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Characterization of coumarins from *Ipomoea mauritiana* Jacq by LC-APCI-MS/MS analysis and evaluation of its anti-amnesic activity

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Abstract

Background: *Ipomoea mauritiana* is one of the source plants of *Vidari*, an Ayurvedic drug used as *Medhyarasayana* (rejuvenating drug). In this current study, coumarins were separated from tuberous root of *I. mauritiana* and characterization of the coumarin fraction was done by LC-MS/MS analysis by atmospheric pressure chemical ionization method. Anti-amnesic activity was evaluated against scopolamine induced amnesia in Wistar rats.

Results: Mass spectroscopic characterization of coumarin fraction directed to the tentative identification of coumarins such as 7-hydroxy-6-methoxy coumarin, 7-hydroxycoumarin, 5-methoxy-6,7-furanocoumarin, 5,7-dimethoxycoumarin, and 6-hydroxy-7-methoxy-4-phenylcoumarin. Aqueous extract of *I. mauritiana* at a dose of 100 and 200 mg/kg showed significant anti-amnesic activity against scopolamine-induced changes in step through latency and working memory errors.

Conclusion: The findings of the study showed that *I. mauritiana* is a rich source of coumarins and possessed significant anti-amnesic activity. The study concluded the scientific basis of using *I. mauritiana* as rejuvenating drug in Ayurveda.

Keywords: *Ipomoea mauritiana*, Coumarin, LC-APCI-MS, Anti-amnesic activity

1 Background

Ipomoea mauritiana Jacq. (IM) is a medicinal plant (Convolvulaceae) used in Ayurveda and Folk medicine. It is one of the source plants of *Vidari*, an Ayurvedic drug which is a component of about 50 Ayurvedic formulations including *Chyawanprash* [1]. The roots are sweet, cooling in action, appetizer, galactagogue, rejuvenating, stimulant, carminative, and tonic. It is also used in emaciation, enteric fever and spermatorrhea [2–4]. It contains phytochemicals such as taraxerol, taraxerol acetate, β-sitosterol, scopoletin, 7-O-β-D-glycopyranosyl scopoletin and caffeoyl glucose [4, 5].

Alzheimer's disease is a progressive neurological disorder that mostly affects the elderly population. Learning and memory impairment as the most distinguishing expression of dementia could be induced chemically by scopolamine, a cholinergic antagonist. Cholinergic deficits are neuropathological occurrences that are consistently associated with memory loss and are correlated with the severity of Alzheimer's disease [6–8].

Chemically coumarins are benzopyrones and are fairly ubiquitous in the plant kingdom; more than 3400 naturally occurring coumarins have been identified [9, 10]. Recently, coumarin chemistry has attracted much attention due to diverse biological and pharmacological properties such as anticoagulant, antibacterial, antifungal, antiprotozoal, insecticidal, fungicide, antimycobacterial, antimutagenic, HIV protease inhibition, monoamine oxidase (MAO) inhibition, and anti-inflammatory activity. Number of evidences also shows the implication of coumarin in the inhibition of acetylcholinesterase enzyme because the planarity and aromaticity of the coumarin ring is the key structural feature that allows its

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interaction easily with PAS through possible π-π stacking [11, 12].

In the present study, coumarins were separated from the tuberous roots of *I. mauritiana*, and their structural characterization was done by LC/MS analysis. Aqueous extract was screened for its antiamnesic activity against scopolamine induced amnesia in Wistar rats.

2 Methods

2.1 Chemicals and instruments

High performance thin layer chromatography (HPTLC) profiling was done on pre-coated silica gel $60F_{254}$ TLC plate (Merck, India). The mobile phase was standardized as toluene:ethyl acetate:methanol in the ratio 8:2:1. The chromatogram was developed in a saturated chromatographic chamber (Camag, Switzerland), and the developed plate was visualized under 366 nm. LC/MS analysis was conducted on Agilent 6520 accurate mass Q-TOF LC/MS coupled with Agilent LC 1200 equipped with Extend-C18 column of 1.8 μm, 2.1 × 50 mm. The APCI source was operated with following settings in positive mode: drying gas (nitrogen) flow 8 L/min; nebulizer pressure 40 psig; drying gas temperature 325 °C; capillary voltage + 3000 V; fragmentor volt 125 V; Oct Rf Vpp 750 V. Gradient elution was performed with water/0.05% formic acid (solvent A) and acetonitrile (solvent B) at a constant flow rate of 0.9 ml/min. Column temperature was maintained at 30 °C.

2.2 Plant material

Mature tubers of *I. mauritiana* were collected from Herb garden, Kottakkal, Kerala, India, and authenticated by Dr. K M Prabhukumar, Plant Systematics and Genetic Resources Division, Centre for Medicinal Plants Research (CMPR), Kerala, India, and a voucher specimen was deposited in CMPR herbarium.

2.3 Extraction of coumarins

Coumarins were separated by specific method. Briefly, 50 g of the material was extracted with methanol by soxhlet extraction method for 48 h. After evaporating the extract under reduced pressure, the residue was refluxed with 5% HCl for 2 h. After filtration two fractions were obtained, residue and aqueous—acid phase. The residue was extracted with methanol and evaporated (Fr 1) and aqueous—acid phase was extracted with diethyl ether and was evaporated (Fr 2). The fractions (Fr 1 and Fr 2) were kept under refrigerator until further analysis.

2.4 Acute oral toxicity study

2.4.1 Plant extracts

The pharmacological evaluation was done in aqueous extract. Two hundred fifty grams of the tubers of IM was extracted with water using soxhlet extraction method for 72 h. The final extract was concentrated to dryness under reduced pressure using rotary evaporator (Heidolph, Germany).

2.4.2 Target animals

The experiment was conducted on Wistar rats (females) weighing 147 to 204 g and aged 8 to 9 weeks obtained from the Animal House, J.S.S. College of Pharmacy, Ootacamund-Tamil Nadu. The rats were distributed into 5 groups with 6 animals in each group. The experimental procedures relating to the animals were authorized by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Approval No: JSSCP/IAEC/OT/Ph.D/Ph.Cology/06/2017-18) before starting the study and were conducted under the internationally accepted principles for laboratory animal use and care.

The extracts were prepared from a plant material having a high safety margin, and hence, it was decided to use 2000 mg/kg (limit test) for this study. The test item was prepared immediately prior to administration on respective treatment days. A quantity of 2 g of the test item was dissolved in distilled water, and the volume made up to 10 ml to get a test item concentration of 200 mg/ml. Homogeneity of the test item in the vehicle was maintained during treatment by constant stirring and mixing. The test substance was administered soon after preparation. The prepared test item solutions were administered once orally as gavage to the fasted (16–18 h) rats at the dose volume of 10 ml/kg b.wt. to deliver a dose of 2000 mg/kg b.wt. Food was offered about 3–4 h after dosing. Water was not withheld.

The treated rats were observed five times during day 1 (day of administration), i.e., at 30 min and four times at hourly (post-administration) intervals and once daily and, thereafter, for a total of 14 days. The clinical signs were recorded on all working days. The body weights of rats were recorded on test day 1 (pre-administration), day 8 (7 days post-administration), and day 15 (14 days post-administration). The rats were euthanized by using diethyl ether anesthesia and necropsied.

2.4.3 Evaluation of anti-amnesic activity

The test item was prepared immediately prior to administration on respective treatment days. The extract was prepared as solution in distilled water at concentration equal to 1/10th of the dose and administered at a dose volume of 10 ml/kg, b.wt. Animals were randomly divided into seven groups containing 6 each.

Group treatment

1Normal (vehicle 10 ml/kg, p.o.)

2Control (vehicle 10 ml/kg, p.o.)

3Rivastigmine (1.5 mg/kg., p.o.)

4IM (100 mg/kg., p.o.)
5IM (200 mg/kg., p.o.)

The above treatment was given for a period of 14 days, and a day before the memory test, all the animals were given appropriate training. On 14th day, 1 h after the respective treatments, scopolamine (3 mg/kg., i.p.) was administered to all the groups, except group 1, normal. Step down and step through latency were evaluated 45 min after the scopolamine administration to assess the memory, and the radial arm maze memory test was carried out on day

Step through latency was evaluated by the standard procedure. Each rat was individually placed in the bright part of a two-chambered apparatus for training. The door was closed once rat enters the dark chamber to prevent it from escaping. Later a foot shock (1 mA, 1 s) was applied through the grid floor, and the rat was then returned to the home cage.

Twenty-four hours later, testing was repeated by placing the animal again in the bright chamber. The latency period to enter the second darker chamber was measured. A prolonged latency indicates that the animal remembers that it has been punished and, therefore, does avoid the darker chamber.

Step down latency was assessed using a rectangular box (50 × 50 cm) with electrifiable grid floor and 35 cm fits over the block. The grid floor is connected to a shock device which delivers scrambled foot shocks. The experiment was conducted in three phases: (1) familiarization—the animal was placed on the platform, released after raising the cylinder, and the latency to descend is measured. After 10 s of exploration, it was returned to the home cage. (2.) Learning—immediately after the animal has descended from the platform, an unavoidable foot shock was applied (foot-shock 50 Hz; 1.5 mA; 1 s) and the animal is returned to the home cage, (3) retention test—24 h after the learning trial the animal was again placed on the platform, and the step-down latency was measured. The test was finished when the animal steps down or remains on the platform (cut-off time 60 s).

Radial arm maze test was performed in a eight arm radial maze. Animals were placed in wooden elevated eight-arm radial maze with the arms extending from a central platform 26 cm in diameter. Each arm is 56 cm long and 5 cm wide with 2 cm high rails along the length of the arm. The maze was well illuminated and numerous cues are present. Food pellets (reward) will be placed at the end of the arms. During the test, rats were well fasted to motivate them to run the maze. Animals were trained on a daily basis in the maze to collect the food pellets. The session was terminated after 8 choices, and the rat has to obtain the maximum number of rewards with a minimum number of errors.

The number of errors (entries to non-baited arms) will be counted during the session: reference memory error:

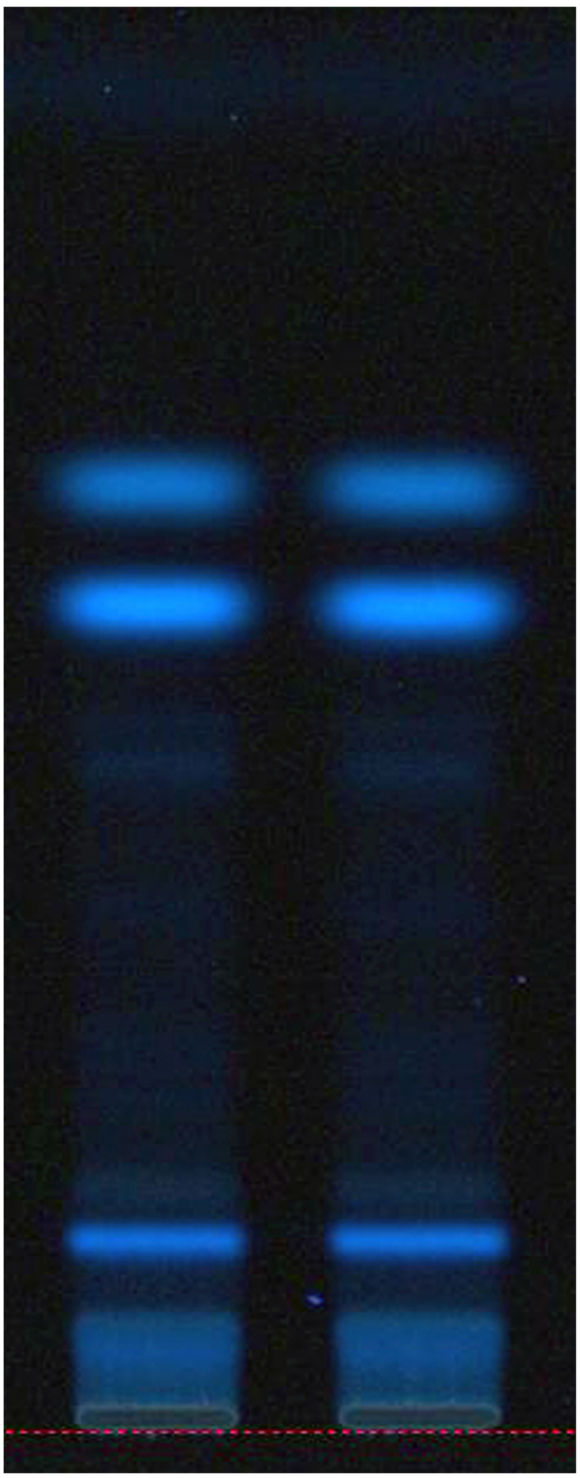

Fig. 1 HPTLC analysis of IM extract

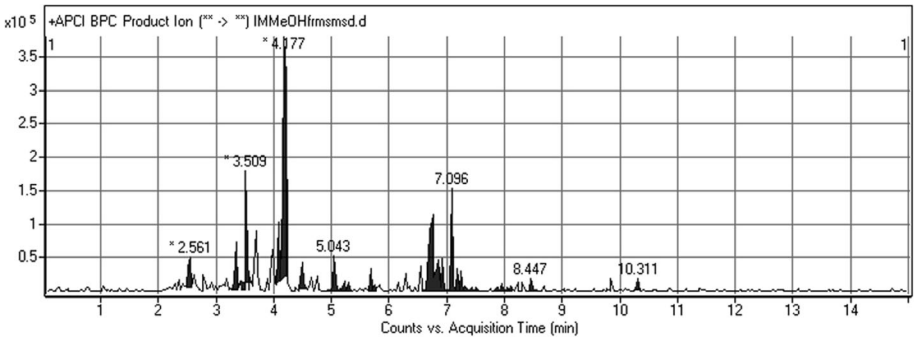

Fig. 2 LC-APCI-MS base peak chromatogram of fraction 1

Visit to unbaited arm more than once; working memory error: Visit to baited arm more than once.

2.5 Statistical analysis

For determination of significant intergroup differences of each parameter one-way analysis of variance (ANOVA) was carried out. Dunnet's test was used for individual comparisons after significant ANOVA results. The differences with $p < 0.05$ were considered statistically significant. Graph pad Prism-6 software (Graph pad software, Inc., USA) was used for the statistical analysis.

3 Results

3.1 Chemical profiling

Chemical profiling of crude extract was done by HPTLC for the preliminary chemical characterization. HPTLC profile at 366 nm showed several bands with blue fluorescents. The presence of coumarins was confirmed by derivetizing with alcoholic KOH. Various intensified blue fluorescence bands were observed on UV-366 evaluation (Fig. 1).

3.2 LC-MS analysis

Two separated coumarin fractions (Fr1 and Fr2) were subjected to LC/MS analysis in APCI positive mode. The total ion chromatogram (TIC) was extracted with molecular feature extraction (MFE) using Agilent Mass hunter software. The base peak chromatogram of fraction 1 and fraction 2 are presented as Figs. 2 and 3. On evaluating the various protonated ions, ms/ms analysis was conducted by selecting preferred precursor ion on the basis of abundance in m/z values. Mass fragmentation was achieved by collision-induced dissociation (CID) by assigning collision energy 4 eV/100 Dalton with an offset voltage of 6 eV. Consistency of fragments was confirmed by targeted ms/ms analysis with fixed collision voltage as obtained from auto ms/ms analysis. The tentative structure of coumarins was assigned based on mass fragmentation pattern in correlation with previously reported data [13, 14]. The coumarins identified by APCI-MS/MS analysis are presented in Table 1 and Fig. 4.

3.3 Acute oral toxicity studies

The acute oral toxicity results of IM extract on administration once orally to Wistar rats at a dose of 2000 mg/kg b.wt. are presented in Table 2.

Based on the results of the acute oral toxicity (acute toxic class method) of *I. mauritiana* extract in Wistar rats, the LD_{50} of the extract may be classified as GHS

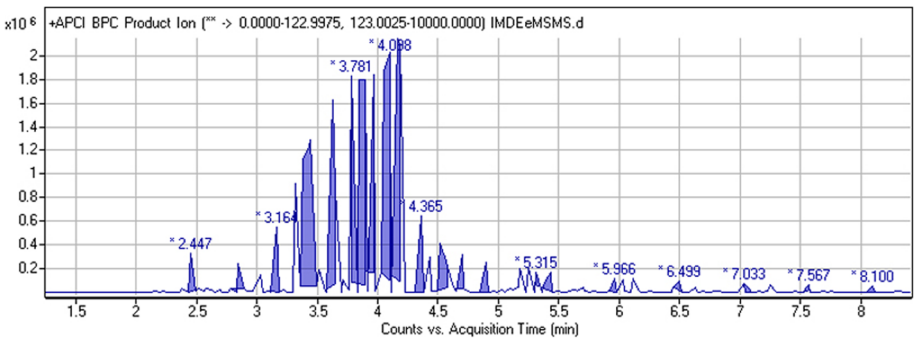

Fig. 3 LC-APCI-MS base peak chromatogram of fraction 2

Table 1 APCI-LC-MS/MS analysis of coumarin fraction of IM

Sl No	*m/z* [M + H]	MS/MS	Molecular formula	Tentative identification	Present in
1	193.0566	133.03	$C_{10}H_8O_4$	7-hydroxy-6-methoxy coumarin	Fr-1 and Fr-2
2	163.0441	107.05	$C_9H_6O_3$	7-hydroxycoumarin	Fr-2
3	217.0593	202.02	$C_{12}H_8O_4$	5-methoxy-6,7-furanocoumarin	Fr-2
4	207.18	151.2	$C_{11}H_{10}O_4$	5,7-dimethoxycoumarin	Fr-2
5	269.1580	152.21	$C_{16}H_{17}O_8$	6-hydroxy-7-methoxy-4-phenylcoumarin	Fr-2

category 5 (LD 50 > 2000 mg/kg) as per OECD Guideline No. 423, December 2001.

3.4 Evaluation of anti-amnesic activity

3.4.1 Effect of test item on body weight

The effect of IM on body weight of the experimental animals is presented in Table 3. There was no significant difference in the body weights were observed with the treated groups when compared to control group ($p > 0.05$).

3.4.2 Effect of test items on step through latency

The effect of IM extracts on scopolamine induced amnesia in Wistar rats is presented in Table 4. The results showed that scopolamine administration (3 mg/kg., i.p.) has significantly decreased the step through latency in passive avoidance test in group 2, control animals when compared with group 1, normal animals ($p < 0.05$), indicating amnesic effect of scopolamine. Groups 4 and 5 animals treated with IM extract at a dose of 100 and 200 mg/kg, p.o., respectively, showed a significant dose dependent

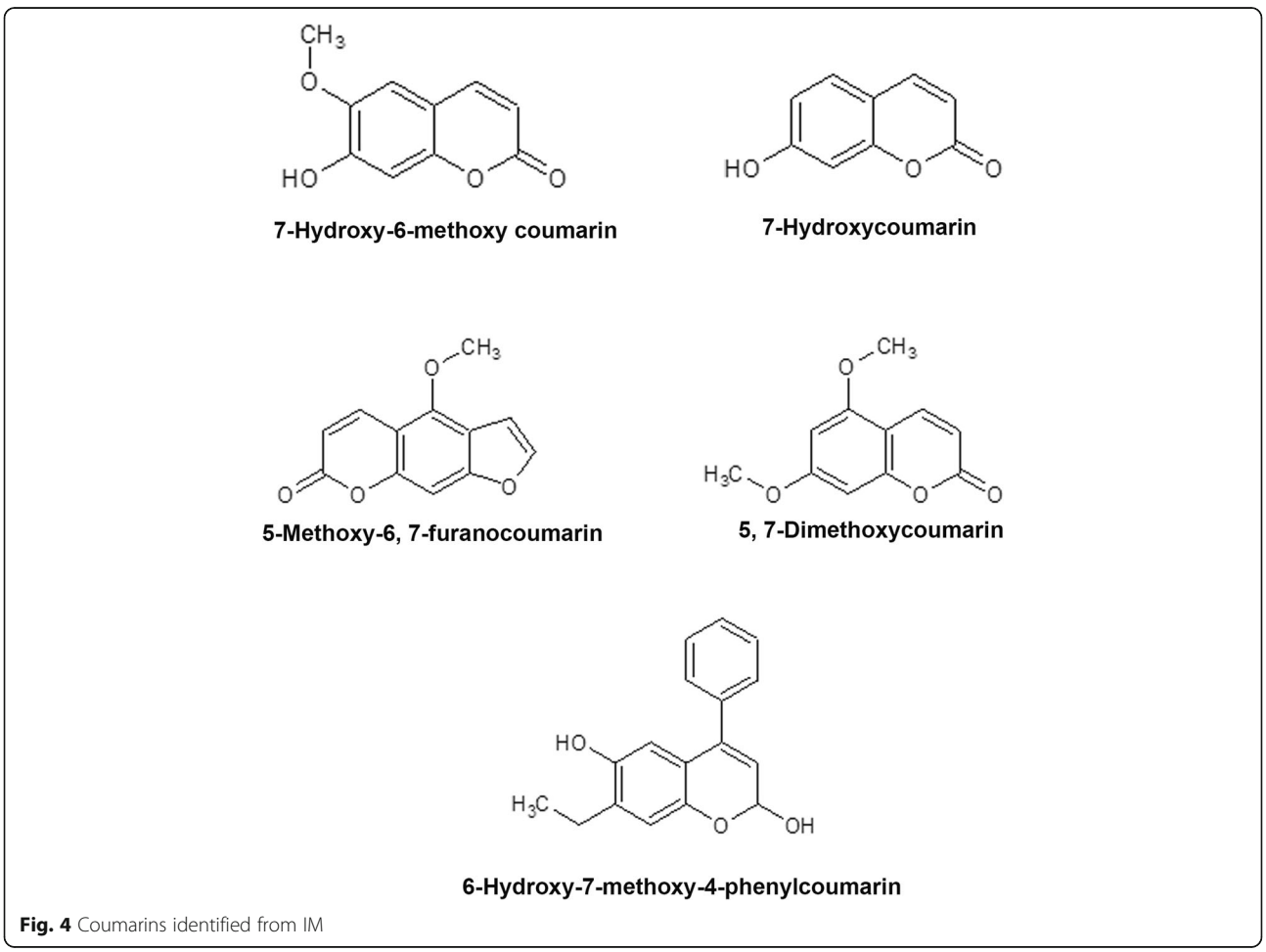

Fig. 4 Coumarins identified from IM

Table 2 Body weight, body weight changes, and pre-terminal deaths

Dose (mg/kg b.wt.)	Rat No.	Sex	Body weight (g)					No. dead /no. tested
			Initial	Day 8	Weight change (day 8–initial)	Day 15	Weight change (day 15–Initial)	
2000	R-013	Female	151	144	− 7	155	4	0/6
	R-014	Female	120	124	4	129	9	
	R-015	Female	170	182	12	203	33	
	R-016	Female	149	152	3	157	8	
	R-017	Female	145	153	8	166	21	
	R-018	Female	141	151	10	162	21	

protection against scopolamine induced memory loss ($p < 0.05$).

3.4.3 Effect of test items on step down latency

The results of the study showed that scopolamine administration (3 mg/kg., i.p.) has significantly decreased the step down latency in passive avoidance test in group 2, control animals when compared with group 1, normal animals ($p < 0.05$), indicating amnesic effect of scopolamine. Groups 4 and 5 animals treated with IM extract at a dose of 100 and 200 mg/kg, p.o., respectively, show no significant protection against scopolamine induced memory loss ($p > 0.05$).

3.4.4 Effect of test item on reference and working memory errors in radial arm maze test

The results of the study showed that scopolamine administration (3 mg/kg., i.p.) has significantly increased reference and working memory errors in radial arm maze test in group 2, control animals when compared with group 1, normal animals ($p < 0.05$), indicating amnesic effect of scopolamine. Group 4 and 5 animals treated with IM extract at a dose of 100 and 200 mg/kg, p.o., respectively, show a significant protection against scopolamine induced working memory errors ($p < 0.05$) and a non-significant protection against scopolamine induced reference memory errors ($p > 0.05$).

4 Discussion

In Ayurveda, Medhya Rasayana is termed for rejuvenating drugs with numerous benefits specifically to improve memory and intellect. *I. mauritiana* is the one of the rejuvenating drugs mentioned in the Ayurvedic texts. The preliminary chemical screening of I. mauritiana was done HPTLC analysis. Crude alcoholic extract showed presence of coumarins, and the same was confirmed by derivetizing the chromatographic plate with alcoholic potassium hydroxide, a common TLC reagent for the easy identification of coumarins [15]. Coumarins are large group of natural compounds isolated from many plants and are still being reported each year. Many coumarins with remarkable biological activities such as anticoagulant, antiproliferative, anti-microbial, etc., have been reported from many medicinal plants [16–19].

Method has been optimized for the separation of coumarins from the crude extract. Two fractions were separated, and the same was subjected to tandem mass spectroscopic analysis. Mass fragmentation based on collision induced dissociation led to the identification of five coumarins from the two fraction of *I. mauritiana*. Learning and memory enhancement activity of coumarin like scopoletin has been reported earlier [20, 21].

The effect of aqueous extract of *I. mauritiana* was evaluated on scopolamine induced amnesia in Wistar rats. It has been established that the increased activity of acetylcholinesterase enzyme in brain decreases the acetylcholine levels leading to memory loss [22, 23]. Scopolamine, a muscarinic antagonist, has shown to improve the acetylcholinesterase activity, and it has also been reported that decreased muscarinic activity causes formation of β-amyloid plaques in brain leading to memory impairment in Alzheimer's disease (AD) [24].

Table 3 Effect of IM extract on weekly body weight of Wistar rats

Group	Treatment	Week 0	Week 1	Week 2
1	Normal (vehicle 10 ml/kg, p.o.)	214.7 ± 30.1	220.3 ± 31.0	226.0 ± 29.1
2	Control (vehicle 10 ml/kg, p.o.)	207.5 ± 29.3	215.0 ± 28.5	222.5 ± 27.8
3	Rivastigmine (1.5 mg/kg., p.o.)	225.7 ± 30.7	231.5 ± 31.4	237.3 ± 32.2
4	IM (100 mg/kg., p.o.)	222.1 ± 36	228.3 ± 36.9	234.5 ± 37.8
5	IM (200 mg/kg., p.o.)	206 ± 33.9	212.8 ± 34.8	219.6 ± 35.9

Values are Mean ± SD, $n = 6$, $^{\#}p < 0.05$when compared with G1, normal, $^{*}p < 0.05$ when compared with G2, control

Table 4 Effect of IM extract on scopolamine-induced amnesia in Wistar rats

Group	Step through latency (sec)	Step down latency (sec)	Reference memory error	Working memory error
Normal (vehicle 10 ml/kg, p.o.)	101.22 ± 12.07	9.05 ± 1.16	4.16 ± 0.75	6.33 ± 1.36
Control (vehicle 10 ml/kg, p.o.)	44.05 ± 8.53$^{\#}$	3.44 ± 1.25$^{\#}$	8.38 ± 1.04$^{\#}$	9.22 ± 2.93$^{\#}$
Rivastigmine (1.5 mg/kg., p.o.)	88.05 ± 17.78*	7.89 ± 2.20*	5.94 ± 1.06*	6.16 ± 1.37*
IM (100 mg/kg., p.o.)	77.33 ± 7.06*	3.72 ± 0.60	7.11 ± 1.43	6.05 ± 0.92*
IM (200 mg/kg., p.o.)	76.61 ± 12.47*	5.44 ± 1.58	7.66 ± 1.72	6.66 ± 1.41*

Values are Mean ± SD, $n = 6$, $^{\#}p < 0.05$when compared with G1, normal, $^{*}p < 0.05$ when compared with G2, control

Moreover, increased acetylcholinesterase activity has also been reported to enhance the aggregation of β-amyloid proteins into more toxic amyloid plaques, worsening the condition especially in AD [25]. IM has shown to reverse scopolamine-induced amnesia imparted significant protection against Scopolamine induced memory loss

5 Conclusion

The present study was conducted to identify active principles of *I. mauritiana* and to evaluate its memory enhancing activity by phytochemical and pharmacological analyses. Preliminary chromatographic analysis of *I. mauritiana* extract showed presence of coumarins, and subsequent tandem mass spectroscopic characterization of the same led to the identification of five coumarins in which four are new report for this species. Anti-amnesic activity was evaluated against scopolamine-induced amnesia in Wistar rats. The result showed significant protection against scopolamine-induced working memory errors and a non-significant protection against scopolamine-induced reference memory errors. The outcome of the present study is a scientific validation of traditional knowledge.

Abbreviations

IM: *Ipomoea mauritiana*; APCI-LC-MS/MS: Atmospheric pressure chemical ionization liquid chromatography mass spectrometer; HPTLC: High performance thin layer chromatography

Authors' contributions

SCT Designed the study, carried out LC/MS and pharmacological evaluation and drafted the manuscript. DM Carried out the HPTLC analysis and coumarin separation. SAR Carried out the extraction. LKR Participated in the extraction of coumarins. IB Participated in designing the study and edited the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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References

1. Sivarajan VV, Balachandran I (1994) Ayurvedic drugs and their plant sources. Oxford and IBH Publishing, New Delhi, India, pp 510–512
2. Karthik S, Chandrakala C, Venkatasubramanian P (2009) Phytochemical and microscopic analysis of tubers of *Ipomoea mauritiana* Jacq. (Convolvulaceae). Pharmacog Mag 5(19):272–278
3. Udayan PS, Balachandran I (2009) Medicinal plants of Arya Vaidya Sala herb garden. AVS, Kottakkal, p 207
4. Sulaiman C, Sivadasan PG, Balachandran I (2014) Identification of phenolic antioxidants in Ipomoea *mauritiana jacq*. using spectrophotometric and mass spectroscopic studies. Avicenna J Phytomed 4(2):89–96
5. Sajjad KM, Nema N, Kharya MD, Salma K (2009) Chromatographic estimation of maturity based phytochemical profiling of Ipomoea mauritiana. Int J Phytomed 1(1):22
6. Parle M, Bansal N (2011) Antiamnesic activity of an Ayurvedic formulation Chyawanprash in mice. Evid Based Complement Alternat Med 898593:1–9
7. Alikatte KL, Akondi BR, Yerragunta VG, Veerareddy PR, Palle S (2012) Antiamnesic activity of Syzygium cumini against scopolamine induced spatial memory impairments in rats. Brain & Develop 34(10):844–851
8. Rahnama S, Rabiei Z, Alibabaei Z, Mokhtari S, Rafieian-Kopaei M, Deris F (2015) Anti-amnesic activity of Citrus aurantium flowers extract against scopolamine-induced memory impairments in rats. Neurol Sci 36(4):553–560
9. Yu D, Suzuki M, Xie L (2003) Recent progress in the development of coumarin derivatives as potent anti-HIV agents. Med Res Rev 23(3):322–345
10. Smyth T, Ramaehandran VN, Smyth WF (2009) A study of the antimicrobial activity of selected naturally occurring and synthetic coumarins. Int J Antimicrob Agents 33(5):421–426
11. Karatas MO, Uslu H, Alici B, Gokce B, Gencer N, Arslan O (2016) Some coumarins and benzoxazinones as potent paraoxonase 1 inhibitors. J Enzyme Inhib Med Chem 31(6):1386–1391
12. Singla S, Piplani P (2016) Coumarin derivatives as potential inhibitors of acetylcholinesterase: synthesis, molecular docking and biological studies. Bioorg Med Chem 24(19):4587–4599
13. Tine Y, Renucci F, Costa J, Wélé A, Paolini J (2017) A Method for LC-MS/MS Profiling of coumarins in Zanthoxylum zanthoxyloides (Lam.) B. Zepernich and Timler Extracts and Essential Oils. Molecules 22(1):174
14. Yang W, Ye M, Liu M, Kong D, Shi R, Shi X, Zhang K, Wang Q (2010) A practical strategy for the characterization of coumarins in Radix Glehniae by liquid chromatography coupled with triple quadrupole-linear ion trap mass spectrometry. J Chromatogr A 1217(27):4587–4600
15. Koshevoi ON, Komissarenko AN, KovalevaYa M, D'yakonova V (2009) Coumarins from Eucalyptus viminalis leaves. Chem Nat Compd 45:532
16. Alente EJ, Porter WR, Trager WF (1978) Conformations of selected 3-substituted 4-hydroxycoumarins in solution by nuclear magnetic resonance. Warfarin and phenprocoumon. J Med Chem 21:231–234
17. Morii K, Murai O, Hashimoto S, Nakamura Y (1996) Highly regio- and stereoselective photocycloaddition between coumarin and thymine by molecular recognition. Tetrahedron Lett 37:8523–8526
18. Kimura Y, Okuda H, Arichi S, Baba K, Kozawa M (1985) Inhibition of the formation of 5-hydroxy-6,8,l 1,14-eicosateraenoic acid from arachidonic acid in polymorphonuclear leukocytes by various coumarins. Biochim Biophys Acta 834:224–229
19. Matern U (1999) Medicinal potential and biosynthesis of plant coumarins. In: Romeo JT (ed) Phytochemicals in Human Health Protection, Nutrition, and Plant Defense, Recent Advances in Phytochemistry (Proceedings of the Phytochemical Society of North America), vol 33. Springer, Boston, MA, pp 161–183
20. Hornick A, Lieb A, Vo NP, Rollinger J, Stuppner H, Prast H (2008) Effects of the coumarin scopoletin on learning and memory, on release of acetylcholine from brain synaptosomes and on long-term potentiation in hippocampus. BMC Pharmacol 8(1):A36
21. Hornicka A, Lieba A, Vo NP, Rollinger JM, Stuppner H, Prasta H (2011) The coumarin scopoletin potentiates acetylcholine release from synaptosomes,

amplifies hippocampal long-term potentiation and ameliorates anticholinergic- and age-impaired memory. Neuroscience 197(1):280–292
22. Ibach B, Haen E (2004) Acetylcholinesterase inhibition in Alzheimer's disease. Curr Pharm Des 10:231–251
23. Malika J, Karana M, Vasisht K (2015) Attenuating effect of bioactive coumarins from Convolvulus pluricaulis on scopolamine-induced amnesia in mice, Natural Product Research. doi: https://doi.org/10.1080/14786419.2015.1025398.
24. Liskowsky W, Schliebs R (2006) Muscarinic acetylcholine receptor inhibition in transgenic Alzheimer-like Tg2576 mice by scopolamine favours the amyloidogenic route of processing of amyloid precursor protein. Int J Dev Neurosci 24:149–156
25. Campos EO, Alvarez A, Inestrosa NC (1998) Brain acetylcholinesterase promotes amyloid beta peptide aggregation but does not hydrolyze amyloid precursor protein peptides. Neurochem Res 23:135–140

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