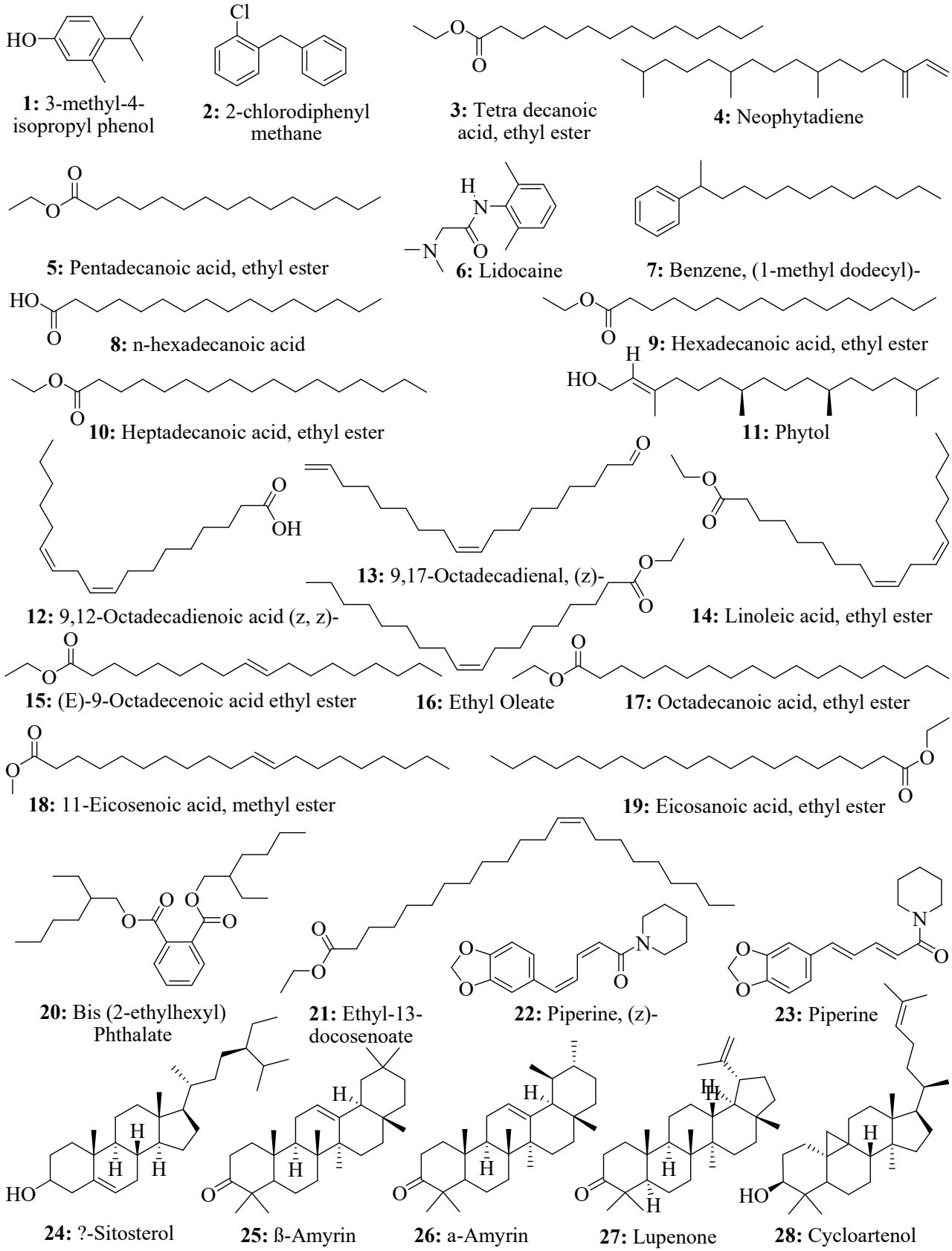

Fig. 6: Structures of phytoconstituents detected by GC-MS analysis

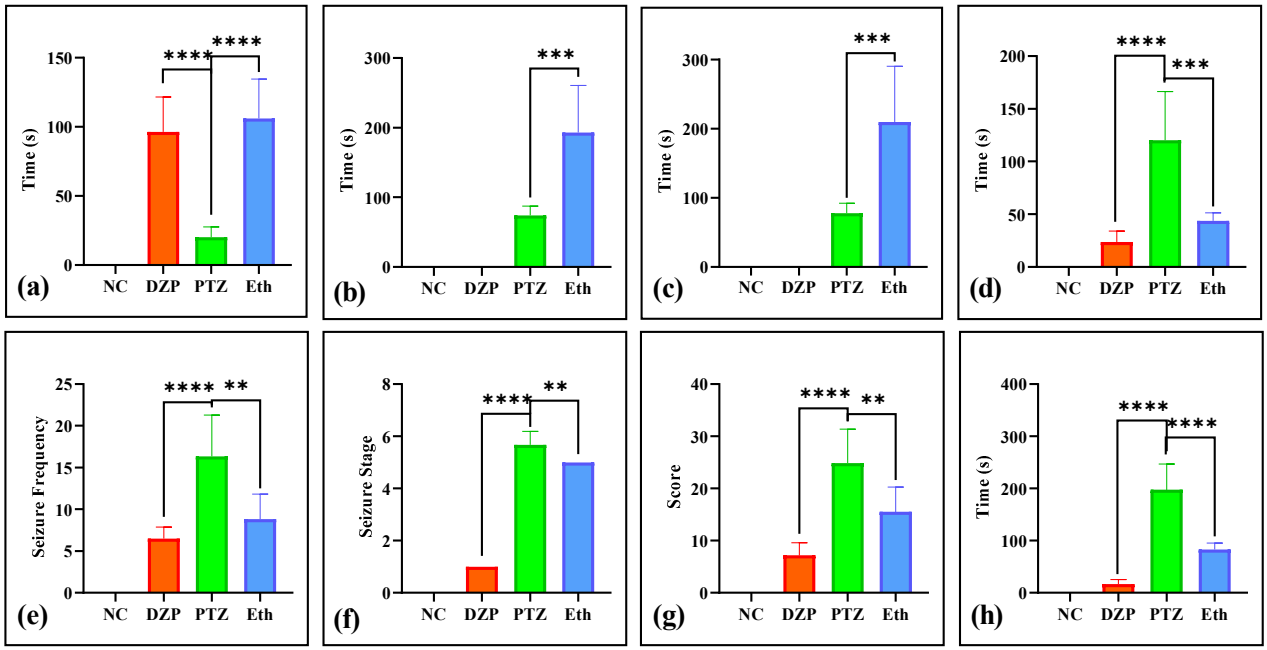

Fig. 7: Characteristics of Seizures (a) Latency to first twich, (b) Latency to first hind-limb extension, (c) Latency to first tonic-clonic seizure (d) Duration of seizures (e) Number of Seizures (f) Stages of seizures (g) Total seizure score (h) Recovery from seizures after PTZ-induced epilepsy
NC: normal group, DZP: diazepam treated control group, PTZ: Negative control group, Eth: Ethanol extract treatment group. The data is presented as mean ± SD (n=6)

The UV-Visible spectra revealed the presence of chromophores containing π-bonds and lone pair of electrons. The lambda maximum in UV regions indicates the presence of π to π* and n to π* transitions in conjugated double bonds that would be an aromatic system. The lambda maximum of the extract near 400nm exhibits the π to π* transition, probably due to isolated or nonconjugated double bonds. The appearance of absorbance bands in ultraviolet and visible regions specifies the presence of secondary metabolites, such as polyphenols and flavonoids, in the plant (Karpagasundari and Kulothungan, 2014). The peaks at 402nm and 664nm indicate strong porphyrin absorption exhibiting the typical π to π* transition in tetrapyrroles and characteristics for chlorophylls (Pandey *et al*., 2024). The regions of the IR spectra describe the existence of double and single bonds in the functional group regions, while the area indicating the presence of triple bonds is devoid of any absorbance band (Nandiyanto *et al*., 2019). O-H stretching between 3300 to 3400 cm^{-1} and C=O stretching around 1700 cm^{-1} are characteristic of the carboxylic group and the broadness of O-H stretching indicates H-bonding of the compound (Coates, 2000). The strong peaks revealed the presence of a number of fatty acids and their esters in the extract, as apparent from the GC-MS results. Similarly, the appearance of multiple peaks around 2930 cm^{-1}, 2860 cm^{-1}, 1460 cm^{-1} and 700 cm^{-1} indicates the presence of linear long-chain aliphatic compounds, while a prominent peak between 870 and 950 cm^{-1} revealed the para-substituted aromatic compound and C-N stretching vibration at 1329 cm^{-1} revealed aromatic tertiary amine. (Coates, 2000). Overall, the extract appeared to have good quantities of terpenoids (that might be α- & β-amyrins, γ-sitosterol, lupenone and cycloarterol, etc.), phenolic compounds (lutein, caffeic acid, gallic acid, quinic acid, etc.), flavonoids (apigenin, carpachromene, kaempferol, quercetin, etc.), long-chain aliphatic and aromatic carboxylic acids and their esters. Other functional groups identified in the extract include ketone (C=O stretching near 1700), alkenyl group (C=C stretching at 1641 cm^{-1}), ether, alcohol and glycosidic linkage (C-O stretching between 1000-1300 cm^{-1}) (rutin, rhein, furostanol, etc.). Many of these phytochemicals have also been found in other Ficus species as well (Yaqub *et al*., 2025). Among these compounds, β-amyrin, apigenin, caffeic acid, chlorogenic acid, gallic acid, kaempferol, naringenin, protocatechuic acid, quercetin, rutin and ursolic acid from different plant species have been established to modulate $GABA_A$-related neurotransmission in PTZ-seizure models (Khan *et al*., 2016, Çiçek, 2018, Rabidas *et al*., 2023, Althagafi, 2024, Yazğan, 2024, Li *et al*., 2025). Several of these substances have been found in numerous plants of the genus Ficus (Barot and Barot, 2025). The GC-MS data validated the presence of bioactive phytochemicals and the relative heights of peaks confirmed their relative quantities. The most probable active phytochemical for antiepileptic activity, identified by GC-MS, was piperine (Mishra *et al*., 2015), phytol (Costa *et al*., 2012), lidocaine (DeToledo,

2000), α- & β-amyrins (Aragão *et al*., 2015), neo-phytadiene (Gonzalez-Rivera *et al*., 2023) and a thymol derivative; 3-methyl-4-isopropyl phenol (Waris *et al*., 2024).

Further, we investigated the antiepileptic potential of the plant extract via an acute study on a murine epilepsy model using pentylenetetrazol (PTZ). The study revealed the neuroprotective effects and resultant anti-epileptic propensities of ethanol extract of *Ficus benghalensis* against PTZ-induced seizures in the guise of delay in the onset of seizures, decline in the stage, number and duration of seizures and reduction in the post-ictal phase. PTZ is a bicyclic tetrazole derivative that stimulates epileptiform seizures by blocking GABA-mediated transmission in cortical neurons by interacting with the benzodiazepine (BZP) recognition sites of the $GABA_A$ receptor complex. No endogenous agonist of this site is known; however, BZP and non-BZP agonists act as positive allosteric modulators of the $GABA_A$ receptors at this site (Wang *et al*., 2024). Long-term treatment of PTZ increases the glutamate-mediated excitation in the brain by upregulating the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors and N-methyl-D-aspartate (NMDA) receptors (Samokhina and Samokhin, 2018). It also causes metabolic disruptions in the temporal lobe, including low blood perfusion, decreased glucose metabolism and depletion of hippocampal neurons. PTZ-generated seizure induction is linked with increased glutamate concentration and production of free radicals in neurons. Thus, such seizures imitate the elevated oxidative and nitrosative stress in the brain. Reactive oxygen species from the mitochondria of neurons increase lipid peroxidation, thereby altering the membrane phospholipid metabolism, which results in the emission of free radicals in different regions of the brain, such as the hippocampus, cerebral cortex and striatum, during seizures (Ramalingam *et al*., 2013, Samokhina and Samokhin, 2018). Two key contributors involved in epilepsy are oxidative stress and damaged blood-brain barrier (BBB), which result in neuro-inflammation and ultimately neurodegeneration (Firdous *et al*., 2021). Inflammatory processes and mediators such as interleukins (IL), such as IL-1β, IL-6, nuclear factor-kappa B (NF-κB) and tumor necrosis factor-α (TNF-α) are the primary contributors to seizure induction and progression as more free radicals are generated during inflammation and these free radicals result in more glutamate release, which further worsens the seizures (Duan *et al*., 2024). The herbal anticonvulsant drugs demonstrate their antiepileptic effects via five well defined mechanisms: 1) enhancement of GABAergic synaptic transmission, 2) scale-down membrane excitability by inhibiting cation channels (e.g. Na^+ and Ca^{2+} channels), 3) refinement in mitochondrial function by antioxidants to decrease oxidative stress, 4) boost the immune response with anti-inflammatory actions and 5) reduction in metabolism and transport protein synthesis (Tabassum *et al*., 2024).

Several other *Ficus* species, such as *F. sur, F. carica, F. hispida, F. racemose, F. platyphylla, F. benjamina, F. religiosa, F. sycomorus* and *F. abutilifolia*, have been documented to suppress seizures by different mechanisms, including increased GABAergic synaptic transmission and decreased GABA-transport protein synthesis (Singh *et al*., 2023b, Alam and Mazumder, 2025). The rich phytochemistry of the plant suggests that *Ficus benghalensis* may exert its antiepileptic aptitude via almost all of these mechanisms. GC-MS analysis also verifies the reported phytochemical constituents such as fatty acids, glycosides, terpenoids, saponins, tannins, flavonoids and polyphenols (Murugesu *et al*., 2021, Singh *et al*., 2023a). The antiepileptic activity of the plant can be attributed to the presence of polyphenols, flavonoids and terpenoids on GABA receptors and gated ion channels (Schachter, 2015). Some detected compounds have been reported to have antiepileptic potential, such as lidocaine and piperine that exert their anticonvulsant action by inhibiting sodium channels at low concentration and $GABA_A$ receptors, respectively (Mittal *et al*., 2011, Kaur *et al*., 2021, Santos *et al*., 2021). Moreover, the terpenes present in the plant, such as α- & β-amyrins, phytol and γ-sitosterol, may also possess antiepileptic potential by acting as antioxidants to reduce neuroinflammation (Holanda Pinto *et al*., 2008, Rabidas *et al*., 2023).

Numerous reported flavonoids from *Ficus benghalensis*, such as ursolic acid, quercetin and rutin etc., are known to truncate convulsions by activating $GABA_A$ receptors and suppressing the inflammatory mediators like IL-1β, IL-6, TNF-α and NF-κB in PTZ-induced kindling in rodents, thereby exhibiting anti-epileptic potential (Khan *et al*., 2016, Al-Khayri *et al*., 2022, Amin *et al*., 2022). Sterols and saponins such as β-sitosterol, γ-sitosterol and stigmasterol found in the plant are also capable of seizing the GABAergic transmission by inhibiting $GABA_A$ receptors (Karim *et al*., 2021, Wang *et al*., 2022). Saponins from other Ficus species have also been reported to exhibit antiepileptic response by inhibiting the voltage-gated sodium and calcium channels without altering ligand-gated sodium and calcium channels function. (Singh and Goel, 2016). Other anti-inflammatory polyphenols, flavonoids and terpenoids of *Ficus benghalensis,* such as apigenin, kaempferol, catechin, gallocatechin, naringenin, cyanidin and pelargonidin, might also have a role in seizure suppression by decreasing the neurodegeneration through the inhibition of inflammatory mediators (Al-Khayri *et al*., 2022). However, a detailed study based on chromatographic isolation and purification of its constituents and their concomitant antiepileptic exploration is required to evaluate and ascertain the *in vivo* propensities of these phytochemicals. Thus, our findings suggest that *Ficus benghalensis* contains bioactive compounds with the potential to mitigate the severity of epileptic seizures, making it a promising source for identifying and discovering new lead compounds for epilepsy treatment.

CONCLUSION

The present study demonstrates that *Ficus benghalensis* contains diverse phytochemicals, particularly polyphenols, flavonoids, terpenoids, saponins, and sterols, which may collectively contribute to its observed anticonvulsant effects in the acute PTZ-induced seizure model. By attenuating the seizure onset, frequency and severity, these phytoconstituents appear to exert neuroprotective, antioxidant and GABA-modulating actions, positioning *Ficus benghalensis* as a promising source of lead compounds for novel antiepileptic drug discovery.

Acknowledgement
Not applicable.

Authors' contribution
MI and HS conceived the idea and designed the experiment. SY performed the experiment, and MI, HS and AA contributed to analysing and the interpretation of the data. SY and AA contributed to drafting and reviewing of the manuscript.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Data availability statement
All data generated or analysed during this study are included in this published article

Ethical approval
The experiment techniques and procedures were used in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Punjab University Institutional Ethics Review Board, University of the Punjab (ethical approval number: No.D/13/FIMS, dated 28-02-2024).

Conflict of interest
The authors declare no conflict of interest

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