

Research Article



GC-MS, Molecular Docking and In vitro Studies of Methanolic Extract of Leaves of *Jatropha gossypifolia* as Potent Anti-alzheimer's

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ABSTRACT

The aim of this study is to explore anti-alzheimer's effect of phytochemicals identified from methanolic extract leaves of *Jatropha gossypifolia* using GC-MS and Molecular docking studies. Acetylcholinesterase inhibitors improved cholinergic deficit in the brain which are beneficial in the treatment of Alzheimer's disease. In this research work we studied the AChE antagonistic effect of phytochemicals identified from *Jatropha gossypifolia* using an in silico approach. This work reported the phytochemical analysis of the methanolic extract of the leaves of *Jatropha gossypifolia*. Eleven compounds were revealed through GC-MS analysis and screened using GC-MS QP2010PLUS, Shimadzu, Japan. Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST). 1,1,5-Trimethyl-1, 2-dihydro naphthalene, Methyl 10,12-pentacosadiynoate, Dodecanoic acid, methyl ester, Methyl tetradecanoate, 3,7,11,15-Tetramethyl-2-hexadecen-ol, Cyclopropanepentanoic acid 2-undecyl, methyl ester-trans, N-hexadecanoic acid, 9,12,15- Octadecatrienoic acid, Heptadecanoic acid, 16-methyl methyl ester, 7,8 Epoxylanostan 11-ol, 3-acetoxy, Cis,Cis, Cis – 7,10,13 – Hexadecatrienal compounds identified from methanolic leaf extract of *Jatropha gossypifolia* using GC-MS analysis. Molecular docking of *Jatropha gossypifolia* on human AChE protein was determined by using AutoDock Vina software and compared with Donepezil a known reversible acetylcholinesterase inhibitor. The interactions of Human Acetylcholinesterase-ligand conformations, including hydrogen bonds and various other bonds were analyzed using BIOVIA Discovery Studio 2016. Cis, Cis, Cis – 7,10,13-Hexadecatrienal existing phytochemicals from the leaves of *Jatropha gossypifolia* had the highest fitness score of -7.6 kcal/mol comparable with donepezil having -9.2 kcal/mol and hence could be a potent antialzheimer's drugs.

Keywords: *Jatropha gossypifolia*, GC-MS, Docking studies, Acetylcholinesterase.

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INTRODUCTION

Alzheimer disease is characterized by the accumulation of A β plaques around the neurons extracellularly and formation of neurofibrillary tangles by hyperphosphorylation of Tau proteins intracellularly that results decrease in the level of Ach neurotransmitter.¹ In brain cholinergic neurons synthesise acetylcholine a neurotransmitter involved in the signal transmission and delivery of messages in the brain.^{2,3} It plays an important role in learning and memory.^{4,5} Deposition of plaques and neurofibrillary tangle in the brain which leads to degradation of the cholinergic neurons in the hippocampal and cortical part of the brain and decrease in the level of acetylcholine.⁶⁻¹⁰ Decrease acetylcholine level is responsible for unregulated signal transmission of the cholinergic pathway which is accompanied by AD.

Hence the AChE inhibitors are beneficial in the treatment of Alzheimer's disease. Currently two pharmacotherapy's are approved by Food and Drug Administration (FDA) i.e. Acetyl cholinesterase inhibitor like Tacrine, Donepezil, Rivastigmine, Galantamine and N-methyl D-aspartate, glutamate antagonist (NMDA) - Memantine.

Jatropha gossypifolia, commonly known as bellyache bush. It grows up to 2.5-4m (8.2-13.1 ft) height. The three lobed leaves are purple and sticky when young and become bright green with age. The small red flowers with yellow centres appear in clusters. These are followed by cherry-sized seed pods that are poisonous. It possess different biological activities like Analgesic activity, Neuropharmacological activity, Anxiolytic activity, Anti-diarrhoeal activity, Anti-cancer activity, Hepatoprotective activity, Hypotensive and Vasorelaxant effect, Anti-microbial activity, Haemostatic agent, Anti-diabetic activity, Anti-inflammatory activity.

MATERIALS AND METHODS

SELECTION OF THE PLANT

Drug discovery from medicinal plants has evolved to include numerous fields of inquiry and various methods of analysis. The process typically begins with a botanist, ethno botanist, ethno pharmacologist, or plant ecologist who identifies the plant of interest. Collection may involve



species with known biological activity for which active compounds have not been isolated or may involve taxa collected randomly for a large screening program. On the basis of intensive literature survey; *Jatropha gossypifolia*, leaves were selected for present study.

Collection and Authentication of Plant Material:

The plant *Jatropha gossypifolia* leaves had been collected from ABS botanical garden, Salem, Tamil Nadu, India. The plant was identified and authenticated by the botanist Mr.A.Balasubramanian (consultant – central siddha research) Executive Director ABS botanical, Salem, Tamil Nadu.

Extraction of Plant Material

The fresh leaves of *Jatropha gossypifolia* are collected and dried under shade, sliced into small pieces and ground into powder with mechanical grinder and the powder was sieved by Sieve no.30 and preserved in a container.

Solvents for Extraction

Petroleum ether (60-80°C)

Chloroform

Methanol

Extraction procedure

The dried powder of leaves of *Jatropha gossypifolia* was defatted with petroleum ether in Soxhlet apparatus by hot percolation. The defatted powder material (marc) thus obtained was further extracted with Chloroform and methanol. The solvent was removed by distillation under reduced pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary flash evaporator.

Preliminary Phytochemical Studies¹¹⁻¹⁴

The extract was subjected to preliminary phytochemical investigations to identify various phytoconstituents present in the leaves of *Jatropha gossypifolia*.

Identification of Phytochemical Constituents using Gas Chromatography.¹⁵

GC-MS analysis was carried out by GC-MS QP2010PLUS, Shimadzu, Japan coupled with mass spectrometry as detector. The temperature was adjusted to -30°C to 280/300°C, the HP-5MS column with dimensions 30 m X 0.32 mm X 0.25 µm was used for analysis. The oven temperature was adjusted to 35°C and hold time 5 min and, ramp 10°C/min up to 220°C. Column flow is 1.2 ml. The inlet temperature was kept at 250°C and the source temperature of 230°C and MS Quad temperature of 150°C.

Molecular docking studies.¹⁶

Human Acetyl cholinesterase binding mode and interaction with individual Phytoconstituent was performed using AutoDock Vina software. Possible conformations and orientations for the ligand at the binding site were obtained by Molecular docking. The PyRx

software used to load the protein, creating a PDBQT file which contains a protein structure with hydrogens in all polar residues. All ligand bonds were set to be rotatable. The protein-fixed ligand-flexible docking calculations were done using the Lamarckian Genetic Algorithm (LGA) method. The docking site on protein target was defined by establishing a grid box with the dimensions of X: 38.0729 Y: 33.3208 Z: 25.0000 Å, with a grid spacing of 0.375 Å, centered on X: 20.2892 Y: 10.3219 Z: 32.3218 Å. The lowest docked energy was chosen as best conformation, after the docking search was completed. Eight runs with AutoDock Vina were performed in all cases per each ligand structure, and for each run the best pose was saved. The best poses average affinity was taken as the final affinity value. The interactions of Human Acetylcholinesterase-ligand conformations, including hydrogen bonds and various other bonds were analyzed using BIOVIA Discovery Studio 2016.

Preparation of protein

The water molecules, cofactors, and other ligands were removed from Crystal Structure of Recombinant Human Acetylcholinesterase in complex with Donepezil (PDB ID 4EY7) through Molegro molecular viewer. Then they were used for molecular docking studies

Preparation of ligands

The crystal 3D structures of the active compounds were retrieved from PubChem database. Energy minimization was done using Open babel version 2.4.1

In vitro study (Aβ₁₋₄₂ intoxicated SH-SY5Y cell lines model)

Cell viability assessment – MTT assay¹⁷

SH-SY5Y human neuroblastoma cell lines were obtained from National Centre for Cell Science (NCCS), Pune, India and maintained in Minimum Essential Medium (MEM) as per the standard protocol supplemented with 10% fetal bovine serum (FBS), streptomycin (250µg/ml), gentamycin (400µg/ml), amphotericin (3µg/ml), and penicillin (250units/ml) in a carbon dioxide incubator at 5% CO₂. The viability of neuron in terms of mitochondrial metabolic function was evaluated by MTT [(3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide)] assay based on the principle involving reduction of MTT to formazan. To assess the protective effect, the SH-SY5Y cells were incubated with Aβ₁₋₄₂ (10µM) for 24hrs and then post-treatment were made with methanolic extract of leaves of *Jatropha gossypifolia* using its IC₅₀ value. Then SH-SY5Y cells were washed with phosphate-buffered saline (PBS) and incubated with MTT (5mg/ml) in PBS for 3 hours at 37°C in 5% CO₂. After further washing, the formazan crystals were dissolved with isopropanol and the absorbance was measured at 570nm. The percentage of viability of SH-SY5Y cells were calculated by the standard formula.



AB₁₋₄₂ intoxication and extract treatment

The SH-SY5Y cell lines were maintained in Minimum Essential Medium (MEM) as per the standard protocol. 5000-10000 cells/well were seeded in 96 well plates and the viability was tested using trypan blue dye and 95% of viability was confirmed with help of haemocytometer. The cells were then incubated with Aβ₁₋₄₂ (10μM) for 24hrs. Then post-treatment with methanolic extract of leaves of *Jatropha gossypifolia* using IC₅₀ values were made. After 24 hours of treatment with respective extracts the following evaluations were performed.

Cell morphological observation

The phase contrast microscopy (Motic) was used to observe morphological changes in the SH-SY5Y cell lines.

Preparation of cell lysates

After study period, the medium was aspirated and cells were washed with the ice cold phosphate buffer saline, scraped, and were centrifuged at 5000 rpm for 5 min at 4 °C. The cell pellets were resuspended in 2000 μl of lysis buffer (10mM Tris - HCl, pH 7.5, 50mM NaCl, 5mM EDTA, 1% Triton X-100, 100μM Na₃VO₄, 50mM NaF, 1mM PMSF, 10μg/ml aprotinin 10μg/ml and leupeptin,) and incubated on ice for 30min. The cell lysates were obtained by centrifugation at 12000 rpm for 20 min at 4 °C. Then obtained cell lysates were stored at -20 °C until use.

Lipid peroxidation (LPO) assay¹⁸

Lipid peroxidation was evaluated in cell lysates by measuring the malondialdehyde content according to the TBA test described by ohkawa et al., with slight modification. 0.8 ml saline, 0.5 ml of BHT and 3.5 ml TBA reagent (0.8%) were added to 0.2 ml of the cell lysate, and incubated at 60 °C. After cooling, the solution was centrifuged at 2000 rpm for 10 minutes. The absorbance of the supernatant was measured at 532 nm using spectrophotometer against the blank.

Nitric Oxide (NO) assay¹⁹

This assay was done by taking 0.2 ml of medium with 1.8 ml of saline and 0.4 ml of 35% sulphosalicylic acid (SSA) for protein precipitation. And the precipitate was removed by centrifugation at 4000 rpm for 10 min. To 1 ml of aliquot of supernatant, 2 ml Griess reagent (1g of sulphanilamide dissolved in small volume of water, 2 ml of orthophosphoric acid and 100 mg of naphthyl ethyldiamine were added and the final volume was made upto 100 ml with distilled water) was added and the mixture was allowed to stand for 20 min under dark conditions. The colour intensity of the chromogen was read at 540 nm. Standard calibration curve was plotted using sodium nitrite in the concentration range 200-1000ng.

Reduced glutathione (GSH)²⁰

GSH content was determined by following the method of Jollow et al. (1974). To 0.25ml of cell lysate an equal

volume of ice-cold 5% TCA was added. The precipitate was removed by centrifugation at 4000 rpm for 10 mins. To 1ml aliquot of supernatant, 0.25ml of 0.2M phosphate buffer (pH 8.0) and 0.5ml of DTNB (0.6mM in 0.2M phosphate buffer, pH 8.0) were added and mixed well. The absorbance was measured at 412nm using spectrophotometer (UV, Shimadzu, Japan).

Superoxide dismutase (SOD)²¹

To 0.05ml of cell lysate, 0.3ml of sodium pyrophosphate buffer (0.025M, pH 8.3) was added. To this mixture, 0.025ml and 0.075ml of PMS (186μM) and NBT (300μM in buffer, pH 8.3) were added. The reaction was initiated by the instillation of 0.075ml of NADH. The mixture was then incubated at temperature of 30°C for a period of 90 seconds. To this 0.25 ml of glacial acetic acid was added in order to arrest the ongoing reaction. N-butanol (2ml) was shaken vigorously along with the reaction mixture and the mixture was centrifuged at 4000 rpm for 1 min. The colorimetric analysis with n-butanol (1.5ml) serving as blank was carried out at 560nm using spectrophotometer.

Catalase (CAT)²²

A small quantity of cell lysate (100μl) or sucrose (0.32M) was subjected to incubation with potassium phosphate buffer (2.25ml) 65 mM at pH 7.8 for 30 min at 25°C. The reaction was initiated by the addition of hydrogen peroxide (7.5mM; 650μl). The absorbance change was recorded for a period of 2 to 3 min at 240nm (UV, Shimadzu, Japan).

Protein estimation²³

Protein content in the cell lysate was estimated as described by Lowry et al., 1951 to quantify the above parameters per mg protein.

Measurement of IL-1β & TNF-α (Pro-inflammatory cytokines) by ELISA²⁴

The levels of IL-1β and TNF-α were assessed in cell lysates by using respective ELISA kits (Invitrogen, R&D systems & Alpha diagnostics, USA) as per the manufacturer's protocol. Briefly, 150μl distilled water was added to the standard and blank wells for standard calibration and 100 μl distilled water and 50 μl of each supernatant were added in duplicate into the wells. After incubation for 3 hours at room temperature, the wells were emptied and washed three times with 150 μl of wash buffer. TMB substrate (100 μl) was added to each well and incubated for 15 min at room temperature, followed by addition of 100μl stop – solution to all wells including blank wells. The absorbance was determined at 410 nm using above mentioned ELISA reader.

RESULTS

The preliminary phytochemical studies of methanolic extract of leaves of *Jatropha gossypifolia* showed the presence of phenolic compounds & flavonoids, Carbohydrates & glycosides, Protein & aminoacids, Alkaloids, Phytosterol, Saponins.



Table 1: Preliminary phytochemical studies of methanolic extract of leaves of *Jatropha gossypifolia*

Phytochemical constituents	Test	Petroleum ether extract	Chloroform extract	Methanol extract
Carbohydrates	Molisch's test	-	+	+
	Fehling's test	-	+	+
	Barfoed's test	-	+	+
	Bortrager's test	-	+	+
Glycosides	Molisch's test	-	-	+
Protein & aminoacids	Biuret's test	+	-	-
	Ninhydrin test	+	-	-
	Xanthoprotein test	+	-	-
	Millon's test	+	-	-
Alkaloids	Mayer's test	-	+	++
	Hager's test	-	+	++
	Wagner' test	-	+	++
Phytosterol	Liebermann's sterols test	+	+	++
Saponins	Salvoski's test	+	-	++
Phenolic compound and Tannins	Extract + FeCl ₃	-	++	++
	Gelatin test	-	++	++
	FeCl ₃	-		
Flavonoids	AqNaOH	-	++	+++
	Shinoda test	-	++	+++
	Conc.H ₂ SO ₄	-	++	+++

GC-MS analysis of MEJG

The crude methanolic extract of leaves of *Jatropha gossypifolia* was injected for screening of total active compounds by using GC-MS analyser. Eleven compounds were identified from the methanolic leaves extract *Jatropha gossypifolia*. The retention for bioactive compounds of leaves extract ranged from 8.468 to 43.117. The compound identified from the methanolic extract of *Jatropha gossypifolia* using full mass spectrometry showed the presence of 1,1,5-Trimethyl-1,2-dihydronaphthalene, Methyl 10,12-pentacosadiynoate, Dodecanoic acid, methyl ester, Methyl tetradecanoate, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-, n-hexadecanoic acid, 9,12,15-Octadecatrienoic acid (Z,Z,Z)-, Heptadecanoic acid, 16-methyl-methyl ester-, 7,8-Epoxy lanostan-11-ol, 3-acetoxy, Cis, Cis, Cis- 7,10,13 Hexadecatrienal. Mass spectrum of a few compounds like Heptadecanoic acid, 16-methyl-methyl ester-, and Cis, Cis, Cis- 7, 10, 13 Hexadecatrienal were discussed in the present study.

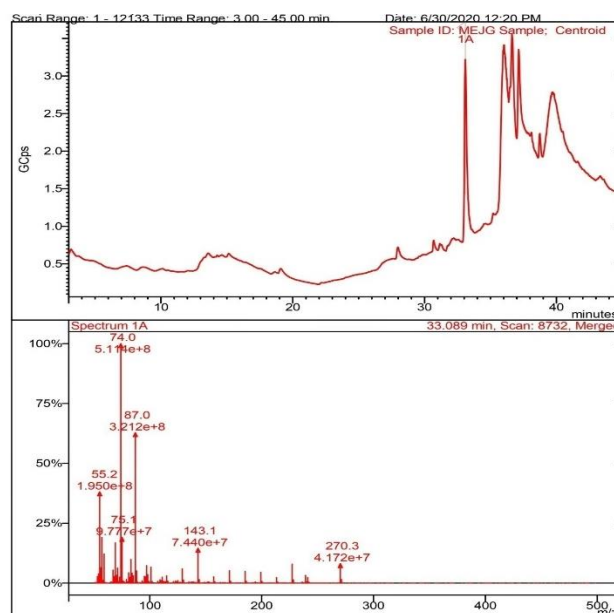
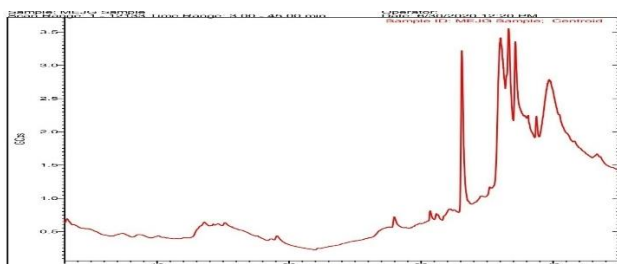


Figure 1: Mass Spectrum of methanolic extract of leaves of *Jatropha gossypifolia*

Molecular docking Studies

Structure based drug design

From PDB acetylcholinesterase enzyme (PDB ID 4EY7) was downloaded, it was prepared by removing chain B. Water molecule present in both the chain are removed. Energy minimization was done. Molecules drawn using

chemsketch were converted to mol format and ligprep was created.

Screenshot showing protein – ligand interactions

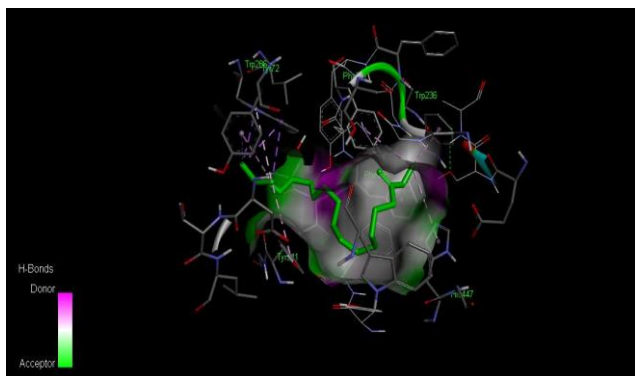


Figure 2: Stereoview of compound BV1 on enzyme 4EY7

Compound BV1 shows their pi-sigma interactions over the enzyme TRP A: 286, pi-alkyl interactions in PHE A: 297, 295, TRIP A: 236, HIS A 447, TYR A: 341, 72, and has a vanderwaals interactions in AGR A: 296, VAL A: 294, TYR A: 124, GLY A: 121, 122. PHE A: 338, ALA A: 204, SER A: 203, TYR A: 337.

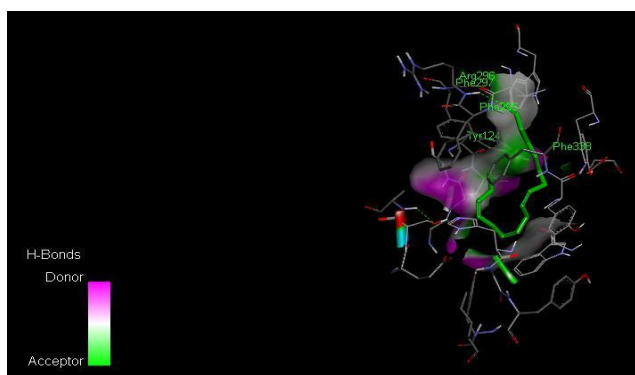
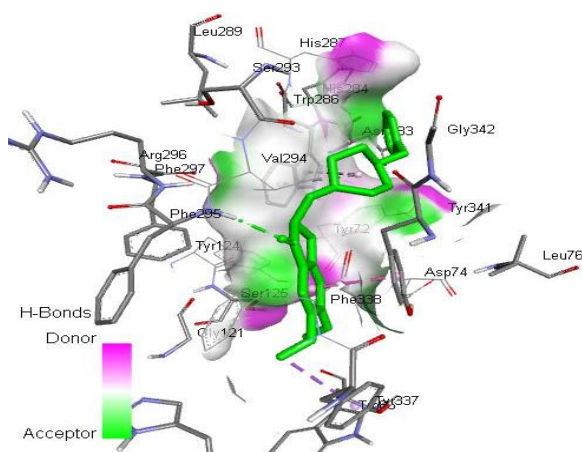


Figure 3: Stereoview of Compound BV2 on enzyme 4EY7

Docking analysis of compound BV2 shows their covalent hydrogen bond interactions over their enzyme ARG B: 296, PHE B: 295 and pi-alkyl interactions with PHE B: 338, 297, TYR B: 124 and has a vanderwaals interactions in VAL B: 294, TRP B 286, 86, GLY B: 448, SER B: 203, GLY B: 121, 122, TYR B: 341, 337, HIS B: 447.

Donepezil



Binding affinity of the compounds with Donepezil (PDB ID 4EY7)

Table 2: Binding affinity of the compounds with Donepezil (PDB ID 4EY7)

Ligand	Target Protein	Binding Affinity	RMSD
Heptadecanoic,16-Methyl acid methyl ester (BV1)	Recombinant Human Acetylcholinesterase in complex with Donepezil (PDB ID 4EY7)	-7.0	3.232
Cis, Cis, Cis 7,10,13-Hexadecatrienal (BV2)		-7.6	0
Standard (Donepezil)		-9.2	0

In Vitro cytotoxicity MTT assay of methanolic extract of leaves of *Jatropha gossypifolia* in SH-SY5Y cell lines

Table 3: MTT assay results

SI No.	Sample Description	SHSY5Y IC ₅₀ µg/ml
1	Methanolic extract of leaves of <i>Jatropha Gossypiifolia</i>	55.35789

Cell viability assessment – MTT assay [in presence of amyloid-beta₁₋₄₂ (Aβ₁₋₄₂)]

Table 4: Cell viability assessment

S. No	Groups	% viability of cells
1	Control	99.87 ± 0.143
2	Aβ ₁₋₄₂ (10µM)	11.22 ± 0.473***
3	met-L-JG	77.96 ± 1.321###

Effect of methanolic extract of leaves of *Jatropha Gossypiifolia* (met-L-JG) on cell viability level in Aβ₁₋₄₂ intoxicated SH-SY5Y cell lines. Statistical significance was determined by one way ANOVA followed by Tukey's multiple comparison tests using Graph pad prism Version 6.0. Values are represented as Mean ± SEM, Superscript *** denotes p<0.001 vs control, ### denotes p<0.001; vs Aβ₁₋₄₂ groups respectively, (Aβ: Amyloid beta).

Anti-oxidant evaluation – Spectroscopy methods.**Table 5:** Effect of methanolic extract of leaves of *Jatropha Gossypifolia* (met-L-JG) on LPO, NO, GSH, SOD, CAT level in A β 1-42 intoxicated SH-SY5Y cell lines.

S.NO	Groups	LPO (nmoles/ml)	NO (nmoles/ml)	GSH (μ M/mg protein)	SOD (U/mg protein)	CAT(U/mg protein)
1	Control	12.93 \pm 1.74	24.20 \pm 2.10	85.55 \pm 4.33	25.17 \pm 2.28	0.907 \pm 0.090
2	A β ₁₋₄₂ (10 μ M)	95.30 \pm 3.38 ^{***}	147.63 \pm 2.39 ^{***}	11.23 \pm 1.44 ^{***}	3.54 \pm 0.87 ^{***}	0.172 \pm 0.035 ^{***}
3	Met-L-JG	53.62 \pm 2.33 ^{###}	88.81 \pm 4.25 ^{###}	71.28 \pm 4.87 ^{###}	17.58 \pm 0.84 ^{###}	0.723 \pm 0.017 ^{###}

Statistical significance was determined by one way ANOVA followed by Tukey's multiple comparison tests using Graph pad prism Version 6.0. Values are represented as Mean \pm SEM, Superscript *** denotes p<0.001 vs control, ### denotes p<0.001; vs A β 1-42 groups respectively, (A β : Amyloid beta).

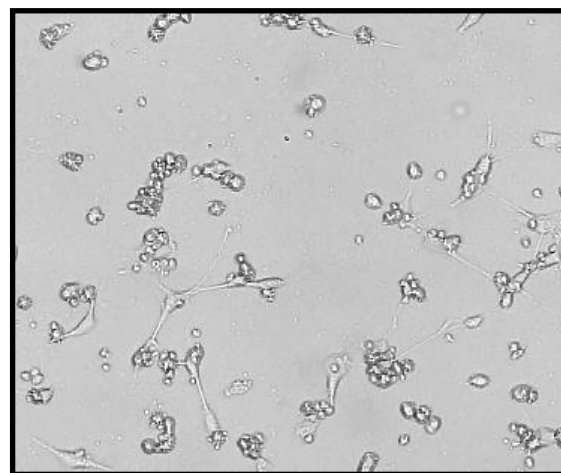
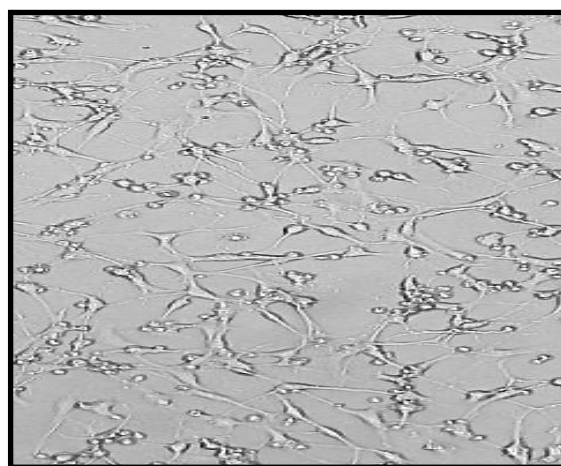
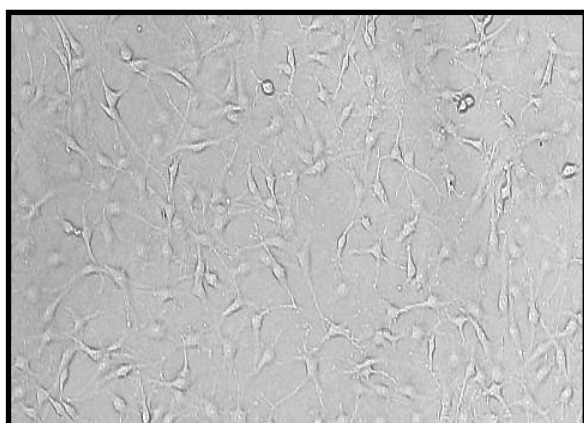
Anti-Inflammatory assessments- ELISA**Table 6:** Effect of methanolic extract of leaves of *Jatropha Gossypifolia* (met-L-JG) on IL-1 β and TNF- α level in A β ₁₋₄₂ intoxicated SH-SY5Y cell lines.

S.NO	Groups	IL-1 β (pg/ml)	TNF- α (pg/ml)
1	Control	105.23 \pm 1.22	133.71 \pm 5.37
2	A β ₁₋₄₂ (10 μ M)	889.87 \pm 17.89 ^{***}	977.84 \pm 36.52 ^{***}
3	Met-L-JG	511.69 \pm 9.54 ^{###}	475.83 \pm 20.30 ^{###}

Statistical significance was determined by one way ANOVA followed by Tukey's multiple comparison tests using Graph pad prism Version 6.0. Values are represented as Mean \pm SEM, Superscript *** denotes p<0.001 vs control, ### denotes p<0.001; vs A β ₁₋₄₂ groups respectively, (A β : Amyloid beta).

Morphology results

The control SH-SY5Y cells are tiny, highly light retractile, fibroblast-like and good morphological features and growing densely. A β ₁₋₄₂ (10 μ M) intoxication caused morphological changes with condensed cell density, swollen, decreased proliferation and differentiation of cells in comparison to the control cells. Post-treatment with met-L-JG extracts have significantly protected the cells from the A β ₁₋₄₂ toxicity which is evidenced by increased cell density and proliferation, in comparison to A β ₁₋₄₂ (10 μ M) treated cell lines. However, met-L-JG treatment has shown better morphological restoration against A β ₁₋₄₂ *in vitro* model of Alzheimer's diseases.

**A β ₁₋₄₂****Met-L-JG****Control****DISCUSSION**

The preliminary phytochemical studies of methanolic extract of leaves of *Jatropha gossypifolia* showed the presence of phenolic compounds & flavonoids, Carbohydrates & glycosides, Protein & aminoacids, Alkaloids, Phytosterol, Saponins. These phytochemical constituents identified were further subjected to GC-MS analysis. The GC-MS analysis has shown the presence of 1,1,5-Trimethyl-1,2-dihydronaphthalene, Methyl 10,12-pentacosadiynoate, Dodecanoic acid, methyl ester, Methyl tetradecanoate, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Cyclopropanepentanoic acid, 2-undecyl-, methyl ester,

trans-, n-hexadecanoic acid, 9,12,15- Octadecatrienoic acid (Z,Z,Z)-, Heptadecanoic acid, 16-methyl-methyl ester-, 7,8-Epoxy lanostan-11-ol, 3-acetoxy, Cis, Cis, Cis- 7,10,13 Hexadecatrienal. From this some important compounds of Cis, Cis, Cis 7,10,13 Hexadecatrienal, Heptadecanoic acid 16-methyl-methylester-, only subjected for molecular docking studies. The results have shown that donepezil had the highest binding score of -9.2 followed by Cis, Cis, Cis- 7,10,13 Hexadecatrienal (7.6), Heptadecanoic acid 16-methyl-methylester (7.0). The docked donepezil and the compound molecules were almost found to occupy the same binding site and form similar interactions to those formed by donepezil in the crystal structure, thus confirming the similar activity of the compounds. Inhibition concentration (IC₅₀) of *Jatropha gossypifolia* was found to be 55.35µg/ml by MTT assay. The percentage of viability of cells was found to be 77.96 in treatment with met-L-JG. Effect of methanolic extract of leaves of *Jatropha gossypifolia* (met-L-JG) in Aβ₁-42 intoxicated SH-SY5Y cell lines was found to be LPO level 53.62, NO level (88.81), GSH level (71.28), SOD (17.58), CAT (0.723), IL- 1β (511.69), TNF-α (475.83). The in vitro results showed that the met-L-JG had the potent activity against alzheimer's.

CONCLUSION

The protein and ligand plays an important role in structural based drug design. In this study phytochemicals obtained from the leaves of *Jatropha gossypifolia* by Gas Chromatography Mass spectrometry (GC-MS) analysis showed the presence of different bioactive compounds provides effective use of plant parts for various ailments by traditional specialists. In this work AChE docked against the phytochemical constituents of *Jatropha gossypifolia* leaves such as Cis, Cis, Cis – 7,10,13 Hexadecatrienal, Heptadecanoic acid 16-methyl-methyl ester-, were found to be promising lead targets against alzheimer's based on molecular docking analysis and in vitro study. So, in vivo approaches are therefore recommended to elucidate the molecular mechanism of these compounds to act as potent drugs against alzheimer's.

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REFERENCES

- Mecocci P, Boccardi V, Cecchetti R, Bastiani P, Scamosci M, Ruggiero C and Baroni M: A long journey into aging, brain aging, and alzheimer's disease following the oxidative stress tracks. *Journal of Alzheimer's Disease* 2018; 62(3): 1319-1335.
- Ferreira-Vieira HT, Guimaraes MI, Silva RF and Ribeiro MF: Alzheimer's disease: targeting the cholinergic system. *Current Neuropharmacology* 2016; 14(1): 101-115.
- Pope CN and Brimijoin S: Cholinesterases and the fine line between poison and remedy. *Biochemical Pharmacology* 2018; 19: 433-446.
- Blokland A: Acetylcholine: A neurotransmitter for learning and memory? *Brain Research Reviews* 1995; 21(3): 285-300.
- Francis PT, Palmer AM, Snape M and Wilcock GK: The cholinergic hypothesis of Alzheimer's disease: a review of progress. *Journal of Neurology, Neurosurgery & Psychiatry* 1999; 66(2): 137-147.
- Arendt T, Bruckner MK, Morawski M, Jager C and Gertz HJ: Early neuron loss in Alzheimer's disease: cortical or subcortical? *Acta Neuropathologica Communications* 2015; 3(1): 10.
- Carter J and Lippa CF: β-Amyloid, neuronal death and Alzheimer's disease. *Current Molecular Medicine* 2001; 1(6): 733-737.
- Geula C and Mesulam M: Cholinesterases and the pathology of Alzheimer disease. *Alzheimer disease and associated disorders* 1995; 9(2): 23-28.
- Lombardo S and Maskos U: Role of the nicotinic acetylcholine receptor in Alzheimer's disease pathology and treatment. *Neuropharmacology* 2015; 96: 255-262.
- Paul S, Jeon WK, Bizon JL and Han JS: Interaction of basal forebrain cholinergic neurons with the glucocorticoid system in stress regulation and cognitive impairment. *Frontiers in Aging Neuroscience* 2015; 7(43): 1-11.
- Kokate, C.K., "Practical Pharmacognosy" 2002, p: 107-114, 123-125, 130. 2002: 1st edition; 405-406, 426-489.
- Kokate, C.K., Evaluation of crude drug, *Practical Pharmacognosy*, 1985; 1st edition; 122-135.
- Kokate, C.K., Purohit A.P., Gokhale S.B., Fluorescence analysis and qualitative Phytochemical analysis, *Practical Pharmacognosy*, 2000: 4th edition; 107-111.
- Khandelwal K.R., "Practical Pharmacognosy" 1996, P: 15-148, 149-153, 157-159, 162-166.
- Hossain M., Al-Hdhami S., Weli A., Al-Riyami Q., Al-Sabahi J. Isolation, fractionation and identification of chemical constituents from the leaves crude extracts of *Menthapiperta L* grown in Sultanate of Oman. *Asian Pacific J. Trop. Biomed.*, 2014;4(1):368-372.
- Lu S., Wu J., Liu H., Zhao J., Liu K., Chuang C., Lin H., Tsai W., Ho y., The discovery of potential acetylcholinesterase inhibitors; A combination of pharmacophore modelling, virtual screening and molecular docking studies, *Journal of Biomed Sci.*, 2012;(1)6:1-13.
- Kaja S, Duncan RS, Longoria S, Hilgenberg JD, Payne AJ, Desai NM, Parikh RA, Burroughs SL, Gregg EV,



- Goad DL, Koulen P. Novel mechanism of increased Ca²⁺ release following oxidative stress in neuronal cells involves type 2 inositol-1,4,5-trisphosphate receptors. *Neuroscience* 2011;175:281-291.
18. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;95(2):351-358.
 19. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Anal Biochem* 1982;126(1):131-138.
 20. Kakkar, P., Das, B., Viswanathan, P.N., A modified spectrophotometric assay of superoxide dismutase. *Indian J BiochemBiophys.* 1984;21(2): 130-132.
 21. Beers, R.F. Jr., Sizer, I.W., 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem.* 1952;195(1):133-140.
 22. Jollow, D.J., Mitchell, J.R., Zampaglione, N., Gillette, J.R., 1974. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology.* 1974;11(3):151-169.
 23. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., Protein measurement with the Folin phenol reagent. *J Bio Chem,* 1951;193(1):265-275.
 24. Roeske-Nielsen A, Fredman P, Mansson JE, Bendtzen K, Buschard K. Beta-galactosylceramide increases and sulfatide decreases cytokine and chemokine production in whole blood cells. *ImmunolLett.* 2004;91(2-3):205-211.

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