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Network pharmacology-based prediction and experimental validation of *Mimosa pudica* for Alzheimer's disease

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ABSTRACT

Mimosa pudica is a traditional folk medicine and has been reported to improve the memory in experimentally induced amnesia. However, present literature lacks the data for *M. pudica* compound-protein interaction with targets related to Alzheimer's disease (AD) including cytotoxic profile. Hence, the present study aims to evaluate *in silico* and *in vitro* antioxidant, cytotoxicity, and acetylcholinesterase inhibitory activity and network evaluation of *M. pudica* with targets related to Alzheimer's disease. The whole plant of *M. pudica* was collected, authenticated and hydroalcoholic extract/fractions were prepared. The antioxidant activity of the hydroalcoholic extract was evaluated by DPPH˙ free radical scavenging assay (*in vitro*) and xanthine oxidase binding affinity (*in silico*). Acetylcholinesterase (AChE) inhibitors from *M. pudica* were identified from an open-source database and analyzed using a network among compounds, proteins, and modulated pathways. Docking was performed using autodock4 and AChE inhibitory activity of extract/fraction(s) was carried using the *in vitro* method. The cytotoxicity of *M. pudica* was assessed using CLC-Pred (*in silico*) and MTT assay (*in vitro*). Quercetin-3-O-β-D-xylopyranoside and myricetin-3-O-β-D-xylopyranoside showed the highest binding affinity with xanthine oxidase. AChE was majorly targeted by multiple phytoconstituents of *M. pudica*. Quercetin showed the highest binding affinity with AChE. Luteolin interacted with maximum proteins involved in the pathogenesis of AD. The compounds were predicted to be more cytotoxic in cancer cells compared to normal. The phytoconstituents from *M. pudica* were found to be safe in normal cells, and were potent anti-oxidants. Flavonoids showed highest binding affinity with xanthine oxidase and fraction rich in flavonoids showed the highest AChE inhibitory capacity.

Keywords: Acetylcholinesterase, Alzheimer's disease, Anti-oxidant, Cytotoxicity, *Mimosa pudica*

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease that impairs memory and psychiatric functions. Multiple theories have been proposed to explain the pathogenesis of AD e.g. cholinergic theory [1], autophosphorylation of tau protein [2] and accumulation of β- amyloid [3]. However, AD occurs due to multiple etiological factors like a physical injury in neuronal cells, an increase in oxidative stress, aging and genetic factors [4]. These multiple etiological factors trigger several proteins generating the complex network among them [5]. Further, the current pharmacotherapy of AD employs single-target agents like cholinesterase inhibitors (tacrine, donepezil, galantamine), nootropic agents (piracetam, aniracetam) and N-methyl-D-aspartate receptor (NMDA) receptor antagonist (memantine) [6]; prolong usage of these agents lose their efficacy and are often associated with multiple side effects [7].

Mimosa pudica L. belonging to family Fabaceae is a traditional folk agent that is utilized in the management of various diseases [8] including cognitive dysfunction [9]; demonstrated the improvement of memory in experimentally induced amnesia [10]. The beneficial effects of *M. pudica* have been reported due to the presence of flavonoids, saponins, alkaloids, polyphenols, and steroids [8]. Although this agent has been reported for its effectiveness in the amelioration of AD, the probable mechanism has not been proposed due to insufficient evidence for an interaction of phytoconstituents from *M. pudica* with proteins involved in the pathogenesis of AD. Further, the potency of a particular group of phytoconstituents to inhibit acetylcholinesterase (AChE) has not been reported yet. Hence, the present study aimed to identify the group of phytoconstituents for its beneficial effect in the treatment of cognitive dysfunction including cytotoxicity assessment using both *in silico* and *in vitro* approach.

MATERIALS AND METHODS

Plant authentication, extract preparation and fractionation

The whole plant of *M. pudica* was collected from local areas of Belagavi and authenticated by a botanist in ICMR-NITM, Belagavi and the herbarium (accession number: RMRC-1421) was deposited for the same for future reference. The collected plant was washed under running water, shade dried and crushed into a coarse powder. Then the extraction and fractions were performed as explained by Cos *et al.*, 2006 [11]. The coarse powder was subjected for maceration with 70% ethanol for seven days, and filtered; marc was shade dried and subjected for soxhlet extraction using 95% ethanol and the combined extract was fractioned. For the fractionation process different solvents like dichloromethane, petroleum ether, water, methanol, and acetone were used. The outline for the extraction and fractionation steps is represented in Figure 1.

Total polyphenol and flavonoid content

Total polyphenol and flavonoid content of the hydroalcoholic extract of *M. pudica* was performed as explained by Ainsworth *et al.* [12] and Chang *et al.* [13] with equivalent to gallic acid and quercetin respectively. All the experiments were performed in triplicates.

***In silico* and *In-vitro* anti-oxidant activity**

X-ray crystallographic protein of xanthine oxidase (PDB ID:3NRZ) was retrieved from the RCSB database (https://www.rcsb.org/); was in a complex with hetero atoms; hence, removed using Discovery studio 2019. Similarly, all the ligand molecules were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/); energy was minimized using the mmff94 force field [14] and docked using autodock4[15]. After the completion of docking, the pose scoring minimum binding energy was chosen to visualize the ligand-protein interaction in Discovery studio 2019. Further, the DPPH˙ free radical scavenging assay of the hydroalcoholic extract was performed as explained by Choi *et al.* [16]. Ascorbic acid was used as standard and all the experiments were performed in triplicates. Suitable controls were taken for test and reference compounds and IC_{50} was calculated. All the values were expressed in Mean±SD.

***In silico* prediction of AChE inhibitors from *M. pudica* (Network pharmacology)**

All the phytoconstituents reported in *M. pudica* were mined from open source database; Canonical SMILES of each compound was retrieved from the PubChem database and their targets were predicted using BindingDB[17] at 70% similarity index. The proteins involved in the memory impairment, primarily in AD were identified with respect to the Therapeutic Target database [18]. Further, protein-protein interaction was assessed using the STRING database [19] and the pathways modulated by protein-protein interaction were identified using the KEGG pathway. The network among phytoconstituents, proteins and pathways were constructed using cytoscape [20]. The constructed network was analyzed by treating it as directed and setting the node size as "*low values to small size*" and "*low values to bright colors*" based on edge count for both settings.

***In silico* and *In vitro* AChE inhibitory activity**

The homology model of human AChE protein was prepared using accession number: AAA68151.1 as a query sequence and PDB ID:4PQE as a template using Modeller9.10 (https://salilab.org/modeller/). Water molecules and other heteroatoms were removed using Discovery Studio 2019 to avoid docking interference. Hydroalcoholic extract of *M. pudica* and its fractions were tested for *in- vitro* AChE activity as explained by Chowdhury S *et al.* [21]. Donepezil was taken as standard and suitable controls for all tests. All the experiments were performed in triplicates and values were expressed in Mean±SD.

In silico* and *in vitro* cytotoxicity of *M. pudica

The cell line toxicity was predicted using CLC-Pred [22] at the probability activity (Pa) > 0.5 in both tumor and normal cell lines. Similarly, *in vitro* cytotoxicity assay was performed in Chinese hamster ovary (CHO) (normal cell line) and A549 (tumor cell line) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described by Mosmann *et al.* [23]. The cells were treated with different concentrations (2.0, 2.30, 2.47, 2.60, 2.69, 2.77, 2.84, 2.90 µg/ml; log concentration) of hydroalcoholic extract, incubated for 48hrs in humidified environment (37ºC and 5%CO_2) followed by addition of 10µl of MTT and absorbance was measured 570 nm and IC_{50} was calculated. The experiment was performed in triplicate.

RESULTS

Preliminary phytochemical investigation

The preliminary phytochemical investigation identified the presence of flavonoids, saponins, alkaloids, tannins, steroids, and polyphenols in a hydroalcoholic extract of the whole plant of *M. pudica*. Similarly, the phytochemical investigation of fractions identified a group of compounds which is summarized in Figure 1.

Total polyphenol and flavonoid content

The total polyphenol content and flavonoid content in the hydroalcoholic extract were found to be 98.4±1.50 and 147±1µg/ml respectively.

In silico* and *In-vitro* antioxidant activity of *M. pudica

Quercetin-3-O-β-D-xylopyranoside and myricetin-3-O-β-D-xylopyranoside were predicted to possess the highest binding affinity with xanthine oxidase i.e. -8.2 kcal/mol by interacting with eleven hydrogen bonds with GLN:144, ALA:142, ASN:146, GLY:145, THR:52, LSN:71, SER:123, and LEU:127 and eight with THR:52, ASN:146, SER:69, ASN:130, HIS:67, and ASP:141 respectively. The binding energy of each ligand with xanthine oxidase with hydrogen bond count and residues is summarized in Table 1. Similarly, the interaction of quercetin-3-O-β-D-xylopyranoside and myricetin-3-O-β-D-xylopyranoside with xanthine oxidase is represented in Figure 2. Further, the DPPH free radical scavenging capacity of hydroalcoholic extract (IC_{50}) of *M. pudica*, and ascorbic acid were found to be 546.79±68.06 µg/ml and 25.88±4.847 µg/ml respectively.

***In silico* prediction of AChE inhibitors from *M. pudica* (Network pharmacology)**

Twenty-seven phytoconstituents from *M. pudica* were identified in which seventeen compounds modulated eighteen proteins (AChE, ADRA2A, ADRA2C, App, BACE1, CHRM2, CHRM5, DRD2, GSK3B, HTR1A, HTR4, MAOA, MAOB, PTGES, PTGS1, PTGS2, SNCA, and TNF) involved in the pathogenesis of AD. Network analysis identified AChE as a primarily targeted protein by maximum phytoconstituents i.e. 5,3'-di-O-methylluteolin, hernancorizin, quercetin, 7,3',4'-trihydroxy-3,8-dimethoxyflavone, 2'-hydroxy-3,7,8,4',5'-pentamethoxyflavone, diplotrin A, diplotrin B, diplotrin C, and luteolin. Among them, luteolin, a flavonoid was predicted to interact with the highest number of protein molecules i.e. AChE, MAOA, MAOB, App, BACE1, PTGS1, PTGS2, GSK3B, HTR1A, and HTR4 (Figure 3). Further, enrichment analysis identified thirty-five different pathways in which serotonergic synapse and neuroactive ligand-receptor interaction were majorly modulated by affecting the highest number of genes. Table 2 summarizes the modulated pathways with their respective modulated genes by phytoconstituents of *M. pudica*.

***In silico* and *In vitro* AChE activity**

Docking study predicted quercetin to possess the highest binding affinity with AChE i.e. -9.4 kcal/mol with four hydrogen bonds by interacting with HIS:478, TRP:117, GLN:102, and TYR:103. Table 3

summarizes the binding affinity of each phytoconstituents with AChE including hydrogen bond. Figure 4 represents the interaction of quercetin with AChE. The IC_{50} of hydroalcoholic extract of *M. pudica* was found to be 398.45±30.19 µg/ml. Similarly, among the different fractions and hydroalcoholic extract, fraction rich in flavonoids showed the highest AChE inhibitory activity 124.26±1.73 µg/ml compared to others fractions. Table 4 summarizes AChE inhibitory activity of hydroalcoholic extract/ fractions of *M. pudica*.

***In silico* and *In vitro* cytotoxicity study**

Cytotoxicity prediction identified the phytoconstituents from *M. pudica* were more cytotoxic in tumor cells compared to normal cells. Figure 5 summarizes cluster of cytotoxic potential of phytoconstituents from *M. pudica* in normal (WI-38 VA13) and tumor (786-0, 8505C, A498, ACHN, BT-549, CAKI-1, DMS-114, FaDu, HCC 2998, HCT-15, `HL-60, HOP-92, Hs 683, IGROV-1, KM12, LOX IMVI, M14, Malme-3M, MCF7, MDA-MB-468, MOLT-4, NCI-H23, NCI-H460, NCI-H522, NCI-H838, OVCAR-4, OVCAR-5, OVCAR-8, PC-3, PC-9, RPMI-8226, RXF 393, SF-295, SK-MEL-1, SK-MEL-2, SK-MEL-28,SK-MEL-5, SNB-75, SR, SW-620, TK-10, UACC-257,and UO-31) cell lines. Similarly, *in vitro* MTT test pointed higher cytotoxic potency (IC_{50}) of hydroalcoholic extract in A549 (cancer) compared to CHO (normal) cell lines i.e. 282.56±6.76 µg/ml and 468.10±12.27 µg/ml respectively.

DISCUSSION

The present study was performed to investigate various phytoconstituents of *M. pudica* for anti-oxidant, cytotoxicity and AChE inhibitory activity. The study reports the hit phytoconstituents to possess the highest anti-oxidant capacity using both *in silico* and *in vitro* models. Additionally, the compound-protein interactions of phytoconstituents of *M. pudica* with the proteins involved in the pathogenesis of AD were reported to produce a synergistic effect. Similarly, for the first time, the present study reports the multiple interactions of phytoconstituents from *M. pudica* with the proteins involved in the pathogenesis of AD.

Network pharmacology and enrichment analysis are the bioinformatic approaches which have contributed a lot in the elucidation of lead molecules, targets and molecular mechanism of herbal formulations [24, 25]. Similarly, *in silico* docking study also helps to predict the binding affinity of compounds with respective targets [24]. In the present study, network pharmacology identified the lead molecule (luteolin), highly targeted protein (AChE) and majorly regulated pathways (serotonergic synapse and neuroactive ligand-receptor interaction) which are well-recognized synapse in the progression of AD [26-27].

The beneficial effects of flavonoids from various traditional medicines were identified in memory improvement [28]. Over database mining, the majority of phytoconstituents were flavonoid which was also confirmed by the estimation of total flavonoid content. The network interaction also predicted luteolin (flavonoid) to target the maximum number of pathogenic proteins. Further, the *in vitro* study also showed the high AChE inhibitory activity by flavonoids compared to others. The overall result suggests the potency of flavonoids from *M. pudica* to inhibit the AChE which could be utilized in the management of cognitive impairment.

A docking study helps to predict the binding affinity of small ligand molecules with their targets [29, 30]. This approach utilizes multiple algorithms to assess the pose and interactions of the ligand with the respective targets. However, the obtained docking hit could be falsely positive if the targets were not identified [31]. Hence, to minimize this effect, a cheminformatic tool, BindingDB was utilized to predict the target for each compound; network was constructed between proteins and compounds and analyzed, in which AChE was targeted by maximum phytoconstituents i.e. 2'-hydroxy-3,7,8,4', 5'-pentamethoxyflavone, 7,3',4'-trihydroxy-3,8-dimethoxyflavone, diplotrin A, diplotrin B, diplotrin C, hernancorizin, 5,3'-di-O-methylluteolin, luteolin, and quercetin. This approach has been previously utilized to evaluate multiple compounds-protein interactions and identify multiple lead molecules and targets [32]. The present study identified the majority of flavonoids to have a strong binding affinity with AChE in which quercetin was identified to possess the highest binding affinity. Hence, the beneficial effect of quercetin with donepezil in cognitive dysfunction [33] could be due to the combined inhibition of both molecules over AChE.

Likewise, the anti-oxidant potential of the majority of phytoconstituents also possesses a beneficial effect in the management of AD [34]. For this hypothesis, the potency of hydroalcoholic extract of *M. pudica* for DPPH free radical scavenging assay was performed. Xanthine oxidase is identified to produce free radicals in various cell lines including epithelial, endothelial, connective and polymorphonuclear tissues [35]. Similarly, docking study also revealed compounds from *M. pudica* do not limit only for free radicals scavenging capacity but they also inhibit free radical generators.

The single-target pharmacotherapeutic agents of AD i.e. cholinesterase inhibitors, nootropic agents and NMDA receptor antagonists [6] lose their efficacy for prolonged use and are associated with multiple side effects [7, 36] limiting for further use. More often, these single-target drug molecules are also more cytotoxic since the administered dose is high. However, in the present study, the compounds from *M. pudica* were found to be more cytotoxic in tumor cell lines compared to normal; reflects for safety in human consumption. Further, the study identified the IC_{50} value of hydroalcoholic extract to inhibit the acetylcholinesterase was found to be lower compared to the IC_{50} value for MTT assay in normal cell lines. However, the IC_{50} of DPPH scavenging capacity was found to be higher than IC_{50} of MTT cytotoxicity. This suggests the amount of hydroalcoholic extract required to inhibit AChE could be safer in normal cells compared to scavenging free radicals. However, the assessment of safety of fraction rich in flavonoids needs to still investigated which is the future scope of present study. Likewise, the study also suggests investigating the fraction rich in flavonoids from *M. pudica* for its DPPH scavenging capacity.

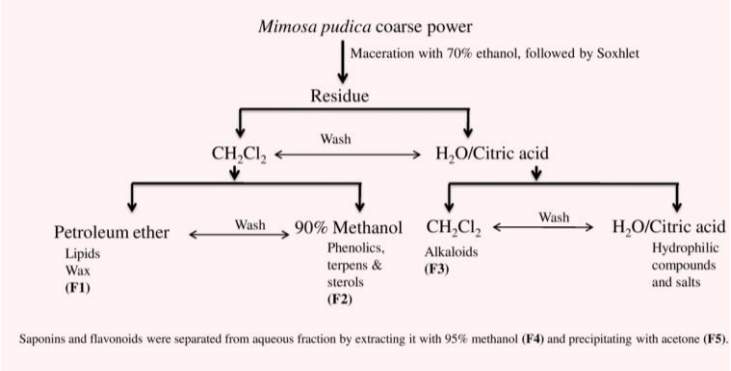

Figure 1: Flow diagram for extraction and fractionation

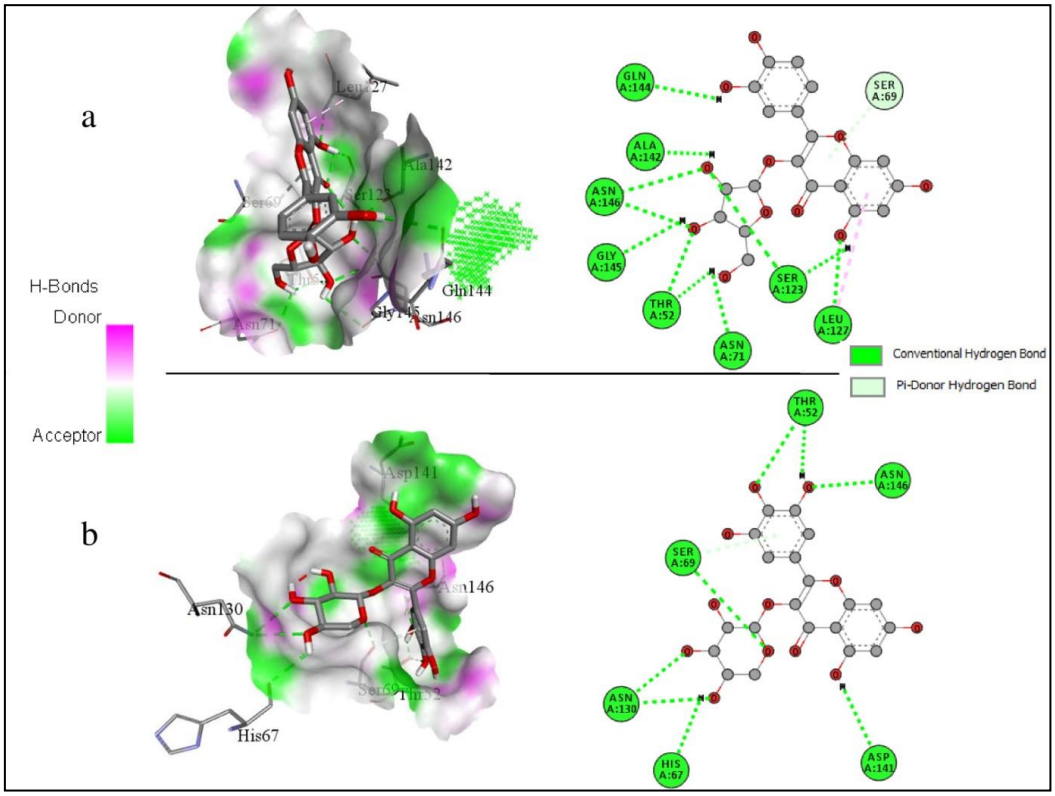

Figure 2: Interaction of (a) Quercetin-3-O-ß-D-xylopyranoside and (b) myricetin-3-O-β-D-xylopyranoside with xanthine oxidase

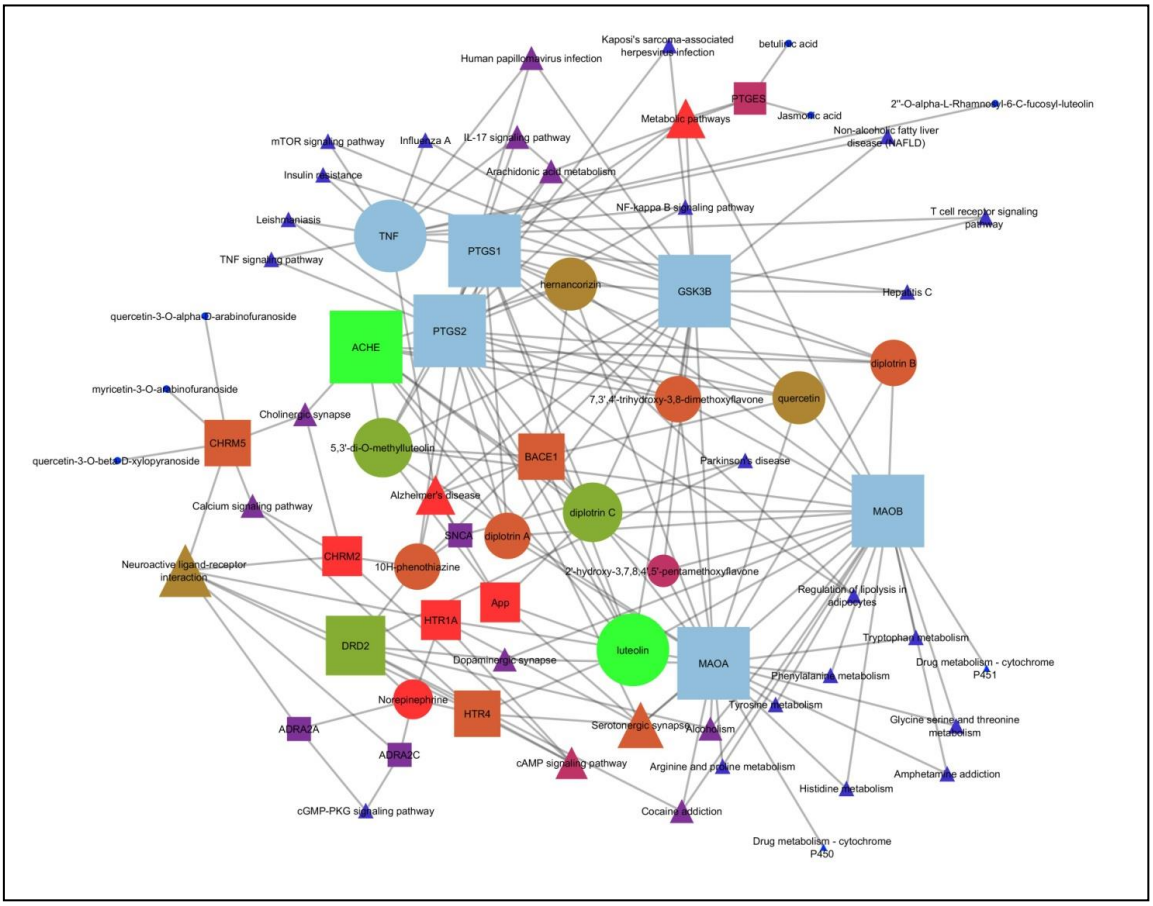

Figure 3: Interaction of phytoconstituents with targets and respective pathways

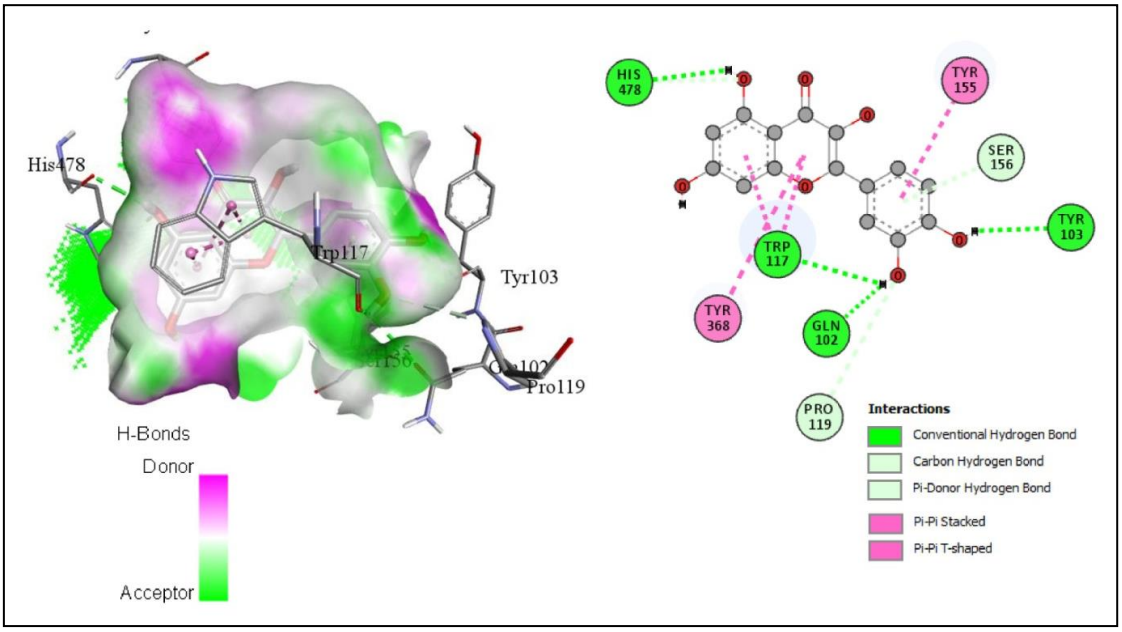

Figure 4: Interaction of quercetin with acetylcholinesterase

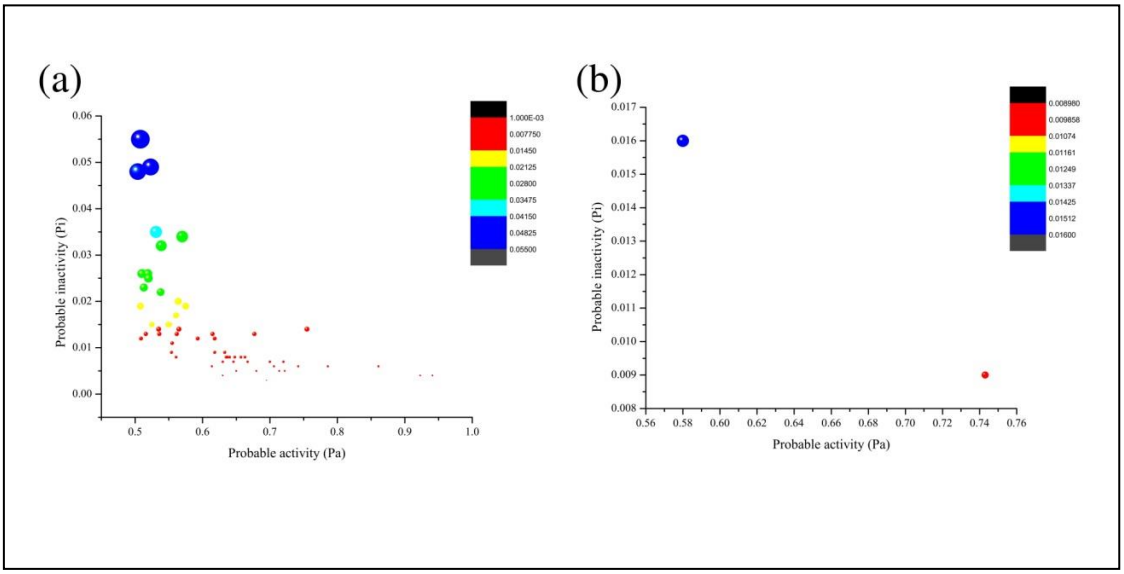

Figure 5: Cytotoxicity of *Mimosa pudica* in (a) cancer and (b) normal cell lines

Table 1: Binding energy of ligand molecules with xanthine oxidase (PDB ID:3NRZ)

Ligands	Binding energy (kcal/mol)	NHBD	H-bond residue
10H-phenothiazine	-7	2	PHE:143, ASN:146
2''-O-α-L-Rhamnosyl-6-C-fucosyl-luteolin	-7.7	2	GLU:89, THR:94
2'-hydroxy-3,7,8,4',5'-pentamethoxyflavone	-6	2	SER:69, ASN:71
5,3'-di-O methylluteolin	-6.9	3	SER:69, GLU:138
7,3',4'-trihydroxy-3,8-dimethoxyflavone	-6.9	5	THR:52, SER:123, ASN:71, SER:69
Betulinic acid	-7.6	-	-
Diplotrin A	-6.4	2	THR:86
Diplotrin B	-6.4	4	THR:86, VAL:85, GLY:12
Diplotrin C	-6.6	1	VAL:88
Hernancorizin	-6.9	5	ASN:146, SER:123, THR:52, GLY:145, ASP:141
Luteolin	-7.3	5	SER:123, THR:52, GLY:145, SER:69, ASN:130
Myricetin-3-O-β-D-xylopyranoside	-8.2	8	THR52, ASN146, SER69, ASN130, HIS67, ASP141

Norepinephrine	-5.2	4	ASP:141, SER:123, THR:52
Quercetin-3-O-α-D-arabinofuranoside	-7.7	4	SER:123, ASN:146, THR:52
Quercetin-3-O- β-D-xylopyranoside	-8.2	11	GLN144, ALA142, ASN146, GLY145, THR52, 1SN71, SER123, LEU127
Quercetin	-7.3	5	SER:69, SER:123, THR:52, ASN:71, ASP:141
Jasmonic acid	-6.5	0	-NA-

NHBD: Number of Hydrogen Bond Donor, NA: Not Applicable

Table 2: Gene-set enrichment analysis of modulated pathways

Pathway	Description	Count in gene set	Gene codes	False discovery rate
hsa04726	Serotonergic synapse	7	HTR4, MAOB, MAOA, HTR1A, APP, PTGS2	8.94e-10
hsa04080	Neuroactive ligand-receptor interaction	7	HTR4, DRD2, HTR1A, ADRA2A, ADRA2C, CHRM2, CHRM5	1.78e-07
hsa05010	Alzheimer's disease	5	TNF, SNCA, GSK3B, BACE1, APP	1.53e-05
hsa04728	Dopaminergic synapse	4	GSK3B, MAOB, MAOA, DRD2	0.00016
hsa05030	Cocaine addiction	3	MAOB, MAOA, DRD2	0.00032
hsa04024	cAMP signaling pathway	4	HTR4, DRD2, HTR1A, CHRM2	0.00051
hsa00590	Arachidonic acid metabolism	3	PTGES, PTGS2, PTGS1	0.00051
hsa04657	IL-17 signaling pathway	3	TNF, PTGS2, GSK3B	0.0012
hsa04725	Cholinergic synapse	3	ACHE, CHRM2, CHRM5	0.0017
hsa00360	Phenylalanine metabolism	2	MAOA, MAOB	0.0017
hsa00340	Histidine metabolism	2	MAOA, MAOB	0.0025
hsa05034	Alcoholism	3	MAOA, MAOB, DRD2	0.0029
hsa04020	Calcium signaling pathway	3	CHRM5, CHRM2, HTR4	0.0049
hsa00350	Tyrosine metabolism	2	MAOA, MAOB	0.0049
hsa00380	Tryptophan metabolism	2	MAOA, MAOB	0.0050
hsa00260	Glycine, serine and threonine metabolism	2	MAOA, MAOB	0.0050
hsa00330	Arginine and proline metabolism	2	MAOA, MAOB	0.0065
hsa04923	Regulation of lipolysis in adipocytes	2	PTGS1, PTGS2	0.0074
hsa05031	Amphetamine addiction	2	MAOA, MAOB	0.0104
hsa00982	Drug metabolism - cytochrome P450	2	MAOA, MAOB	0.0104
hsa05140	Leishmaniasis	2	TNF, PTGS2	0.0108
hsa05165	Human papillomavirus infection	3	TNF, PTGS2, GSK3B	0.0155
hsa04064	NF-kappa B signaling pathway	2	TNF, PTGS2	0.0171
hsa04660	T cell receptor signaling pathway	2	TNF, GSK3B	0.0185
hsa04931	Insulin resistance	2	TNF, GSK3B	0.0206
hsa04668	TNF signaling pathway	2	TNF, PTGS2	0.0206
hsa01100	Metabolic pathways	5	PTGES, PTGS1, PTGS2, MAOA, MAOB	0.0206
hsa05160	Hepatitis C	2	TNF, GSK3B	0.0270
hsa05012	Parkinson's disease	2	SNCA, DRD2	0.0304
hsa04932	Non-alcoholic fatty liver disease (NAFLD)	2	TNF, GSK3B	0.0318
hsa04150	mTOR signaling pathway	2	TNF, GSK3B	0.0318
hsa04022	cGMP-PKG signaling pathway	2	ADRA2A, ADRA2C	0.0346
hsa05164	Influenza A	2	TNF, GSK3B	0.0368
hsa05167	Kaposi's sarcoma-associated herpesvirus infection	2	PTGS2, GSK3B	0.0419

Table 3: Binding energy of ligand molecules with acetylcholinesterase

Ligand	Binding Affinity (kcal/mol)	NHBD	H-bond residue
2'-hydroxy-3,7,8,4', 5'-pentamethoxyflavone	-6.7	2	TRP:563, HIS:436
5, 3'-di-O-methylluteolin	-7.3	2	THR:269, ASN:564
7, 3', 4'-trihydroxy-3,8-dimethoxyflavone	-7.4	2	PRO:67, ASN:217
Diplotrin A	-6.7	2	THR:269, GLY:271
Diplotrin B	-6.6	2	THR:269
Diplotrin C	-7	0	-NA-
Hernancorizin	-7.2	1	ARG:277
Luteolin	-7.7	2	TRP:563, THR:117
Quercetin	-9.4	4	HIS:478, TRP:117, GLN:102, TYR:103

NHBD: Number of Hydrogen Bond Donor, NA: Not Applicable

Table 4: Acetylcholinesterase inhibitory activity of extract and fractions of *M. pudica*

S. No.	Test agents	Major Phytoconstituents	IC_{50} (µg/ml)
1	HAE	Flavonoids, Saponins, Alkaloids, Tannins, Steroids, And Polyphenols	398.45±30.19
2	Fraction 1	Lipid and Wax	221.54±5.19
3	Fraction 2	Phenolics, terpens and sterols	191.85±9.32
4	Fraction 3	Alkaloids	257.40±5.48
5	Fraction 4	Flavonoids	124.26 ±1.73
6	Fraction 5	Saponins	182.16 ±11.62
7	Donepezil*	-NA-	89.04±0.67

Values are expressed in mean±SD (n=3). IC_{50}: Inhibitory Concentration50, HAE: Hydroalcoholic extract, NA: Not applicable, *gold standard for AChE inhibition.

CONCLUSION

Multiple compounds from *M. pudica* were identified to inhibit the AChE; demonstrated via *in silico* and *in vitro* approach. The hydroalcoholic extract of *M. pudica* was identified to be a potent free radical scavenger. Further, the compounds from *M. pudica* were more cytotoxic in cancer cell lines compared to normal cell lines. The potency of the compounds to inhibit the AChE could be primarily due to the flavonoids which need to be further proven via *in vivo* animal models.

Abbreviation

AD: Alzheimer's Disease, BindingDB: Binding database, CHO: Chinese hamster ovary, CLCPred: Cell line toxicity predictor, DPPH: 2,2-diphenyl-1-picrylhydrazyl, IC_{50}: Inhibitory concentration 50, ICMR-NITM: Indian Council of Medical Research – National Institute of traditional medicine, KEGG: Kyoto Encyclopedia of Genes and Genomes, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, NMDA: N-methyl-D-aspartate receptor, PDB: Protein Data Bank, RCSB: Research Collaboratory for Structural Bioinformatics, RMRC: Regional Medical Research Center, SMILES: Simplified molecular-input line-entry system, STRING: Search Tool for the Retrieval of Interacting Genes/Proteins.

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Authors' contributions

Taaza Duyu contributed in the completion of work, manuscript preparation and review of literature. NA Khatib suggested the modification in study protocol and reviewed the manuscript. Pukar Khanal designed the study, involved in manuscript preparation and drafting it. Prof. BM Patil, and KK Hullatti has equal contribution in reviewing and editing the manuscript.

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Competing interests

Nil

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