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Original Article

Phytochemical and Network Pharmacology Based Evaluation of Antiepileptic Potential of Identified Metabolites in *Argimone mexicana*

Vinod Gahlot[1], Dinesh Kumar Yadav[2,*]

ABSTRACT

Background: Quality-based assessment of herbal drugs or products is being more concerning for their quality, safety and regulatory purpose. *A. mexicana* is a traditional herbal medicine that has a long history in the treatment of arthritis, anti-fungal anti-cancer and brain disorders. **Aim:** Due to lack of scientific evidence based on phytopharmacology, the study is aimed for phytochemical and antiepileptic evaluation of identified metabolites in *A. mexicana*. **Materials and Methods:** Phytochemical identification was done using MS, FTIR and ^{1}H-NMR and quantitated using HPTLC densitometric analysis. Further, ADME and network pharmacology studies were performed to evaluate biological response of identified metabolites. **Results:** The resulted outcomes of the spectral analysis suggest isolated compounds as ferulic acid, caffeic acid, berberine and angoline. In HPTLC quantitative analysis, the content of ferulic acid, caffeic acid, berberine and angoline was found as 3.475 ± 0.028, 1.036 ± 0.013, 0.714 ± 0.014 and 0.738 ± 0.081 µg/mg of A. mexicana extract. In ADME analysis, berberine and angoline showed good bioavailable response while in network pharmacology analysis, except angoline, all the metabolites significantly interacted with several genes (SOD1, NOS, MAPK3, UGT1As, G6PD, ACOT2, BAAT etc) associated with brain ischemia, oxidative and inflammatory stress or the genes response for elimination of toxins from the body. **Conclusion:** Hence, the study enlightens that *A. mexicana* possess several major and minor metabolites and which can be the key parameter for further quality and regulatory based assessment of *A. Mexicana* and biological role against oxidative and inflammatory stress induced epileptic seizure.

Key words: *Argemone mexicana* L., Phytoconstituents, HPTLC, ADME analysis, Network pharmacology.

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INTRODUCTION

The application for authentication and reliable quality studies are the principal requirement for herbal raw materials or medicinal plants used under the traditional system of medicine including the Indian system of medicine, the Chinese system of medicine etc.[1,2] Historically, medicinal plants have been used in the treatment of various disorders from a long time while many of the traditional scripture classify them as per their applicability, accessibility and admissibility to cure, treat and nullify the disease or any deleterious effects arise in the body's system.[3] Due to the complex matrix of phytoconstituents in medicinal plants works with multidimensional therapeutic approaches. Quality assessment of the medicinal plants becomes the major challenge for the current official quality control mode.[4] A few bioactive markers were chosen only for qualitative and quantitative assay which characterize the authenticity of the targeted or selected medicinal plant of interest. Referring to many minor and major bioactive constituents present in herbal preparations, it is impossible to pinpoint every single bioactive constituent in the herbal drug, qualitatively and quantitatively.[5] However, the analytical techniques such as high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LCMS), gas chromatography-mass spectrometry (GCMS), ion-exchange chromatography and gel-permeation (molecular sieve) chromatography, affinity chromatography and paper chromatography are associated with qualitative and quantitative evaluation of medicinal plants and provides the standard and authentic scientific with aspects to their safety and regulatory purpose.[6,7] Moreover, chromatographic techniques such as column chromatography and HPTLC are one of the widely used and cost-effective methods for qualitative and quantitative evaluation of medicinal plants or even

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provide the best methods over the quality control techniques. Major advantages of HPTLC are its reliability in the quantification of analytes at micro and even in nanogram levels and even useful to detect the huge number of compounds with high resolution and detection approaches. Automation of sample application, detection (spraying or dipping), closed developing chambers providing standardized conditions (relative humidity, saturation, drying), and digital documentation systems for capturing chromatograms are the essential tools in HPTLC.[8,9] Because of easy handling and great progress in instrumentation leading to the reproducibility of data, HPTLC will remain the most important technique for qualitative analysis of plants. With its extreme flexibility concerning choices of mobile phases and detection methods, the technique can cope with the diversity of substances from primary and secondary metabolism.[10]

A. mexicana commonly known as Ghamoya or Satyanashi (family: Papaveraceae) is found in almost every part of the world and distribution in many tropical and sub-tropical countries including Asia and Africa. In India, It is commonly found on roadsides and fields as well and it is considered one of the important medicinal plants in India.[11] The yellow juicy part was exuded when the plant is injured, has long been used in India as traditional medicine for dropsy, scabies, ophthalmic, jaundice, and cutaneous affections. In the modern system of medicine, it is widely used for brain disorders, anti-fungal and anti-cancer etc.[12,13] Not only juice but different parts of this plant are used in chronic disorders. The seeds and their oil are frequently employed as a remedy for asthma, ulcers, dysentery, and other intestinal affections.[11] The leaves and seeds have been used in snakebites or anti-venom properties.[14] Flowers are found to be expectorant and have been used in the treatment of coughs.[15] Besides, its infusion finds application against hypertension and inflammatory.[13] However, quality control evaluation of *A. mexicana* still evaded its authenticity. Quality control analyses based on qualitative and quantitative evaluation of phytoconstituents make us far formalized from its safety, efficacy and regulatory purpose and generate such scientific evidences which help us to protect the drug origin from spurious or adulterants belongs to the same species even pharmaceuticals also.

Taking all these into consideration, the present study is aimed for isolation and characterization of phytoconstituents using modern chromatographical and spectroscopical tool and development of simple, accurate and rapid HPTLC method for quantification of phytoconstituents in the hydroalcoholic extract of *A. Mexicana* at a single platform. The method has been validated as per the ICH guidelines. Further, *in-silico* analysis was conducted to explore the biological relevance of identified metabolites. Hence, the present prospective of the study is to generate scientific evidences which do not only oblige the quality standardization of plant/its plant-based herbal formulations but also biological mechanistic relevance in terms to treat or cure epileptic seizure induced by oxidative and inflammatory stress.

MATERIALS AND METHODS

TLC Silica gel 60 F_{254} aluminum sheet, double distilled water, HPLC water were purchased from sigma whereas 60-120# size silica gel and 230- 400# size silica gel purchased from Sisco Research Laboratories Pvt. Ltd, New Delhi, India. The organic or inorganic solvents were used of analytical grade.

Collection and authentication of plant

A total of 1 kg fresh plant material was collected from the local region of Delhi NCR and authenticated by CSIR- National Institute of Science Communication and Information Resources. The plant specimen was submitted to the Raw Material Herbarium and Museum with the authentication number NISCAIR/RHMD/Consult/2020/3660-61. The collected plant material has been displayed in Figure 1.

Figure 1: *A. mexicana* plant.

Preparation of extract

The plant material was shade dried for one week and the whole plant was coarsely powdered. The powdered material (500 g) was processed for the extraction by the reflux method for 8 hrs at 60°C temperature using 2.8 L of extracting solvent. The hydroalcoholic solvent system was prepared using methanol and distilled water in 7: 3 v/v ratio. After the period of extraction, the content was filtered using a muslin cloth and Whatman's filter paper and the filtrate was dried on a water bath at 60°C temperature till complete evaporation of the solvent. After all, the solid residue was obtained and stored in an airtight container for further analysis. During the same period, the extractive value of the sample was calculated for record purposes.[16,17] The plant material and extracted yield have been displayed in Figure 1.

Column chromatography

Column chromatography (CC) was performed for the separation of phytoconstituents from the complex matrix of *A. mexicana* hydroalcoholic extract and their further separation for pure constituent using different gradients of the solvent system. In brief, 5 g of extract was loaded or adsorbed on 15 g of silica gel (60-120 mesh size) while the size silica gel (230- 400 mesh size) used for column packing, the sample loaded on silica gel was subjected to the column (column length × diameter (40×3 cm)) for separation of phytoconstituents in a gradient manner using chloroform and methanol as the mobile phase. Each collected fraction was of 10 ml

volume capacity. The flow rate of eluted solvent during CC was set at 3ml/min. During CC process, each collected fraction was estimated for the separation of a single constituent by thin-layer chromatography (TLC) using toluene, ethyl acetate and formic acid as mobile phase in different necessary compositions.

Spectral analysis of isolated compounds

Mass spectrometric (MS) analysis of isolated compounds

The isolated compounds from hydroalcoholic extract of *A. mexicana* were analyzed through MS analysis. The compound was identified based on their molecular mass obtained from the spectral data. In brief, The MS analysis was performed on Water's ACQUITY UPLC(TM) system (Waters Corp., MA, USA) adjoined with an auto-sampler, binary solvent delivery system, column manager and a tunable MS detector. Four samples of 1 mg/ml concentration were prepared in LCMS grade solvent, acetonitrile (A: 85%) and water (B: 15%) were used as chromatographic solvent run throughout a monolithic capillary silica-based C_{18} column (ACQUITY UPLC(R) BEH C_{18} 1.7 µm, 2.1 x 100 mm) the injection volume for sample was set at 2µl. The flow rate of the nebulizer gas and cone gas was set at 500 L/h and 50 L/h, respectively. In mass analysis electrospray ionisation (ESI) was used as the ion source and the temperature for mass source was fixed at 120°C while capillary and cone voltage was set at 3.0 and 40 KV, respectively. For collision, argon was active at a pressure of 5.5 x 10^{-5} torr. The obtained spectral data were interpreted and tentatively identified phytoconstituents based on their m/z value from mass data sources such as Mass Bank, ChemSpider, etc as well as literature.[3]

^{1}H-NMR spectroscopic analysis of isolated compounds

NMR spectroscopical analysis of isolated compounds was performed as per the described method using Bruker Avance 400 MHz and 100 MHz NMR spectrometer. Each sample was dissolved in deuterated methanol-d4 (CD3OD) as NMR solvent while tetramethylsilane was used as an internal standard. In brief, the above-isolated sample was dried under high vacuum pressure to remove traces of solvent. The resulting residue was dissolved in dimethyl sulfoxide (DMSO) as a standard solvent then the sample proceeded for spectroscopic analysis by NMR technique.[18,19]

FT-IR spectroscopy analysis of isolated compounds

The spectral analyses of isolated compounds were performed by using Win-IR, Bio-Rad FTS spectrophotometer. In brief, 1 mg of isolated sample was mixed with potassium bromide to form a solid pallet and well ahead proceed for spectroscopical analysis under the range of 4000 to 400 cm^{-1}.[20]

HPTLC analysis of *A. mexicana* hydroalcoholic extract and quantitative analysis for isolated compounds

30 mg of *A. mexicana* hydroalcoholic extract was dissolved in methanol using vortex followed by filtration using a PTFE membrane filter of 0.2 µM. The stock solution (1mg/ml) of ferulic acid, caffeic acid, berberine and angoline were prepared. Thereafter, 6 µL of the sample and different concentration (0.2- 2.0 µL) of each standard was applied with the help of Camag Linomat-V with a 6 mm wide band length to different pre-washed and activated Silica gel 60 F_{254} pre-coated HPTLC plates (10x10 cm). The nitrogen flow delivery speed was set up to 130 nL/s. Toluene, ethyl acetate, glacial acetic acid, methanol (6:3:1:1, v/v/v) were used as a solvent system to develop plate 1 containing 3 markers (ferulic acid, caffeic acid and berberine) while toluene, ethyl acetate, glacial acetic acid, methanol (5:4:1:1, v/v/v) were used as solvent system to develop the TLC plates containing sample and angoline as marker in a pre-saturated TLC development chamber. Each plate was developed to a distance of 7.5 cm at room temperature (25°C). After drying, the spots on the developed plate were visualized under visible (white), short UV (254 nm), and long UV (366 nm) light. The quantification studies of ferulic acid, caffeic acid, berberine and angoline were carried out at 254 nm as per ICH guide line and the content of each marker in the sample was expressed in µg/mg (w/w).[21]

ADME analysis

ADME analysis for ferulic acid, caffeic acid, berberine and angoline was established through the computational tool "SwissADME (http://www.swissadme.ch/index.php)". TPSA (Topological Polar Surface Area (TPSA) for drug integrity, Consensus Log Po/w for drug lipophilicity, Log Kp (skin permeation) and drug-likeness were predicted as the standard parameters for consideration of their bioavailable or ADME response. Further, the comparative analysis of each corresponding parameter was done to determine the relationship between molecular integrity and their bioavailable response.

Network construction of active components common target

In the network construction analysis, the ligation efficacy of each selected gene was predicted. Gene Ontology (GO) analysis through Metascape Gene Analysis (metascape.org) tool was performed to evaluate multiple physiological roles of each gene in the regulation of kidney and associated disorders after the analysis of compound-disease common target. A protein-protein interaction (PPI) network and compound-proteins interaction were obtained based on the STRING platform (https://string-db.org/) and Cytoscape (version 3.8.2) software. The target PPI was constructed and imported to Cytoscape software for interaction information, integration and further protein ligated construction analysis. The analysis covered all the nearly functional interactions among the expressed proteins-proteins and compound-proteins.

Statistical representation

The data were expressed in Mean ± SD for significant evaluation of experimental outcomes. The measurements were taken in triplicate for better consideration of chromatographical outcomes in HPTLC analysis.

RESULTS

The extraction process for *A. mexicana* hydroalcoholic extract was done successfully. The obtained yield was found as 13.27% (w/w) which was used for further studies.

Column chromatography

The column chromatography method was done successively using chloroform (A) and methanol (B) as mobile phases for the separation of phytoconstituents from the complex matrix of *A. mexicana* hydroalcoholic extract. During chromatographic separation, gradient mode of the mobile phase was applied in different ratio as 100% A and 0% B for collection of 20 fractions, 95% A and 5% B for collection of 30 fractions, 90% A and 10% B for collection of 18 fractions, 85% A and 15% B for collection of 33 fractions, 80% A and 20% B for collection of 37 fractions and 75% A and 25% B used or collection of 38 fractions. Each fraction was collected of 10ml volume capacity. During CC process, the solvent polarity was only increased when no separation was occurred which was confirmed by TLC while the individual fraction pointed with single spot of separated compound was collected and concentrated for further analysis. The chromatographic results reveal that the fraction numbers 28 -31, 55-57, 83-87 and 104-109 were confirmed as single compound fractions and the remaining was obtained as a mixture of compounds. In a simple and more understanding schematic representation, the

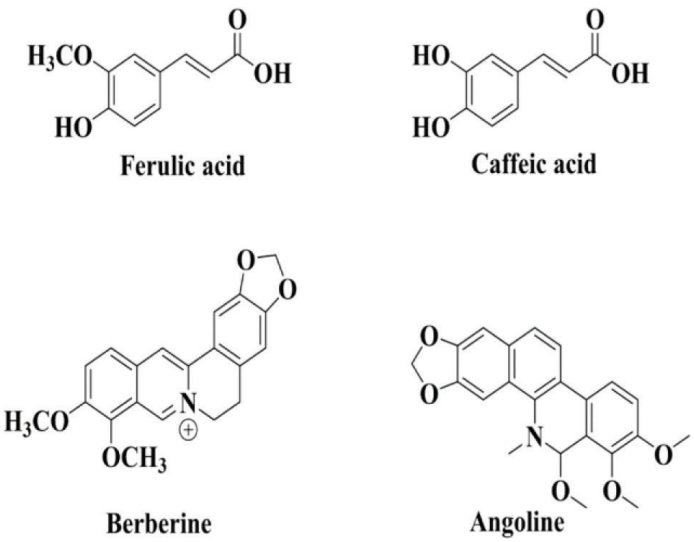

Figure 2: Chemical structure of identified metabolites in *A. mexicana* extract.

outcomes of chromatographic separation have been summarized in Figure 2.

Spectroscopic analysis

The MS, FTIR and ^{1}HNMR spectral analyses of isolated compounds from *A. mexicana* hydroalcoholic extract were performed to identify the compounds. NMR spectroscopy of the isolated compounds was performed using TMS as an internal standard. In spectral analysis, the MS spectral data of compound 1 showed an intense signal of 194.71m/z. In FTIR analysis, the principal absorption peaks were appeared at 3378.13 cm^{-1} due to the presence of-COOH and -OH groups. The identified peaks at the frequency range 2985.20 and 2912.28 cm^{-1} due to the presence of =CH_2, and -CH_3 stretching. The most significant part of the spectrum was bands that appeared at 1659.22 and 1488.97cm^{-1} due to the presence of ketone (-C=O) and C-O-C stretching vibration. The resulted data suggests being the presence of the phenolic compound. The ^{1}HNMR spectral data of compound 1 showed three aromatic protons at δ 7.12, 6.98, 6.38 (3H d, d, m) respectively. The signals showed at δ 11.18 and 9.78 (2H, 2s) represents for carboxyl and phenol group. Two signals for the ethylene group appeared in the aliphatic region at δ 7.47 and 6.28 (2H, 2d) while an intense peak appeared at δ 3.88 (3H, s) for protons of methoxy group which reveals the presence of ferulic acid as reported in the previous findings also.[22]

The MS spectral data of compound 2 showed an intense signal of 180.36 m/z. In FTIR analysis, the main absorption peaks appeared at 3487.29, 3012.28, 1663.84 and 1512.26 cm^{-1} were appeared due to stretching of –COOH, -OH, -CH_2, -C=O and –C-O-C stretching vibration. The resulted data suggests being the presence of the phenolic compounds. The ^{1}HNMR spectral data of compound 2 showed three aromatic protons at δ 7.14, 6.98, 6.35 (3H d, d, m) respectively. The signals showed at δ 11.15 and 9.79 (2H, 2s) represents for carboxyl and phenol group. Two signals for the ethylene group appeared in the aliphatic region at δ 7.47 and 6.28 (2H, 2d). The spectral results and previous findings strongly emphasize to the caffeic acid as compound 2.[23]

The MS spectral data of compound 3 showed an intense signal of 366.52 m/z. In FTIR analysis, the main absorption peaks appeared at 3532.14, 3381.75, 3045.22 and 2934.55 cm-1 due to stretching vibration of –N/N$^+$, -CH_2, and -CH_3 group. Further, the absorption peaks at 1607.27, 1469.39, 1351.98, 600.28 cm^{-1} are due to stretching vibration of –C=C, -C-O-C and asymmetric carbons. The resulted data suggests being the presence of an alkaloid compound. In the ^{1}HNMR spectral data of compound 3, the six protons of the aromatic methoxy group appeared at δ 3.81 (6H, s). The four protons of the isoquinoline ring appeared at δ 6.75 (H, d), 7.04 (H, m), 8.53 (H, d) and 10.19 (H, s), respectively. The two protons of the dioxolane ring appeared at δ 5.92 (2H, s) position. The obtained data were matched with the reported data which strongly supports compound 3 as berberine.[24]

The MS spectral data of compound 4 showed an intense signal of 379.39 m/z. In FTIR analysis, the main absorption peaks appeared at 3522.03, 3107.49, 3002.57 cm^{-1} due to the stretching vibration of tertiary amine (-N), -CH_2, -CH_3 group. the absorption peaks at 1487.51, 1348.67 and 1033.27 cm^{-1} due to stretching vibration of –C-O-C, -C-H or asymmetric carbons. The spectral data of compound 4 reveals the presence of alkaloid compound having ether group skeletal. The ^{1}HNMR spectral data of compound 4 reveals twelve aromatic methoxy and tertiary amine proton at δ 2.98, 3.31, 3.79, 3.89 (12H, 4s), respectively. The two protons of the dioxolane ring appeared at δ 6.15 (2H, s) position. Four –CH proton of appeared in the aromatic region at δ 6.75, 7.12, 7.37 and 7.49 positions. The data was matched with the reported values which reveal the presence of angoline as compound 4.[25,26]

The spectra's of MS, FT-IR, ^{1}HNMR and the predicted structures of isolated compounds have been displayed in Figure 3-6 (supplementary files).

HPTLC profiling and quantitative estimation of ferulic acid, caffeic acid, angoline and berberine

HPTLC profiling and quantitative estimation of ferulic acid, caffeic acid, angoline and berberine in the hydroalcoholic extract of *A. mexicana* were performed successively. The resulted data reveals several numbers of minor and major metabolites at a different wavelength (254 nm and 366 nm) in the complex matrix of hydroalcoholic extract of *A. mexicana* while validation parameters for quantification of ferulic acid, caffeic acid, berberine and angoline was found linear, accurate, and robust in the wide range of 200-2000 ng/spot. The limit of detection (LOD) and the limit of quantitation (LOQ) for ferulic acid, caffeic acid, berberine and angoline were found as 13.501, 17.096, 10.304, 20.119 ng/spot and 40.913, 51.807, 31.226, 60.967 ng/spot, respectively. The inter-day and intra-day precision were determined as percentage relative standard deviation or the coefficient of variation and the results were expressed in the range found as 0.325-2.107, 0.198-2.652, 0.210-1.578, 0.374-2.154% and 0.384-1.925, 0.196-2.538, 0.347-1.682, 0.284-1.219%, respectively. The accuracy of the developed method was determined as the percentage drug recovered by percentage spiking 0, 50, 100, and 150% of the standard to the sample in pre-analyzed samples at 2000 ng/spot which exhibited recovery in the range of 99.186-99.926, 99.290-99.781, 99.815-100.077 and 99.106-99.385 % for ferulic acid, caffeic acid, berberine and angoline. Thereafter, the content of each marker was calculated in the hydroalcoholic extract of *A. mexicana* which was found as 3.475 ± 0.028, 1.036 ± 0.013, 0.714 ± 0.014 and 0.738 ± 0.081 µg/mg of the extract at 0.68, 0.56, 0.16 (plate A'), and 0.27 R*f* (plate B'), respectively. HPTLC plate view at 254 nm and chromatograms for hydroalcoholic extract of *A. mexicana*, marker constituents and their respective chromatograms have been displayed in Figure 7.

ADME analysis

SwissADME is one of the proficient and admirable *in-silico* online tools for computational analysis of drugs (synthetic or natural compounds) to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness.[27] It gives robust data to predict the bio-response of the targeted molecules. ADME analysis of isolated compounds was performed based on SwsisADME online computational tool by using the unique

molecular code of the compound as Canonical smiles. The parameters such as TPSA, iLOGP, XLOGP3, WLOGP, MLOGP, Silicos-IT Log P etc were estimated. The predicted observation of each metabolite has been summarized in Table 1. Consensus Log is the average value of all the lipophilic parameters revealed high gastrointestinal (GI) absorption and lipophilicity while blood-brain barrier (BBB) permeability showed also high. The permeability strength was expressed as log Kp (cm/s) and the bioavailability Score of each targeted compounds was found as 0.85 and 0.55 which represents its availability to the therapeutic site. ADME analysis of each metabolite with their solubility radar plots is summarized in Figure 4.

Network Construction of Compound-Disease Common Targets

Common target network (protein-protein interaction)

Fifty seven putative target genes were used in the process of network construction while 52 genes were interconnected each other and potentially associated in pathophysiology for oxidative and inflammatory stress induced epileptic seizures. A protein-protein interaction network was imported from the STRING database for analysis and network establishment. The selected target interaction network was established with a medium confidence score of 0.400. The established network embodied 52 numbers of nodes, 188 numbers of edges, 7.23 average node degree and 0.631 as average local clustering coefficient. In the constructed network the expected number of edges was found 43 while PPI enrichment *p*-value < 1.0e-16. Besides, the established protein-protein network has significantly more interactions than expected. The significant interaction of each protein/target gene is based on proteins of similar size selected from the genome database and characterized that the proteins are at least partially biologically connected in each other during oxidative and inflammatory stress induced epileptic seizures. In the Figure, the edges characterize the interaction between sets of potential targets, while the nodes characterize the targets. The intensity of target interaction is represented with the target degree value (Figure 5).

Further, the target genes were analyzed for Gene Ontology (GO) through Metascape Gene Analysis (metascape.org) to evaluate the multiple physiological roles of each gene in the regulation of oxidative and inflammatory stress induced epileptic seizures. Out of 94 analyzed genes, the top twenty results were selected which were directly interrelated with the pathophysiology and analyzed for further assessments. The observation of enriched terms across input gene revealed that many targeted genes play a vital role in response to nutrient level, brain ischemia, signaling by interleukins, regulation of lipoprotein particle level and inflammatory and oxidative response, positive regulation of cell death, dopamine metabolism, etc.[28] The bar graph of enriched terms across input gene lists, colored by p-values and the summary of enrichment analysis have been summarized in Figure 6.

DISCUSSION

Herbal medicines are the growing need of the human beings to face several ailments due to their easy assessability, affordability, and least side effects.[3] Besides, qualitative and quantitative evaluation is one of the critical needs for the assessment of herbal medicine or products based on their unique pattern of metabolites which helps to generates scientific evidence comprehended to concern their safety, efficacy and regulatory prospectus.[29] The present prospective of study provides a simple, authentic and robust scientific evidences for quality based assessment of *A. mexicana* using chromatographical and spectroscopical techniques at a single platform which play role in its evation from multiple misleading species as adulterant.[30]

Table 1: ADME analysis of identified molecules.

Molecule	Canonical SMILES	Formula	MW	TPSA	iLOGP	Consensus Log P	ESOL Solubility (mol/l)	Ali Log S	GI absorption	BBB permeant	Bioavailability Score
Ferulic acid	COc1cc(/C=C/C(=O)O)ccc1O	C10H10O4	194.18	66.76	1.62	1.36	7.68E-03	-2.52	High	Yes	0.85
Caffeic acid	OC(=O)/C=C/c1ccc(c(c1)O)O	C9H8O4	180.16	77.76	0.97	0.93	1.29E-02	-2.38	High	No	0.56
Berberin	COc1c(OC)ccc2c1c[n+]1CCc3c(c1c2)cc1c(c3)OCO1	C20H18NO4	336.36	40.8	0	2.53	2.83E-05	-4.16	High	Yes	0.55
Angoline	COC1c2c(ccc(c2OC)OC)c2c(N1C)c1cc3OCOc3cc1cc2	C22H21NO5	379.41	49.39	3.76	3.57	8.28E-06	-4.98	High	Yes	0.55

Table 2: List of selected genes with Uniport ID.

Sr.no.	Gene name	Protein name	Uniport ID
1.	GLXR2	2-hydroxy-3-oxopropionate reductase	G0F006
2.	CYP1A1	Cytochrome P450 1A1	P04798
3.	G6PD	Glucose-6-phosphate 1-dehydrogenase	P11413
4.	UCP2	Mitochondrial uncoupling protein 2	P55851
5.	SULT1A4	Sulfotransferase 1A4	P0DMN0
6.	NFKBIB	NF-kappa-B inhibitor beta	Q15653
7.	SLC22A16	Solute carrier family 22 member 16	Q86VW1
8.	SULT1A1	Sulfotransferase 1A1	P50225
9.	IL17A	Interleukin-17A	Q16552
10.	CXCL2	C-X-C motif chemokine 2	P19875
11.	NOS3	Nitric oxide synthase, endothelial	P29474
12.	CYP1A2	Cytochrome P450 1A2	P05177
13.	PCSK9	Proprotein convertase subtilisin/kexin type 9	Q8NBP7
14.	UTG1A1	UDP-glucuronosyltransferase 1A1	Q64550
15.	TYR	Tyrosyl-DNA phosphodiesterase 2	O95551
16.	GATA2	Endothelial transcription factor GATA 2	P23769
17.	STK11	Serine/threonine-protein kinase STK11	Q15831
18.	PLA2G10	Group 10 secretory phospholipase A2	O15496
19.	HP		
20.	PLA2G2A	Phospholipase A2, membrane associated	P14555
21.	DECR1	2,4-dienoyl-CoA reductase [(3E)-enoyl-CoA-producing], mitochondrial	Q16698
22.	SLC22A8	Solute carrier family 22 member 8	Q8TCC7
23.	AHR	Aryl hydrocarbon receptor	P35869
24.	ACOT1	Acyl-coenzyme A thioesterase 1	Q86TX2
25.	SLC2A4	SLC2A4 regulator	Q9NR83
26.	SULT1C2	Sulfotransferase 1C2	O00338
27.	MAPK3	Mitogen-activated protein kinase 3	P27361
28.	CYCS	Cytochrome c	P99999
29.	BAAT	Bile acid-CoA:amino acid N-acyltransferase	Q14032
30.	BECN1	Beclin-1	Q14457
31.	KCNQ1	Potassium voltage-gated channel subfamily KQT member 1	P51787
32.	DAXX	Death domain-associated protein 6	Q9UER7
33.	ALOX15	Polyunsaturated fatty acid lipoxygenase ALOX15	P16050
34.	HNF4G	Hepatocyte nuclear factor 4-gamma	Q14541
35.	ADIPOQ	Adiponectin receptor protein 1	Q96A54
36.	MMP9	Matrix metalloproteinase-9	P14780
37.	ALDH7A1	Alpha-aminoadipic semialdehyde dehydrogenase	P49419
38.	ACOT2	Acyl-coenzyme A thioesterase 2, mitochondrial	P49753
39.	LPL	Lipoprotein lipase	P06858
40.	SOD1	Superoxide dismutase [Cu-Zn]	P00441
41.	NOS2	Nitric oxide synthase, inducible	P35228
42.	SULT1A2	Sulfotransferase 1A2	P50226
43.	MAU2	MAU2 chromatid cohesion factor homolog	Q9Y6X3
44.	MAPK1	Mitogen-activated protein kinase 1	P28482
45.	UGT1A7	UDP-glucuronosyltransferase 1A7	Q9HAW7
46.	MIF	MIF4G domain-containing protein	A9UHW6
47.	VMP1	Vacuole membrane protein 1	Q96GC9
48.	NRF1	Endoplasmic reticulum membrane sensor NFE2L1	Q14494
49.	HMOX1	Heme oxygenase 1	P09601
50.	AIFM1	Apoptosis-inducing factor 1, mitochondrial	O95831
51.	NME1	Nucleoside diphosphate kinase A	P15531
52.	SLC22A7	Solute carrier family 22 member 7	Q9Y694
53.	LDLR	Sortilin-related receptor	Q92673
54.	COMT	Catechol O-methyltransferase	P21964
55.	PGD	Prostaglandin-H2 D-isomerase	P41222

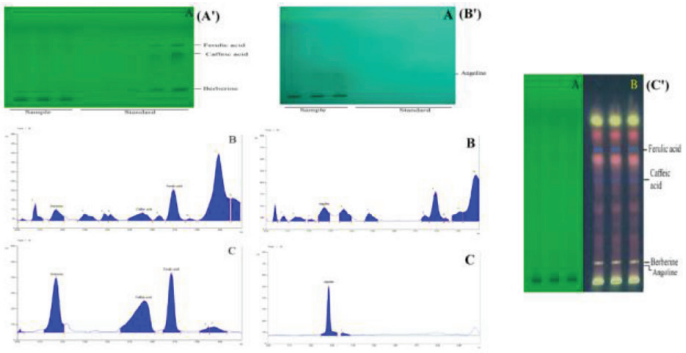

Figure 3: HPTLC profiling and quantification of ferulic acid, caffeic acid, angoline and berberine in the hydroalcoholic extract of *A. mexicana*. Figure A' (A) showing the developed HPTLC plate at 254 nm for ferulic acid, caffeic acid and berberine. Figure A' (B and C) showing the chromatograms of *A. mexicana* extract and standards. Figure B' (A) showing the developed HPTLC plate at 254 nm for sample and angoline. Figure B' (B and C) showing the chromatograms of *A. mexicana* extract and angoline. Figure C' (A and B) showing fingerprinting of hydroalcoholic extract of *A. mexicana* at 254 and 366 nm.

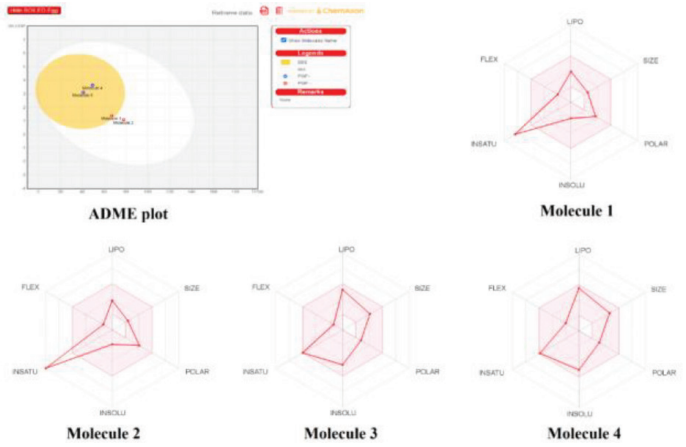

Figure 4: ADME analysis of identified metabolites, molecule 1 represents radar plot for ferulic acid, molecule 2 represents the radar plot of caffeic acid, molecule 3 represents the radar plot of berberine and molecule 4 represents the radar plot of angoline.

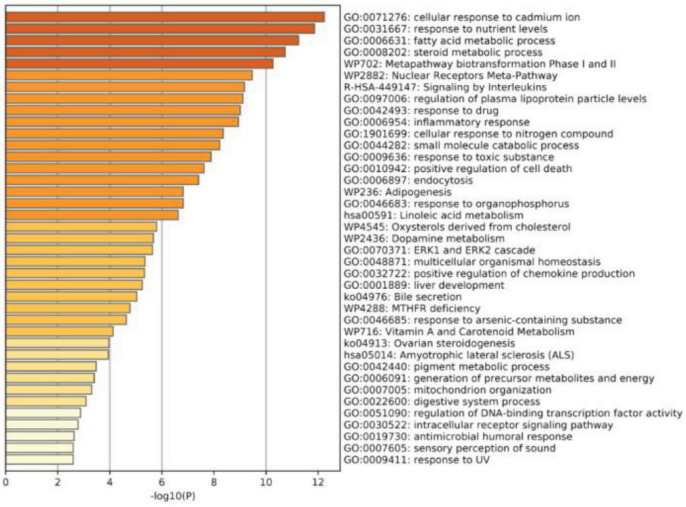

Figure 5: Gene ontology (biological process) of the selected genes.

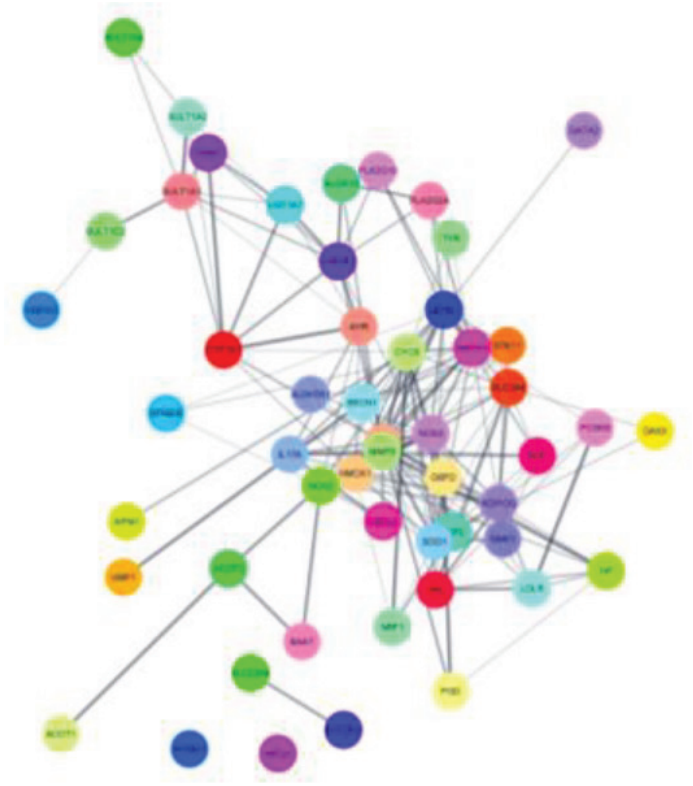

Figure 6: Protein-protein network of selected genes

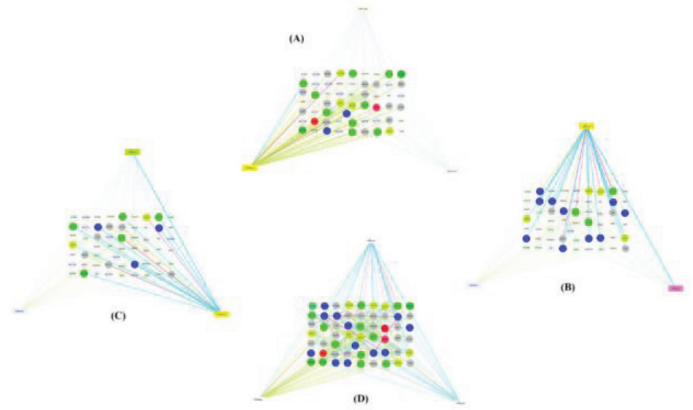

Figure 7: Interaction of active metabolites with selected genes, Figure (A) represents targeted genes of berberine, Figure (B) represents targeted genes of caffeic acid Figure (C) represents targeted genes of ferulic acid where Figure (B) represents the common network of each metabolites and targeted genes.

Computational analyses are the advance tools to explore biological response of drug or plant metabolites based on their gene interaction. Network pharmacology is one of the novel technique to screen hundreds of metabolites based on their biological activity concerning to the targeted sites.[31] The observation of our study revealed the interaction between active metabolites and targeted genes. The analysis showed ferulic acid with significantly interaction with the genes such as MAPK1, MAPK3, UGT1As, G6PD, ACOT2 BAAT etc which regulates inflammation.[32] Glucose-6-Phosphate Dehydrogenase (G6PD) protects these cells from oxidative chemicals while the lack of G6PD causes hemolysis of red blood

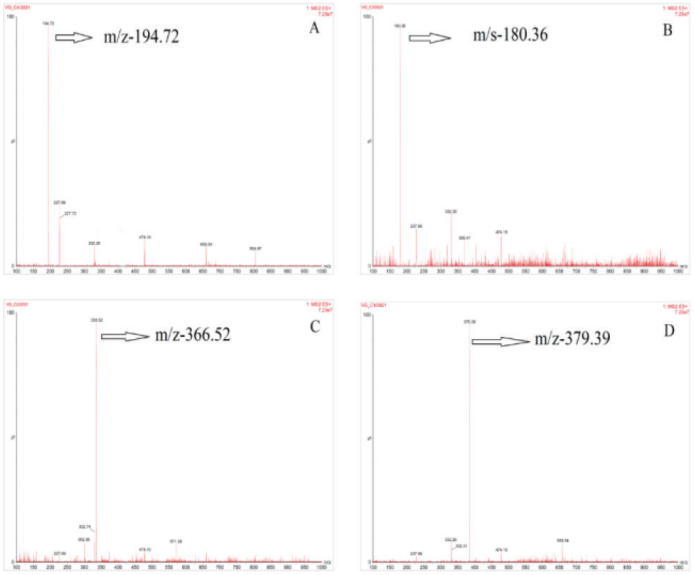

Figure 8: Mass spectra of isolated compounds where (A) showing for compound 1, (B) for compound 2, (C) for compound 3 and (D) for compound 4.

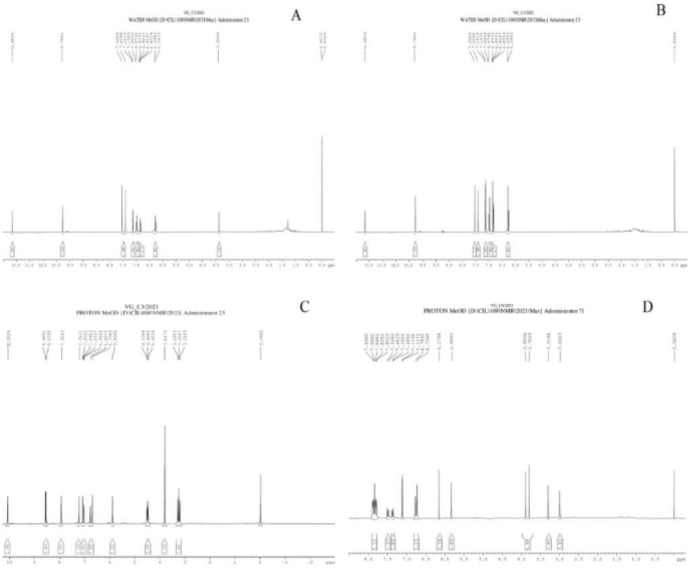

Figure 10: 1HNMR spectra of isolated compounds where (A) showing for compound 1, (B) for compound 2, (C) for compound 3 and (D) for compound 4.

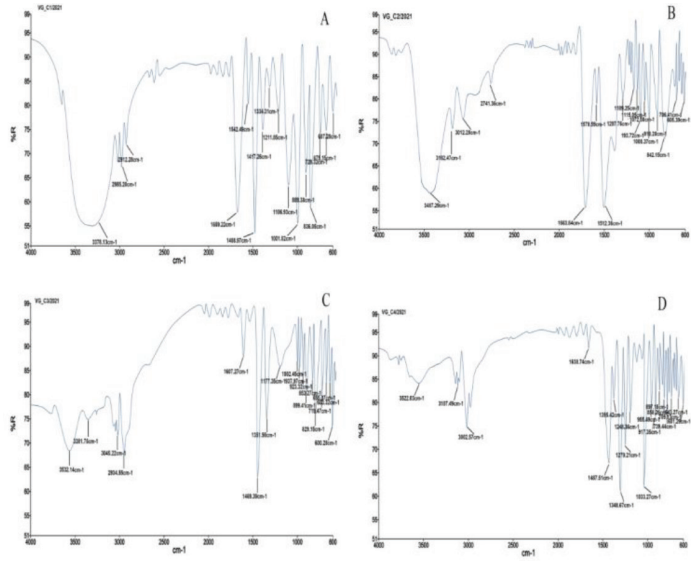

Figure 9: FT-IR spectra of isolated compounds where (A) showing for compound 1, (B) for compound 2, (C) for compound 3 and (D) for compound 4.

cells and represents acute hemolysis. However, the deficiency of G6PD cause Sevier problem of convulsion in the patients chronically associated with hemolysis.[33] The genes of cytosolic sulfotransferases associated with SULTs (SULT1A4, etc) exclusively expressed in neurons with no known function.[34] However SULT4A1 protects against oxidative-stress induced mitochondrial dysfunction in neuronal cells.[34] Ferulic acid, caffeic acid and berberin are the strong candidate which protects the neuronal cells against oxidative and inflammatory stress induced epileptic seizure. In a study cited by revealed that polyphenols such as ferulic acid possess neuroprotective effects against oxidative and inflammatory stress.[35-38] Besides, in the study conducted by Li *et al.*, revealed that berberine strongly attenuates the toxic effects of oxidative and inflammatory stress by amelioration of SOD and reduction of NOS oxidative enzyme, MAPKs and interleukins.[39]

CONCLUSION

The study concludes that *A. mexicana* is rich in several major and minor metabolites which characterized its chemical complexity. Further, four compounds (ferulic acid, caffeic acid, berberine and angoline) were found as major metabolites and the content of each metabolites found as 3.475 ± 0.028, 1.036 ± 0.013, 0.714 ± 0.014 and 0.738 ± 0.081µg/mg of the extract, respectively. In computational analysis, it can might be reported that ferulic acid, caffeic acid and berberine are the strong candidate for regulation of epileptic seizure induced by oxidative and inflammatory stress. In view of its study exploration, further experimental analysis are necessary to acknowledge the above biological facts strongly.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

A. mexicana: Argimone mexicana, **HPTLC:** High-performance thin-layer chromatography, **HPLC:** High-performance liquid hromatography, **LCMS:** Liquid chromatography-mass spectrometry, **GCMS:** Gas chromatography-mass spectrometry, **MS:** Mass spectrometric, NMR: Nuclear magnetic resonance, **FTIR:** Fourier transform infrared spectroscopy, **TLC:** Thin layer chromatography, **CC:** Column chromatography, **ESI:** Electrospray ionization, **ADME:** Absorption, distribution, metabolism and excretion, **GO:** Gene Ontology, **PPI:** Protein-protein interaction

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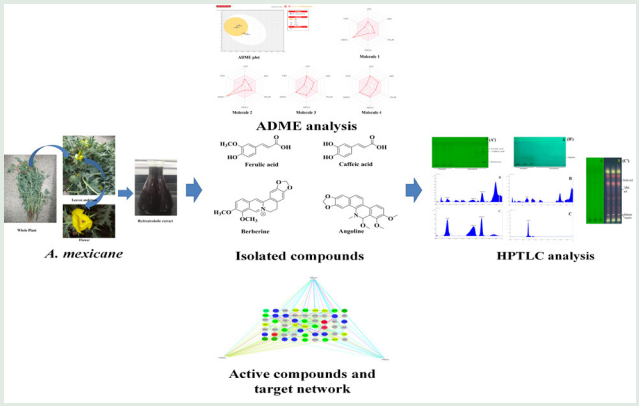
GRAPHICAL ABSTRACT



SUMMARY

Traditional Indian medicines play an important role against several ailments. *A. mexicana* is an Indian medicinal plants used for the treatment of asthma, ulcers, dysentery etc. Due to lack of phytopharmacological evidences and molecular mechanistic evaluation, the study is associated to evaluate the phytochemicals and antiepileptic potential of *A. mexicana* using analytical and computational tools. The study revealed ferulic acid, caffeic acid, berberine and angoline as the major constituents of this plant. These phytochemicals are potentially exhibited to mitigate the oxidative and inflammatory stress induced epileptic seizure. In active compound network analysis it can be clearly define that the identified constituents having significant interaction with the selected targets. In GO analysis, the selected targets are potentially associated with brain disorder, oxidative and inflammatory stress etc. Hence, it can prove that *A. mexicana* possess several metabolites which play a protective role in against oxidative and inflammatory stress induced epileptic seizure.

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