ISSN: 2320-2831

International Journal of Pharmacy and Analytical Research (IJPAR)

IJPAR |Vol.15 | Issue 1 | Jan - Mar -2026

www.ijpar.com

DOI : https://doi.org/10.61096/ijpar.v15.iss1.2026.80-94

Review

Multifunctional Tannins from *Phyllanthus amarus* Exhibit Disease-Modifying Activities Against Alzheimer's Pathogenesis: An *In Vitro* and *In Vivo* Investigation

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Published on: 13.02.2026

Published by:
Futuristic Publications

Abstract

Alzheimer's disease (AD) manifests as a chronic neurodegenerative syndrome featuring cognitive impairment alongside characteristic neuropathological markers: amyloid-beta (Aβ) plaques and tau-associated neurofibrillary tangles. Absence of curative interventions necessitates exploration of novel therapeutic candidates. Phyllanthus amarus, recognized for its phytochemical diversity including tannins and lignans, demonstrates antioxidant and neuroprotective bioactivities warranting AD-focused investigation.

This investigation employed systematic extraction and characterization of tannins from Phyllanthus amarus, subsequently evaluating their anti-Alzheimer properties through cellular and transgenic animal models. Experimental data revealed substantial neuroprotective effects, including suppression of Aβ aggregation and mitigation of neuroinflammatory processes. Behavioral studies in AD-model mice demonstrated cognitive function restoration following tannin administration. Molecular analyses identified modulation of key pathological pathways, specifically amyloid precursor protein processing regulation and oxidative stress amelioration.

Findings validate Phyllanthus amarus tannins as promising disease-modifying candidates for Alzheimer's therapy. This research strengthens the scientific rationale for phytochemical-based neurotherapeutic development, presenting viable strategies for addressing neurodegenerative conditions.

Keywords: Alzheimer's disease, Phyllanthus amarus, Tannins, Amyloid-beta.

INTRODUCTION

Today, deprived memory, sluggish recall, and lesser retention are widespread concerns worldwide. Alzheimer's disease (AD), the most prevalent neurodegenerative condition which impairs memory and the brain, hence is regarded as the main contributor to dementia in the aged population[1-2].AD is attributed to expedited accumulation of Aβ (beta-amyloid) plaque around neurons and neurofibrillary tangles (NFTs) comprised of tau proteins linked to hyper phosphorylated microtubules. The amyloidogenic proposition suggests Aβ as a crucial played in the AD pathologies[3]. The therapy/management of the CNS disorders is a problem due to the complexities of these disorders as well as the limitations of the available allopathic medications that are currently in the market. More than 100 years after the initial description of AD and the identification of Aβ as a key pathologic component, the search for effective anti-Aβ therapies continues[4].

Traditional medicine still contributes significantly to treatment regimen in developing nations. India has a rich history of ayurvedic medicines, which have provided effective treatment for numerous disease-like conditions using plants, plant parts, and plant-derived compounds[5,54]. *Phyllanthus amarus* Schumm and Thonn (Family Euphorbiaceae) generally referred as bhumi amla, is used since years to treat different disease-like conditions such as diabetes, dropsy, flu, and jaundice. It has been reported to exhibit hepatoprotective, diuretic, antiviral, anticancer, anti-inflammatory, and antioxidant activity. One of the *P. amarus* extract has demonstrated potential for the treatment of nervous debility and epilepsy[6]. Also, our laboratory has investigated pain modulatory potential and antifibromyalgic activity of different extract of *P. amarus*[19-20]. This investigation was intended to assess the anti-Alzheimer-like potential of *P. amarus*. We have investigated probable mechanisms involved using molecular docking simulations.

MATERIAL AND METHODS

Animals used

In the current investigation, Swiss albino mice of any sex were employed. We used young mice, 2-3 months old, weighing about 20g, and old mice, 12-15 months old, weighing around 30g. Six mice were housed in each cage under the usual laboratory lighting (12-hour cycles of darkness and light) and temperature regimes. Water and food are available at all times. The studies took place between 9 am and 3 pm. Prior to the series of experimental experiments, animals were fasted for 12 hours (from food but not from water). Mice were given time to adjust to the laboratory setting.

The institutional animal ethical committee, which was established by the Ministry of Environment and Forests of the Government of India, New Delhi, to control and supervise the use of experimental animals, approved the experimental protocols, which were then carried out in accordance with the aforementioned guidelines. IAEC/ABCP/18/2018-19 and RCP/18 19/P-19 were the protocol numbers that were approved.

Drugs, Tannin rich extract, and chemical agents

The *P. amarus* leaf standardized hydromethanolic extract, referred as *Phyllanthus amarus* Tannin rich extract (PATRE), that has a greater than 5% of tannins (Ref.No. FP1102034PA/11LOT/02) was obtained from Natural Remedies Pvt. Ltd., Bangalore. In this study, we employed Flumazenil (Flu) from Neon Laboratories Ltd, Mumbai, Scopolamine (Sco) from Alcon Inc., and sodium chloride (NaCl), potassium bromide (KBr), all from Loba chemicals. Diazepam of Ranbaxy laboratories and Piracetam (Pir) of Dr Reddy's Laboratories Ltd were purchased from a local medical shop.

Characterization of Tannin extract (PATRE) by Fourier-Transform Infrared Spectrometry (FT-IR)- Shimadzu FT-IR-8400 was utilized to record the FT-IR spectrum. The diffusive reflectance gadget (DRS8000, Shimadzu Corp., Japan)-equipped FT-IR spectrometer and a data unit were utilized for recording the spectra. The specimens were made by combining a tiny amount of extract—roughly 2-3 mg per extract—with 100 mg of dry potassium bromide. These samples were scanned at a resolution of 2 cm-1 for the wavelength range of 4000–400 cm–1. The FTIR-8000-SCS program was employed to record distinctive peaks.

Assessment of improvement in cognitive functions using Morris Water Maze test

The Morris Water Maze (MWM) is widely used test that evaluates the drug's potential in improving learning and memory. This model throws light on ability of the phytochemicals in improving cognitive functions impaired by Sco, thereby

emphasizing its utility in cognitive disorders involving dementia. The MWM consists of a large water tank [48 x 28 x 18] cm filled with water, which is made opaque by adding milk. Water helps to eliminate olfactory obstructions if any and to provide an even unvarying environment within the maze. A [7x7] cm rectangular escape platform is constructed of water-resistant material (plexiglass in this study) that when submerged, allows experimental animals to stay on top. The platform is 10 cm in height and water is filled so that it is submerged 2 cm below the level of water surface. The water temperature is maintained at 26 ±1 degree Celsius.

To assess spatial memory, young mouse with head pointing towards side of the pool, was released and the time taken (escape latency [EL]) to reach the submerged platform was noted. With previous exposure to this set up, the time the mouse is taking to find a hidden platform using only available external cues utilized to quantify the spatial memory. For acclimatization, the mice permitted to swim for 90s before beginning of the hidden platform training. Then the platform positioned in the middle of target quadrant of the pool and the animals released in the pool from opposite quadrant. Each mouse was given 90s to reach the platform. If the animal fails to locate the platform in 90s, then the animal was guided to the platform by the researcher. Then the mouse allowed remaining on platform for 20s to rest. Again, the mouse released from same place and time for reaching the submerged platform was recorded. Likewise, totally 4 trials were conducted in a row, in a day, and average time to reach the submerged platform was recorded, keeping similar experimental conditions.

Standard drug (Pir 200mg/kg) and extract PATRE and in three doses, i.e. 100, 200, and 400 mg/kg) were administered orally and after 60 minutes all the groups were exposed to the training schedule. This procedure was repeated at 24 hour interval for three more days until each subject acquired minimum time interval to reach the submerged platform in the pool. On fourth day, all groups were administered Sco (1mg/kg, i.p.) 30 minutes later they were treated with test drugs and after 60 minutes they were tested for spatial memory. Latency to reach the platform in seconds (mean values) was calculated on days 1, 2, 3, 4, and 5. Day 2 is the day from which animals were treated with the drug. The mice were assessed again on the fifth day for spatial memory to check the ability of drug to restore Sco-induced amnesia (retention trial)[40-45].

Assessment of anti-Alzheimer activity in young versus aged mice using Elevated plus maze apparatus and with scopolamine-induced amnesia

Both young and aged Swiss albino mice utilized in this behavioral model. The 3-4 month old mice (young mice) weighing approximately 20g and 12-15 month old mice (aged mice) weighing approximately 30g were utilized. The test drug PATRE and in three doses, i.e 100, 200, and 400 mg/kg was administered orally for eight consecutive days to mice of both age groups. On eighth day, Sco 1mg/kg, was given intraperitoneally post 60 minutes of the last dose of test drugs (PATRE and in three doses) to induce amnesia in young mice. Post 45 minutes of Sco treatment, animals were permitted to the training session on elevated plus maze (EPM) apparatus. The transfer latency (TL), i.e., movement between open and closed arms of EPM apparatus was recorded. The TLs recoded on eighth day are presented as results of acquisition trail. On the ninth day (i.e. after 24 hours) the mice were assessed again on EPM to record retention of memory (Retention trail). Piracetam (200mg/kg, i.p.) was used as reference standard and was injected for 8 consecutive days and procedures outlined in the above paragraph are followed. Similarly, animals in the control group received normal saline for 8 consecutive days.

In this investigation, the EPM equipment as stated for mice by Lister RG and Pellow et al. was used. The EPM test apparatus comprised of enclosed arms sized [37X5X12] cm and open arms sized [37X5] cm and a 12cm high wall linked to 2 closed and 2 open arms, and the wall is placed so that the alike arms were opposite to each other and all four arms are linked to each other by a [5X5] cm of central square. The wooden apparatus was elevated to a height of 25cm above the floor. Each mouse was placed individually in the central square with head pointing towards open arm and TL was recorded for 5 minutes. Each mouse was utilized only once and every test was conducted during scheduled time (and other conditions as specified above in Section 7.2.1). After each test is carried out, the EPM apparatus was cleaned using ethanol. The rationale behind utilizing EPM apparatus included the fear-provoking nature of the open arms and feeling of relative safety towards closed arms and assessing the retention of memory of animals to prefer closed arms over open arms. The EPM test is a widely utilized behavioral animal

model for assessing memory and learning in rodents[7,45].

Assessment of Impact of Tannin extract (PATRE) on Motor Coordination Activity of mice

The complex system of motor coordination involves specific pattern of walking, balancing, and strength of muscle. It is well-established fact that sedatives (such as benzodiazepines, barbiturates, etc.) and other molecules/drugs that interfere with balancing or ambulatory activities or that weaken muscles have demonstrated impaired performance in the tests conducted using Rota-rod apparatus. Hence, Rota-rod apparatus is used popularly to estimate potential impact of test drugs on the motor coordination of rodents. The Rota-rod instrument (from Inco, Ambala,model no. K19616-2) comprised of a central bar (with a constant speed of 22 rpm) subdivided into 3 compartments by disks.A day (24 hours) before actual testing, mice were selected. The animals that failed to remain on the central bar for a period of 150s in two consecutive trials were excluded. Selected mice were treated with test drugs (PATRE and in three doses, ie 100, 200, and 400 mg/kg) or standard drug (Diazepam 2mg/kg) or vehicle as per the group and tests were carried out 30 minutes post treatment. The outcome measure included the time for which mice remained on the revolving bar. The cut-off time for each test was 150s.

Estimation of Acetylcholine [ACh] Levels in Brain by Quantifying Cholinesterase Inhibition

After the completion of Morris Water maze test, mouse from each group was euthanized via cervical detachment. The entire brain was taken right away and chilled in ice-cold phosphate buffer. After washing in ice-cold phosphate buffer it was homogenized in 5ml of phosphate buffer in Glass Teflon homogenizer. The brain homogenate then evaluated for enzyme activity.

Standard Curve of ACh: Aliquots of 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 ml of ACh in buffer added to different test tubes. Phosphate buffer added to each tube to give a volume of 1 ml. 2ml of Alkali added to each tube by shaking vigorously. After not less than a minute, 1 ml of HCL solution (pH of 1.2± 0.2) and 1 ml of $FeCl_3$ solution added. The absorbance of the color in each tube read at 540 nm. The control tube (with 0 ml of Ach) used to adjust the zero reading of the instrument. Plot of milimoles of Ach vs. absorbance obtained.

Determination of Cholinesterase Inhibition: Three types of test tubes were prepared: Tube 1: served as control that contained 1 ml of buffer (instead of ACh solution) and other reagents. Tube 2: served as sample or S that contained 1 ml of ACh solution, 0.1 ml of homogenate and was kept for incubation at 37°C ± 1°C for 1hr. Tube 3: served as STD or S60 that contained 1 ml of ACh solution and 0.1 ml of homogenate was added after the addition of Alk hyd which itself was added after incubation at 37 ºC±1ºC for 1 hr. After the incubation period, 2ml of Alk hyd was added by shaking vigorously to tubes 1and 2. After not less than a minute, 1 ml of HCL solution (pH of 1.2 ± 0.2) and 1 ml of FeCl3 added to all three test tubes. The resultant mixtures centrifuged and the absorbance of the supernatant read at 540 nm. Note: S60 used to correct the determination of non-enzymatic hydrolysis of ACh since the homogenate added after incubation. The control tube (with 1 ml of buffer) used to adjust the zero of the instrument.

Assessment of involvement of GABA receptor

Flumazenil (Flu), a benzodiazepine antagonist, was used in experimental procedures to evaluate if this receptor may conceivably contribute to the test drugs effects (PATRE and at highest dose, ie, 400 mg/kg). Flu 2.5mg/kg was administered to a small group of mice (4 mice per treatment group), along with the test groups and the standard Diazepam (DZP) group, in order to assess the effects using the EPM model equipment in accordance with the above-described methods.

HPTLC analysis

Advanced phytochemical investigations performed on HPTLC for identification and characterization of bioactive extract, with the help and supervision of experts from Anchrom lab Mumbai. The details of the instrumentation parameters and procedure for finger print analysis are outlined below.

Instrumentation and chromatographic conditions utilized for HPTLC analysis:

- Instrument-CAMAG Linomat 5 "Linomat5_080222" S/N 080222 (1.00.12)
- Spotting device - Linomat V Automatic Sample Spotter (Camag, Muttenz, Switzerland).
- Syringe - 100 µl (Hamilton, Bonaduz, Switzerland).

- TLC chamber - Glass twin-trough chamber (20 x10 x 4 cm; Camag).
- Densitometer - TLC Scanner 3 linked to winCATS software (Camag).
- HPTLC plates - 20 x 10 cm, 0.2 mm layer thickness, precoated with silica gel 60 F254, Cat. No. 1.05548, E. Merck KgaA, Darmstadt, Germany.

Linomat 5 application parameters: Spray gas: Inert gas; Sample solvent type: Methanol; Dosage speed: 150 nl/s; Predosage volume: 0.2 ul; Syringe size: 100 µl; Number of tracks: 4-8; Application position: 8.0 mm; Band length: 8.0 mm; Solvent front position: 80.0 mm.

Preparation of Phyllanthus Sample Solutions:

Dried powdered extract of *P. amarus* (200mg) re-extracted exhaustively with methanol using a sonicator for 1 h on a water bath. Themethanol soluble portion filtered used for the further HPTLC analysis. The stock solution ofthe sample, having concentration of 0.4 mg/ml (0.4 µg/µl) was prepared.

Mobile Phases for General Finger Print Analysis: For the creation of typical chromatograms, other mobile phases, such as toluene: ethyl acetate (80:25) and toluene: ethyl acetate: formic acid (60:20:20), were explored. Out of the various mobile phases tried, Toluene:Chloroform: Ethanol (4:4:1, v/v) gave the best resolution for development of commonchromatogram for the analysis of the components of the extract under study from each other.

General Fingerprint Analysis: HPTLC aluminum plates pre-coated with silica gel were as the stationary phase. The plates not pre-washed with any solvent prior to chromatography. The samples spotted in the form of bands, with the help of a Camag 100 micro liter syringe using a Camag Linomat V (Switzerland) sample applicator. A constant application rate 150nL/s employed. The slit dimension was kept at 6 mm × 0.45 mm, with a scanning speed of 20mm/second, and a data resolution of 100 µm/step was employed.

The composition of the mobile phase was toluene: chloroform: ethanol (4:4:1). The linear ascending development carried out in twin trough glass chamber saturated with themobile phase. The optimized chamber saturation time for the mobile phase was 30 minutes at room temperature (25 ± 2°C). The length of the chromatogram run was 80 mm. Subsequently, the plate allowed dry at room temperature. The separated bands on the HPTLC plates scanned over the wavelength of 200 – 540 nm. The source of radiation utilized was the deuterium illumination (D2 lamp) for 254 nm, Mercury (Hg) for 366 nm and for 540 nm. The images captured on Camagreprostar 3 with win-CATS software 4.05.

Statistical analysis

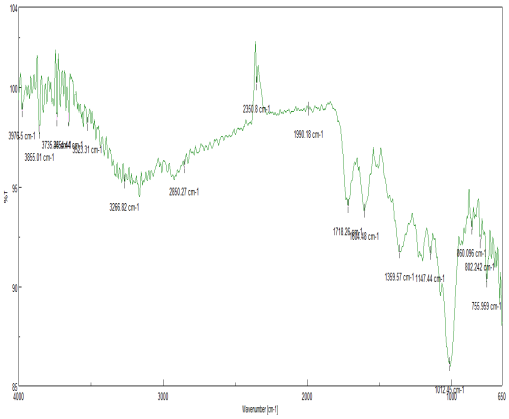
The mean and standard error were used to express every result. One-way ANNOVA was utilized for data analysis and then Dunnett's test to compare the outcomes of the test groups with those of the control group. As six mice were utilized per group, data generated has had 6 values per testing arm, ie, n=6.

3. RESULTS

FT-IR results for Tannin- rich extract (PATRE)

Infrared spectroscopy is one of the most powerful yet simple and reliable analytical techniques that offer rapid identification of molecular structures and information on their chemical classification. The IR spectroscopy also helps in structural elucidation of novel phytochemicals derived from medicinal plants. The FT-IR results confirmed PATRE as tannin-rich and (See Figure 1).

Figure 1: FT-IR spectra of Tannin-rich extract (PATRE)

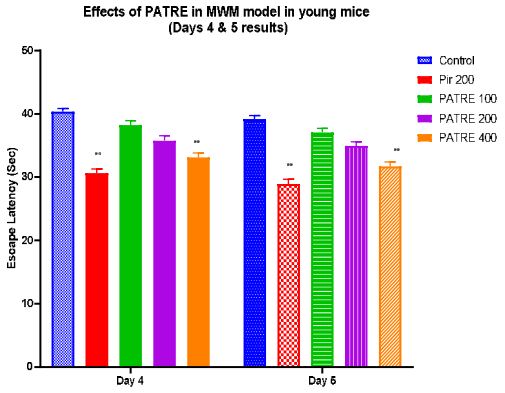


Improvement in cognitive functions using Morris Water Maze test

Pre-treatment with PATRE extract at doses 200 and 400 mg/kg showed moderately substantial ($p<0.05$) fall in the EL to reach the platform on both 4th and 5th day as shown in Figure 2. However, animals in 100mg/kg dose groups of both PATRE extract did not show significant decrease in EL. The

decreased EL on 5th day signifies retention of memory. The results of higher doses (400mg/kg) reflected marked decrease in EL on 4th and 5th day which was comparable to that of piracetam group. Lastly, pre-treatment with extract showed more decrease in EL compared to PATRE extract at all dosage points, i.e., 100/200/400 mg/kg.

Figure 2:Effects of and PATRE extract of *P. amarus* on escape latency in Morris Water Maze model



Values showed above are in Mean ± SEM; N was 6.Comparatively to the control set, by utilizing one-way ANNOVA + Dunnett's test, *P< 0.05, **P < 0.01, were deemed noteworthy; while # denotes P>0.05, i.e., non-significant results.Positive standard was Piracetam 200mg/kg, orally given

Anti-Alzheimer like activity in young and aging animals using Elevated Plus Maze test with scopolamine-induced amnesia

Lowing of TL is an indication of improvement in memory. The PATRE treated young and old mice noted remarkable reduction in TL on 9th day, signifying retention memory. Pre-treatment with standard drug, i.e. Pir 200mg/kg for 8 consecutive days resulted in decreased TL on 8th Day and on 9th Day as compared to control, which validates our model. The PATRE in doses 100, 200, and 400 mg/kg) administered orally for eight consecutive days have resulted in remarkably decreased ($p<0.05$ for 100mg/kg dose groups and $p<0.01$ for 200 & 400 mg/kg dose groups) TL on 8th and 9th day in both aged and young, compared to control groups on EPM test apparatus. And, the results of higher doses of PATRE (400mg/kg) reflected major decrease in TL on 9th day which were comparable to the results of Piracetam (Figures 3 to 6). Treatment with Scopolamine (1mg/kg) resulted in significant increase in TL in young mice on 8th and 9th day as compared to control, signifies memory impairment. Also, it was observed that PATRE 400 mg/kg orally successfully reversed memory deficits induced by Scoin young mice.

The results of aged mice noted higher TL on 8th Day and on 9th Day when compared with TL values of young mice, which signifies impairment of memory and learning abilities in aged mice. The results also noted improved memory and the learning of aged animals compared to Sco-treated young mice as demonstrated by noteworthy decrease in TL when subjected to EPM tests. Lastly, pre-treatment with extract showed more decrease in TL compared to PATRE extract at all dosage points, i.e., 100/200/400 mg/kg.

Figure 3:Effect of PATRE on transfer latency in EPM test in young mice on day 8

Values showed above are in Mean ± SEM; N was 6.Comparatively to the control set by utilizing one-way ANNOVA + Dunnett's test, **P < 0.01was deemed noteworthy; while # denotes P>0.05, i.e., non-significant results.Positive standard was Pir, ie, Piracetam 200mg/kg, orally given **Note** - Scopolamine 1 mg/kg was administered on Day 8 only, intraperitoneal.

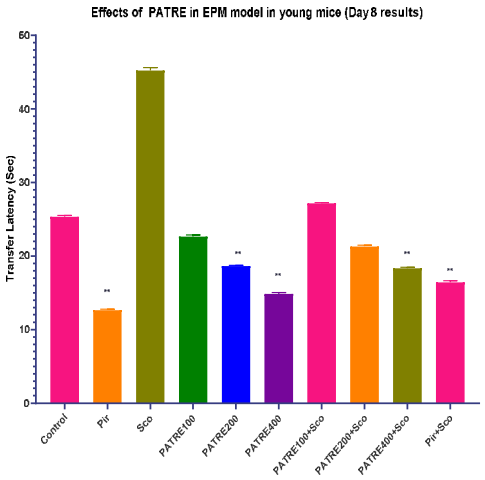


Figure 4:Effect of PATRE) on transfer latency in EPM test in young mice on Day 9

Values showed above are in Mean ± SEM; N was 6.Comparatively to the control set by utilizing one-way ANNOVA + Dunnett's test, **P < 0.01was deemed noteworthy; while # denotes P>0.05, i.e., non-significant results.Positive standard wasPir, ie, Piracetam 200mg/kg, orally given**Note** - Scopolamine 1 mg/kg was administered on Day 8 only, intraperitoneal.

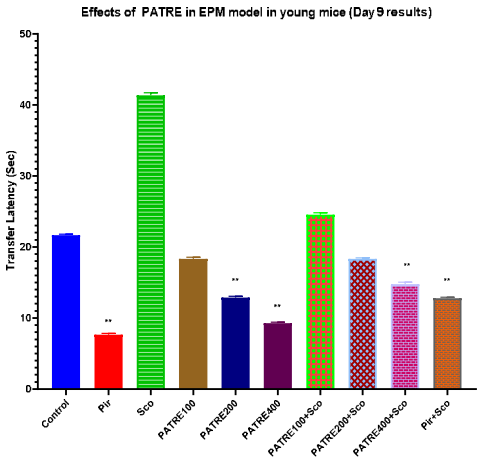


Figure 5: Effect of PATRE on transfer latency in EPM test in aged mice (age-induced amnesia model) on Day 8

Values showed above are in Mean ± SEM; N was 6.Comparatively to the control set by utilizing one-way ANNOVA + Dunnett's test, **P < 0.01was deemed noteworthy; while # denotes P>0.05, i.e., non-significant results.Positive standard wasPir, ie, Piracetam 200mg/kg, orally given

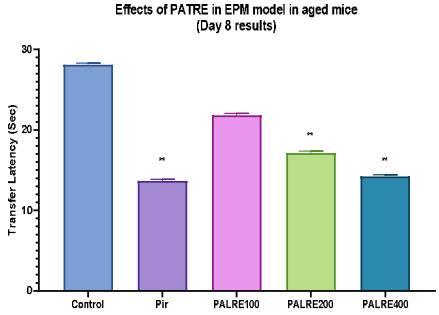
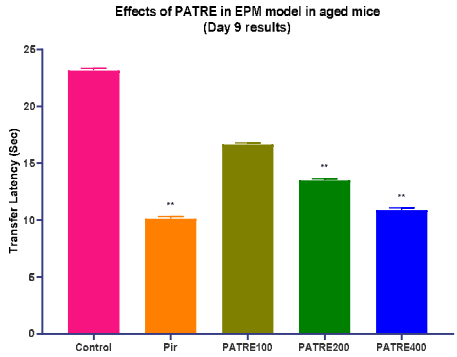


Figure 6: Effect of PATRE on transfer latency in EPM test in aged mice (age-induced amnesia model) on Day 9

Values showedabove are in Mean ± SEM; N was 6.Comparatively to the control set by utilizing one-way ANNOVA + Dunnett's test, **P < 0.01was deemed noteworthy; while # denotes P>0.05, i.e., non-significant results.Positive standard wasPir, ie, Piracetam 200mg/kg, orally given



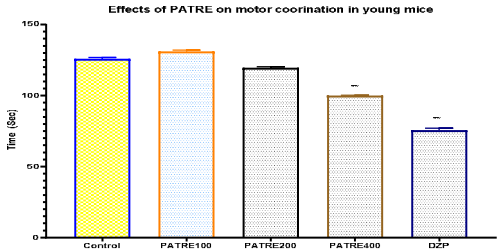
Effect of Tannin-rich extract on motor activity

The results of animals treated with *P. amarus* extract, viz., PATRE 100mg/kg, PATRE 200mg/kg, 100mg/kg, and 200mg/kg does not show significant change in motor coordination activity when compared with the results for control group.

However, at a higher dose (PATRE 400mg/kg and 400mg/kg) the findings of both the extract seems to be sedative ($p<0.01$). Also, PATRE extract showed more impact on motor coordination compared with extract as showed in the Figure 7.

Figure 7: Effect of PATRE on motor coordination

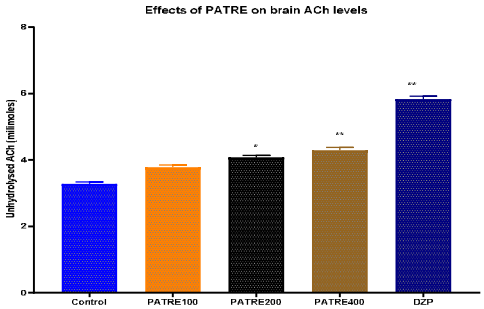
Values showedabove are in Mean ± SEM; N was 6.Comparatively to the control set by utilizing one-way ANNOVA + Dunnett's test, **P < 0.01was deemed noteworthy; while # denotes P>0.05, i.e., non-significant results.Positive standard wasDiazepam 2mg/kg.



Effect of P. amarus extract (PATRE) on Acetylcholine [ACh] levels in mouse brain- Pre-treatment with test drugs (PATRE and in three doses, ie 100/200/400 mg/kg) exhibited significant protective effect against ACh breakdown as indicated by increase in amount of unhydrolysed ACh and decrease in the amount of hydrolysed ACh. Increased levels of unhydrolysed acetylcholine in brain homogenate of treated animals, gives an indication of acetyl cholinesterase inhibitory activity of test drugs (PATRE and in three doses, ie 100, 200, and 400 mg/kg). Also, results presented more rise in unhydrolysed acetylcholine levels in brain homogenate of mice treated with extract compared with PATRE extract at all dose levels, i.e., 100, 200, and 400 mg/kg; which signifies effectiveness of extract over tannin-rich (Figure 8).

Figure 8: Effect of PATRE on Acetlycholinestarase levels in mice brain.

Values showedabove are in Mean ± SEM; N was 6.Comparatively to the control set by utilizing one-way ANNOVA + Dunnett's test,* denotes p<0.05 and**P < 0.01,were deemed noteworthy; while # denotes P>0.05, i.e., non-significant results.Positive standard utilized wasDiazepam 2mg/kg.



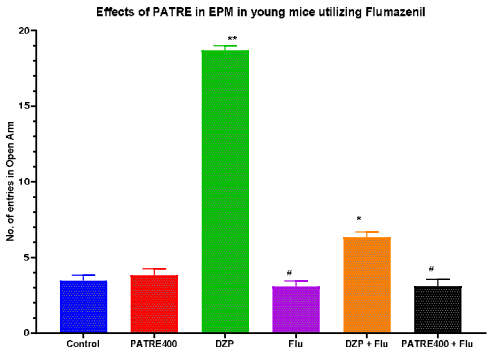
Blockade of the anti-amnesic effect of Tannin rich extract by Scopolamine

In the EPM test, Flumazenil 2.5 mg/kginverted the impact of diazepam 2 mg/kg and extract at the dose of 400 mg/kg on the timeframe spent and amount of entrancesin the open wing of EPM device, suggestive of probablemethod of action of extract via GABA-A receptor as indicative in Figure 9.

However, mice treated with the PATRE extract at a dose of 400mg/kg demonstrated statistically insignificant rise in the time spent and in number of entries in open arm of EPM apparatus. One of the widely utilized tests used to find novel benzodiazepine-like anxiolytic drugs is the EPM testand flumazenil is most-widely used experimental tool to study benzodiazepine antagonism. In this context, the activity of the (PALRE) extract in relieving anxiety-like effects in the EPM model may indicate that the GABA-A/benzodiazepine receptor complex has been positively modulated.

Figure 9: Elevated Plus Maze task effects of Flumazenil on PATRE pretreament

Values showed above are in Mean ± SEM; N was 6.Comparatively to the control setof young mice by utilizing one-way ANNOVA + Dunnett's test,* denotes p<0.05 and **P < 0.01, were deemed noteworthy; while # denotes P>0.05, i.e., non-significant results.Positive standard utilized wasDiazepam 2mg/kg.



HPTLC Finger prints of Tannin-rich extract (PATRE)

HPTLC fingerprinting analyses of the extract of *P. amarus* (PATRE) was conducted for detection class of compounds ie, for the tannins. The peaks observed in the graphs clearly indicate significant content of tannins in PATRE extract, (see Figure 10 to 12).

Figure 10: HPTLC Finger prints of *P. amarus* standardized PATRE

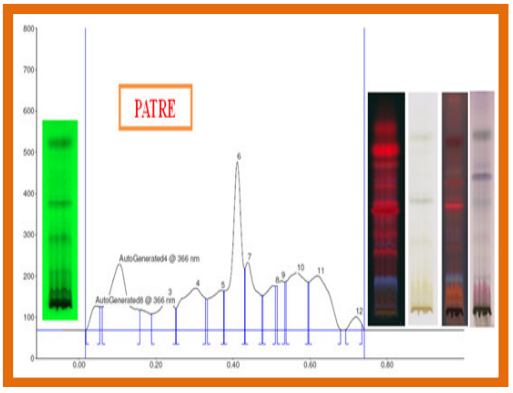


Figure 11: HPTLC chromatogram of PATRE for tannins detection, utilizing corilagin (C) as marker; mobile phase composed of toluene: ethyl acetate: formic acid (6:4:0.3, v/v).

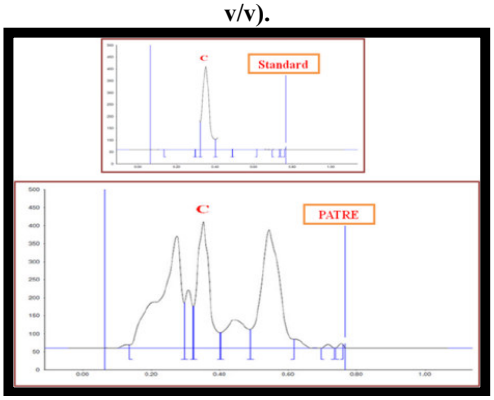
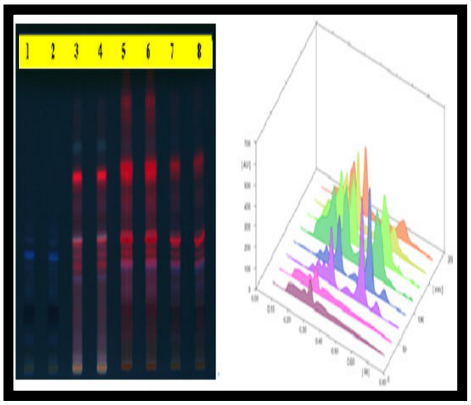


Figure 12: Phytochemical finger print and 3d spectra of phyllanthus extract taken at 254 nm wavelength for detection of class of compounds [Tannins]



Detection of Tannins- For detection of Tannins mobile phase used was toluene: Ethylacetate: formic acid (6:4:0.3, v/v) and the derivatization was carried with the help of Ferric chloride FeCl3. Chromatographic finger print analysis of the PATRE taken at 254 nm wavelength for detection of tannins shows unique Rf loci at 0.13. PATRE extract showed presences of 7 compounds each, of tannin class, with maximum % of area covered by PATRE extract. HPTLC finger print profile and 3d spectra for detection of tannins in phyllanthus extract taken at 254 nm wavelength. The maximum height peak of 487.4 was observed with PATRE extract. The number of auto generated peaks for PATRE extract were 8, each. The Rf range for PATRE was 0.06 - 0.53. The details of Rf range, number of peaks, number of auto-generated tracks, and maximum height of peaks is summarized in Table 1.

Chromatographic finger print analysis of the phyllanthus extract taken at 366 nm wavelength for detection of tannins shows unique Rf loci at 0.54 and 0.27. PATRE extract showed presences of 7 compounds each, of tannin class, with maximum % of area covered by PATRE extract. HPTLC finger print profile and 3d spectra for detection of tannins in phyllanthus extract taken at 366 nm wavelengthis depicted in Figure 12. The maximum height peak of 513.80 was observed with PATRE extract. The number of auto generated peaks for PATRE and PAME extract were 2 and 0 respectively. The Rf range for PATRE was 0.23 - 0.63 and for PAME was 0.28 - 0.76. The details of Rf range, No of Peaks, No of Auto Generated tracks and Maximum height of peaks is summarized in Table 1.

Chromatographic finger print analysis of the derivatised phyllanthus extract taken at 366 nm wavelength for detection of tannins shows unique Rf loci at 0.36. PATRE extract showed presences of 2 and 5 tannins respectively, with maximum % of area covered by PATRE extract. HPTLC finger print profile and 3d spectra for detection of tannins in derivatised phyllanthus extract taken at 366 nm wavelength is depicted in Figure 10. The number of auto generated peaks for PATRE extract were. The Rf range for PATRE was 0.36 - 0.55. The details of Rf range, No of Peaks, No of Auto Generated tracks and Maximum height of peaks is summarized in Table 1.

Chromatographic finger print analysis of the phyllanthus extract taken at 540 nm wavelength for detection of tannins shows unique Rf loci at 0.13 and 0.35. PATRE extract showed presences of 4 compounds of tannin class respectively, with maximum % of area covered by PATRE extract. HPTLC finger print profile and 3d spectra for detection of tannins in phyllanthus extract taken at 540 nm wavelength is depicted in Figure 10. The maximum height peak was 179.7 for PAME. The number of auto generated peaks for PATRE were 8. The Rf range for PATRE was 0.27 - 0.55. While the details of Rf range, No of Peaks, No of Auto Generated tracks and Maximum height of peaks is summarized in Table 1.

Chromatographic finger print analysis of the derivatised phyllanthus extract taken at 540 nm wavelength for detection of tannins shows unique Rf loci at 0.14. PATRE extract showed presences of 4 compounds each, of tannin class, with maximum % of area covered by PATRE extract. HPTLC finger print profile and 3d spectra for detection of tannins in derivatised phyllanthus extract taken at 540 nm wavelength is depicted in Figure 11. The details of Rf range, No of Peaks, The maximum height peak of 514.9 for PATRE extract. The number of auto generated peaks for PATRE extract were 6 for each of them. The Rf range for PATRE was 0.13 - 0.56. No of Auto Generated tracks and Maximum height of peaks is summarized in Table 1.

Table 1: Chromatographic finger print analysis of PATRE extract taken at various wavelength for detection of class of compounds [Tannins]

Details at specific wavelength	Parameters					
	Rf range	Rf of Unique loci	No of Peaks	No of Auto Generated tracks	Max Height	Area % range
254 nm wavelength	0.13 - 0.76	0.48	10	12	23.8 - 124.8	4.33 - 24.57
366 nm wavelength	0.13 - 0.74	0.40	7	12	15.3 - 245.5	1.28 - 61.93
Derivatives 366 nm wavelength	0.13 - 0.47	0.47	3	5	10.4 - 14.4	24.33 - 45.36
540 nm wavelength	0.13 - 0.70	0.13	2	6	10.5 - 11.3	42.81 - 57.19

4. DISCUSSION

This investigation assessed in detail the anti-amnesic activity of standardized extract PATRE of the *P. amarus* in mice by utilizing exteroceptive and interoceptive behavioral rodent models viz. MWM test, EPM test, and Sco-induced amnesia. The HPTLC fingerprint analysis supported the investigational plan and the molecular docking studies helped to see path forward.

Morris water maze

Further, anti-Alzheimer potential of both the plant extract was evaluated using Morris water maze test. Application of scopolamine to block muscarinic acetylcholine receptors causing cognitive deficit is a representative model used to evaluate the anti-dementia activity of herbal extract. Morris water maze model has been extensively utilized to investigate the neurological mechanisms underlying spatial navigation to influence special cognitive processes. The same model can also be used to test working memory by changing the hidden platform from one quadrant to another quadrant. Pre-treatment with the plant extract of *P. amarus* (PATRE) at doses 100mg/kg, 200 mg/kg and 400 mg/kg remarkably ($P<0.05$) reduced the time required reaching the platform post scopolamine treatment. It improved basal as well as scopolamine-impaired performance with respect to acquisition and retention of memory. These results signifying possible anti-AD like activity of PATRE extract may have been mediating via cholinergic pathway.

Elevated Plus Maze

The EPM test is an established rodent model for evoking an approach-avoidance conflict and for assessing the retention of memory of animals to prefer closed arms over open arms. An animal is thought to be in a happy mood, be anxiety-free, have anti-amnesia, anti-Alzheimer's-like activity when it stays for longer time in open arms. In this study, PATRE in three different dose (100, 200, and 400 mg/kg), separately, produced significant effect in a dose dependent manner compared to Piracetam group. Also, results of 100 and 200 mg/kg groups indicated that at these dose levels and PATRE extract did not affect the rodent's motor coordination abilities. The EPM test is well-known model to assist in the quest for novel benzodiazepine-like anti-anxiety drugs and to that point, we have communicated supportive results while demonstrating anxiolytic activity of *P. amarus* extract. Also, in recent years a significant amount of research has been done that is throwing light on the relationship of GABAergic signaling system contributing to AD pathogenesis. In these contexts, the activity PATRE in doses 100, 200, and 400 mg/kg) in relieving dementia/Alzheimer-like behavior in EMP model possible via modulation of the GABA-A/benzodiazepine receptor complex [12,13,14,46].

Study of exteroceptive and interoceptive behavioral models (scopolamine, and ageing induced amnesia) using elevated plus Maze in mice reveals that P. amarus treated mice possess anti-dementia/ anti-Alzheimer-like and anti-amnesic activities. It was noted that the reduction of spontaneous motor activity could be related to the calmness/sedative effect. In this investigation, the motor activity results demonstrated dose-dependent sedative activity of PATRE extract of *P. amarus* [13,15].

Motor coordination test

In this study, PATRE extract in three different doses (100, 200, and 400 mg/kg), separately, produced distinct effects. Comparatively treatment showed more impact on motor co-ordination as compared to PATRE treatment. At lower doses (100 and 200 mg/kg) both the extract did not show significant impact on the motor coordination.

It was evident that at a advanced dose (400mg/kg) PATRE as well as significantly impaired motor coordination of mice. It was also observed that showed more impact on motor coordination compared with PATRE treatment.The calming/sedative effects of *P. amarus* extract may be responsible for the decrease in spontaneous motor coordination activity.

GABAnergic mechanisms of *P. amarus*

It has been demonstrated that the equilibrium amid excitation (glutamate) and inhibition (GABA) in the brain is impacted by the interactivity amidst Aβ, GABAergic signalling, and acetylcholine. Targeting different GABA receptor subtypes has the prospective to provide benefit to people with AD to overcome their memory problems, according to several preclinical interventions reported. The development of anti-AD medications may benefit from targeting GABAergic signalling, according to current research, as multiple preclinical and clinical studies have shown impaired GABAergic cell metabolism in AD. Also, in recent

years a significant amount of research has been done that is throwing light on the relationship of GABAergic signaling system contributing to AD pathogenesis. [56-59]

To aid in the hunt for innovative benzodiazepine-like anxiolytic remedies, the EPM test is a well-known model and to that point, we have communicated supportive results while demonstrating anxiolytic activity of *P. amarus* extract. The actions of *P. amarus* may interact with the GABA/benzodiazepine receptor complex in the brain, according to recent in vivo research in the EPM model and molecular docking studies. Flumazenil negated the effect of diazepam and *P. amarus* on the amount of entrances and timeframe spent in the open wing of the EPM device, as seen in the EPM test, suggesting a potential GABA-A receptor-based mode of action for *P. amarus*. Docking study additionally demonstrates that *P. amarus* possesses most prominent activity against GABA receptors as compared to standard drug Diazepam.

Therefore, additional research was considered essential for determining the precise mechanism (s) by which *P. amarus* shows its disease modifying potential and detailed investigation of its efficacy in neurodegenerative diseases including AD.

Phytochemical screening and HPTLC Fingerprint analysis for class of compounds: Tannins

Investigations on the phytochemical hunt of *P. amarus* extract discovered the occurrence of tannins, lignans, flavonoids, saponins, glycosides, alkaloids, proteins, steroids, and phenolic compounds [13-18]. Lignans were noted to possess antioxidant, analgesic, anti-arthritic, antiinflammatory, and immunomodulatory activity. Bioactive phytochemicals in the class of lignans such as niranthin, nirtetralin, and phyltetralin have reported ant-iinflammatory potential. In addition, niranthin and portion were reported anti-inflammatory like activity [47-53]. The tannins, notably corilagin within P.amarus reported to display antihyperalgesic activity [54].

The phytochemical fingerprint analysis of PATRE extract signifies that the anti-Alzheimer-like anti-amnesic potential noted in this investigation can be considered aligned to the tannins and lignans. The HPTLC fingerprint analysis of revealed significant peaks of and PATRE extract. Additionally, the HPTLC chromatograms of PATRE demonstrated presence of tannins utilizing corilagin as marker compound. The HPTLC investigations on the classes of compounds clearly denoted that the extract has high level of PATRE extract is rich in tannins. Hence, the data from HPTLC chromatograms and assessment of class of compounds utilizing HPTLC as a basis supports the fact that the standardized extract contain substantial amount of tannins in PATRE. So, we can propose that the presence of these phytochemicals might relate to the bioactivity in this investigation.

Proposition of mechanism of action of *P. amarus*

The P. amarus extract and its active constituents were thought to stimulate the brain's central Ach (Acetylcholine) functioning via inhibiting AChE (acetylcholinesterase), despite the fact that the processes behind their anti-amnesic activities continue to be revealed. Additionally, it has been proposed that compounds that improve cognition stimulate cholinergic transmission via having an agonistic or antagonistic impact on the GABAA/benzodiazepine receptor, and that this complex regulates the release of Ach. P. amarus extract could successfully manage memory dearth in a rodent (mouse)process of Sco-led dementia by enhancing cholinergic system performance and perhaps activating GABA neurons.

CONCLUSION

Overall, the outcomes of interoceptive/exteroceptive behavioral rodent models put forward that PATRE showed better anti-Alzheimer-like action whereas at 400mg/kg dose, outcomes suggest sedative potential for PATRE extract. Hence, this investigation hints the outcomes that the *P. amarus* extract could potentially have a role in the AD treatment plan. It also throws light on the need to further investigate role of tannins as potential disease-modifying therapies in AD.

REFERENCES

1. Kessler R, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Archives of General Psychiatry. 2005; 62(6):593-602. DOI: 10.1001/archpsyc.62.6.593.
2. Kumar A. Current and novel therapeutic molecules and targets in Alzheimer's disease. Journal of the Formosan Medical Association.

2016;115(1):3-10. DOI: 10.1016/j.jfma.2015.04.001.

3. Folch J, Ettcheto M, Petrov D, Abad S, Pedrós I, Marin I, Olloquequi J, Camins A. Review of the advances in treatment for Alzheimer disease: strategies for combating β-amyloid protein. Neurología. 2018; 33(1): 47-58. DOI: 10.1016/j.nrl.2015.03.012.
4. Lobello K. Targeting Beta Amyloid: A Clinical Review of Immunotherapeutic Approaches in Alzheimer's Disease. International Journal of Alzheimer's Disease. Volume 2012, Article ID 628070, 14 pages. DOI: https://doi.org/10.1155/2012/628070.
5. Vasudevan M and Parle M. Effect of Anwala Churna (Emblicao‹cinalis GAERTN.): an Ayurvedic Preparation on Memory Deficit Rats. Yakugaku Zasshi. 2007;127(10):1701-7. DOI: 10.1248/yakushi.127.1701.
6. Joshi H and Parle M. Pharmacological evidences for antiamnesic potentials of *Phyllanthus amarus* in mice. African Journal of Biomedical Research. 2007; 10:165-73. DOI: 10.4314/ajbr.v10i2.50622
7. Lister RG. The use of a plus maze to measure anxiety in mouse, Psychopharmacology. 1987; 92: 180-185. DOI: 10.1007/BF00177912.
8. Pillow S, Chopin PN, File SF, Briely M. Validation of open: closed arm entries in an elevated plus maze as a measure of anxiety in rats. Journal of Nueroscience methods. 1985; 14: 149-167. DOI: 10.1016/0165-0270(85)90031-7.
9. Kulkarni SK. Hand Book of Experimental Pharmacology; 3rded; Vallabh Prakashan; Reprints 2005: 36-38.
10. Crawley JN, Goodwin FK. Neuropharmacologic specificity of a simple animal model for the behavioral actions of benzodiazepines Pharmacology Biochemical Behaviour. 1981; 15: 595-599. DOI: 10.1016/0091-3057(80)90067-2.
11. Ambrogi, Lorenzini C, Bucherelli C, Giachetti A. Some factors influencing conditioned and spontaneous behavior of rats in the light-dark box test. Physiolgical Behaviour. 1986; 36(1): 97-101. DOI: 10.1016/0031-9384(86)90080-6.
12. Mesfin M, Asres K, and Shibeshi W. Evaluation of anxiolytic activity of the essential oil of the aerial part of Foeniculumvulgare Miller in mice. BMC Complement Alternative Medicine. 2014; 14:310.
13. Abid M, Gosh AK, and Khan NA. In Vivo Psychopharmacological Investigation of Delphinium Denudatum and AmaranthusSpinosus Extract on Wistar Rats. Basic Clinical Neuroscience. 2017;8(6):503–512. DOI: 10.29252/NIRP.BCN.8.6.503
14. Shastri R, et al. Anxiolytic activity of aqueous extract of Camellia sinensis in rats. Indian Journal of Pharmacology. 2016;48(6):681–686. DOI: 10.4103/0253-7613.194864
15. MoniruzzamanMd, et al. The leaves of CrataevanurvalaBuch-Ham. modulatelocomotor and anxiety behaviors possibly through GABAergic system. BMC Complementary Medicine and Therapies. 2018; 18: 283. DOI: 10.1186/s12906-018-2338-y.
16. Nagaraja T. Evaluation of Anxiolytic effect of Erythrinamysorensis Gamb in mice. Indian Journal of Pharmacology. 2012; 44(4): 489–492.
17. Stephanie NKJ, et al. anxiolytic - like properties of Halleaciliata IN MICE. African Journal of Traditional, Complementary and Alternative Medicines. 2016; 13(4): 1–7. DOI: 10.31887/DCNS.2015.17.3/mbourin.
18. Aman U. Passifloraincarnata attenuation of neuropathic allodynia and vulvodynia apropos GABA-ergic and opioidergicantinociceptive and behavioural mechanisms. BMC Complement of Alternative Medicine. 2016; 16: 77. DOI: 10.1186/s12906-016-1048-6.
19. Chopade A, Sayyad F J, Pain modulation by lignans (phyllanthin and hypophyllanthin) and tannin (corilagin) rich extract of Phyllanthusamarus in carrageenan induced thermal and mechanical chronic muscle hyperalgesia, Phytotherapy Research. 2015; 29 (8): 1202–1210. DOI: 0.1002/ptr.5366.
20. ChopadeA R &Sayyad F J, Antifibromyalgic activity of standardized extract of Phyllanthusamarus and Phyllanthusfraternus in acidic saline induced chronic muscle pain. Biomedicine Aging Pathology. 2014. 4(2): 123–130. DOI: https://doi.org/10.1016/j.biomag.2014.01.005.
21. ChopadeA R & Sayyad F J, Antinociceptive effect of Phyllanthusfraternus extract in complete freund's adjuvant induced chronic pain in mice. Biomedicine Aging Pathology. 2013, 3(4): 235–240.
22. Chopade A R &Sayyad F J, Molecular Docking Studies of Phytocompounds from Phyllanthus Species as Potential Chronic Pain Modulators.

Scientia Pharmaceutica, 2015; 83: 243–267. DOI: https://doi.org/10.3797/scipharm.1408-10

23. Chopade A R, Sayyad F J, Phytocompounds from Phyllanthusamarus as potential cox-2 inhibitors. Int J of Phytochemical Pharmacy Research, 2013, 5(1), 22-29. DOI: 10.4161/mabs.22771.
24. Dong, J., Peters-Libeu, C.A., Weisgraber, K.H., Segelke, B.W., Rupp, B., Capila, I., Hernaiz, M.J., LeBrun, L.A., Linhardt, R.J. Interaction of the N-terminal domain of apolipoprotein E4 with heparin. (2001) Biochemistry 40: 2826-2834. DOI: 10.1021/bi002417n.
25. Pang, Y.P., Ekstrom, F., Polsinelli, G.A., Gao, Y., Rana, S., Hua, D.H., Andersson, B., Andersson, P.O., Peng, L., Singh, S.K., Mishra, R.K., Zhu, K.Y., Fallon, A.M., Ragsdale, D.W., Brimijoin, S. Selective and Irreversible Inhibitors of Mosquito Acetylcholinesterases for Controlling Malaria and Other Mosquito-Borne Diseases. (2009) Plos One 4: E6851
26. Zhang, H., Zhu, X., Pascual, G., Wadia, J.S., Keogh, E., Hoozemans, J.J., Siregar, B., Inganas, H., Stoop, E.J.M., Goudsmit, J., Apetri, A., Koudstaal, W., Wilson, I.A.Structural Basis for Recognition of a Unique Epitope by a Human Anti-tau Antibody. Structure. 2018. 26: 1626-1634.e4
27. www.rcsb.org
28. www.vlifesciences.com
29. Kouémou NE, Taiwe GS, Moto FCO, et al. Nootropic and Neuroprotective Effects of Dichrocephala integrifolia on Scopolamine Mouse Model of Alzheimer's Disease. Front Pharmacol. 2017; 8: 847. DOI: https://doi.org/10.3389/fphar.2017.00847
30. Calixto JB, Santos ARS, Cechinel Filho V, Yunes RA. A review of the plants of the genus Phyllanthus: their chemistry, pharmacology and therapeutic potential. Medicinal Research Review. 1998; 18; 225–258. DOI: 10.1002/(sici)1098-1128(199807)18:4<225::aid-med2>3.0.co;2-x.
31. Bagalkotkar G, Sagineedu SR, Saad MS, Stanslas J. Phytochemicals from Phyllanthus niruri Linn. and their pharmacological properties: a review.J Pharm Pharmacol. 2006; 58: 1559-70. DOI: 10.1211/jpp.58.12.0001.
32. Joseph B, Raj SJ. An Overview: Pharmacognostic Properties of *Phyllanthus amarus* Linn. International Journal of Pharmacology, 2011; 7: 40-45. DOI: 10.3923/ijp.2011.40.45.
33. Patel JR, Tripathi P, Sharma V, Chauhan NS, Dixit VK. *Phyllanthus amarus*: Ethnomedicinal uses, phytochemistry and pharmacology, A review. Journal of Ethnopharmacology, 2011; 138: 286– 313. DOI: 10.1016/j.jep.2011.09.040.
34. Sarin B, Verma N, PedroMartin J, Mohanty A. An Overview of Important Ethnomedicinal Herbs of Phyllanthus Species: Present Status and Future Prospects, Scientific World Journal. 2014; Article ID 839172; 12 pages. DOI: 10.1155/2014/839172.
35. Verghese PB, Castellano JM, and Holtzman DM. Roles of Apolipoprotein E in Alzheimer's Disease and Other Neurological Disorders. Lancet Neurolology. 2011 Mar; 10(3): 241–252. DOI: 10.1016/S1474-4422(10)70325-2.
36. Yiannopoulou KG and Papageorgiou SG. Current and future treatments for Alzheimer's disease. Therapeutic Advances in Neurological Disorders. 2013; 6(1): 19–33. DOI: 10.1177/1756285612461679.
37. Roxana Braga de Andrade Teles, et al. Flavonoids as Therapeutic Agents in Alzheimer's and Parkinson's Diseases: A Systematic Review of Preclinical Evidences. Oxid Med Cell Longev. 2018; 2(11): 7043213. DOI: 10.1155/2018/7043213.
38. Hussain E. Putative Roles of Plant-Derived Tannins in Neurodegenerative and Neuropsychiatry Disorders: An Updated Review. Molecules. 2019; 24(12): 2213. DOI: 10.3390/molecules24122213.
39. Wei M, et al. Systematically Characterize the Anti-Alzheimer's Disease Mechanism of Lignans from S. chinensis Based on In-Vivo Ingredient Analysis and Target-Network Pharmacology Strategy by UHPLC–Q-TOF-MS. Molecules. 2019; 24(7): 1203. DOI: 10.3390/molecules24071203.
40. Vorhees CV and Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nature Protocols. 2006;1(2):848–858. DOI: 10.1038/nprot.2006.116.
41. Reddy DS, Kulkarni SK. Possible role of nitric oxide in the nootropic and antiamnesic effects of neurosteroids on aging and dizoclipine induced learning impairment. Brain Research. 1998; 799: 215-229. DOI: 10.1016/s0006-8993(98)00419-3.

42. Parle M, Singh N. Animal models for testing memory.Asia Pacific Journal of Pharmacology. 2004; 16: 101-120.
43. Dhingra D, Kumar V. Memory-enhancing activity of palmatine in mice using elevated plus maze and Morris water maze. Advances in Pharmacological Science. 2012; 11: 1-7. DOI: 10.1155/2012/357368.
44. Ton J, Nabeshima T, Kameyama T. Utility of an elevated plus maze for the evaluation of nootropics, scopolamine and electro convulsive shock. Psychopharmacology. 1990; 101: 27-33.DOI: 10.1007/BF02253713.
45. Annapurna A, Ramakrishna V, MuraliKrisha K and Krishanakumar V, and Prasad KB. Studies on Nootropicacivity of Ramipril and Losartan on scopolamine induced Amnesia in Rats. Indian Journal of Pharmaceutical Science. 2004; 66(1): 31-35. DOI: 10.4103/2231-4040.161514.
46. Li Y, Sun H, Chen Z, Xu H, Bu G, and Zheng H. Implications of GABAergic Neurotransmission in Alzheimer's Disease. Frontiers in Aging Neuroscience. 2016;8:31. DOI: 10.3389/fnagi.2016.00031.
47. Raphael K R, Kuttan R. Inhibition of experimental gastric lesion and inflammation by *Phyllanthus amarus* extract. Journal of Ethnopharmacology. 2003, 87; 193-7.
48. Ofuegbe S O, Adedapo A A, Adeyemi A A. Anti-inflammatory and analgesic activities of the methanol leaf extract of *Phyllanthus amarus* in some laboratory animals. Journal of Basic and Clinical Physiology and Pharmacology. 2014, 25: 175-80. DOI: 10.1515/jbcpp-2013-0126.
49. Patel JR, Tripathi P, Sharma V, Chauhan NS, Dixit VK. *Phyllanthus amarus*: Ethnomedicinal uses, phytochemistry and pharmacology. A review. Journal of Ethnopharmacology. 2011. 138, 286– 313. DOI: 10.1016/j.jep.2011.09.040.
50. Kandhare A, Ghosh P, Ghule A. E, Protective effect of *Phyllanthus amarus* by modulation of endogenous biomarkers and DNA damage in acetic acid induced ulcerative colitis: role of phyllanthin and hypophyllanthin. Apollo Medicine, 2013. 10: 87–97. DOI: https://doi.org/10.1016/j.apme.2013.01.006.
51. Sarin B, Verma N, PedroMartín J and Mohanty A. An Overview of Important Ethnomedicinal Herbs of Phyllanthus Species: Present Status and Future Prospects. Scientific World Journal. 2014, Article ID 839172; 12 pages. DOI: https://doi.org/10.1155/2014/839172.
52. Kassuya C A, Silvestre A A, Rehder V L, Calixto J B. Anti-allodynic and anti-oedematogenic properties of the extract and lignans from *Phyllanthus amarus* in models of persistent inflammatory and neuropathic pain. European Journal of Pharmacology. 2003, 478; 145– 153 DOI:1016/j.ejphar.2003.08.079.
53. Kassuya C A, Silvestre A, Menezes-de-Lima O, Marotta D M, Rehder V L, Calixto J B. Antiinflammatory and antiallodynic actions of the lignan niranthin isolated from *Phyllanthus amarus*. Evidence for interaction with platelet activating factor receptor. European Journal of Pharmacology. 2006, 546; 182-8. DOI: 10.1016/j.ejphar.2006.07.025.
54. Moreira J, Klein LC, Cechinel Filho V, DeCampos F. Anti-hyperalgesic activity of corilagin, tannin isolated from Phyllanthus niruri L. (Euphorbiaceae). Journal of Ethnopharmacology. 2013, 146; 318-323. doi: 10.1016/j.jep.2012.12.052.
55. Joshi, H. and Parle, M., 2007. Evaluation of the antiamnesic effects of *Phyllanthus amarus* in mice. Colombia medica, 38(2), 132-139.
56. Giovannetti EA, Fuhrmann M. Unsupervised excitation: GABAergic dysfunctions in Alzheimer's disease. Brain Research. 2019; 1707: 216-226.DOI: 10.1016/J.BRAINRES.2018.11.042.
57. Villette V, Dutar P. GABAergic Microcircuits in Alzheimer's Disease Models. Current Alzheimer Research. 2017; 14(1): 30-39.DOI: 10.2174/1567205013666160819125757.
58. Li Y, Sun H, Chen Z, Xu H, Bu G, Zheng H. Implications of GABAergic Neurotransmission in Alzheimer's disease. Frontiers in Aging Neuroscience. 2016; 8: 31. DOI: 10.3389/fnagi.2016.00031.
59. ChopadeAR, et al., An Insight Into the anxiolytic Effects of Lignans (Phyllanthin and Hypophyllanthin) and Tannin (Corilagin) Rich Extract of *Phyllanthus amarus*: An In-Silico and In-vivo approaches. Combinatorial Chemistry & High Throughput Screening 2020. 12(2): 25-34.DOI:10.2174/1386207323666200605150915.