Identification, Isolation and Phytochemical Studies of Mangiferonic Acid From Martynia Annua Leaves for Anti-convulsant action

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ABSTRACT

Herbal medicines are low-cost, safe, and widely accessible to the general public. It's available to anyone who doesn't have access to a modern health-care system. Natural products from medicinal plants, whether as standardized extracts or as pure compounds, provided endless opportunities for new therapeutic discoveries due to the unrivalled availability of chemical variety. As a result of an increasing demand for chemical variety in screening techniques, the quest for medicinal pharmaceuticals from natural materials has increased around the world. HRLCMS was used to identify numerous phytochemicals in Martynia Annua leaves. One of the compound was isolated using a preparative HPTLC approach, and it was identified as Mangiferonic Acid using spectroscopy. Plant extract has anti-convulsant action, according to in-vivo research (MES-induce convulsion).

KEYWORDS : Martynia Annua, Leaves, Isolation, HPTLC, Anti-Convulsant, Herbal Tablet

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1. Introduction

From the very ancient time to till now the plants have been the basis of more traditional medicines systems throughout the world and further continued to furnish mankind with new remedies. The several varieties of medicinal plants and their purified components and natural products from the medicinal plants give immense opportunities for new drug development due to the unparalleled convenience of diverse chemical compounds [1]. The enhancing the awareness about the adverse effects of drugs had made the western pharmaceutical industries to revolve regarding the plant based Indian and Chinese medicine [2]. From the natural sources, nature has been a source of medicinal plants for past few years and an imposing number of modern drugs have been isolated from the natural sources. The several medicinal plants have been used for years in daily life to treat the numerous diseases all over the world. They have used as a remedy and for health care preparations [3].

In medicine, plant offer a great range of natural component belongs to different molecular families which have interesting biological activities which enchant various researchers to their illumination to give knowledge that will lead to advances in medicines [4]. It is crucial that any crude drug for pharmacological and pharmaceutical use required to be subjected to scrutiny for botanical identity. The role of phytochemical analysis are look for at this juncture to give a set of diagnostic features of the drug which will assist to a substantial extent to ascertain the botanical specification of the drug [5]. The phytochemicals are non-nutritive plant chemicals that show the protective and disease preventive properties. To protect itself plants produce these chemicals but the current research revealed that many phytochemicals can protect the humans against diseases [6].

Medicinal plants are another medicine for treatment of several diseases because of their presume acceptability, effectiveness, affordability, safety and low a cost. Currently, there is enhanced utilization of herbal formulations by the people due to the strong belief that these products are natural and safe for the treatment of ailments [7]. Indian government and other institutes throughout the world carry clinical and laboratory research on herbal medicine within the context of the eastern belief system but herbal medicines is not generally studied as part of conventional [8]. Observation is mostly fascinated on the consideration of efficacy of plant-based drugs used in the traditional medicine because they are economical and have less side effects. According to W.H.O about 80% of the world population relies mostly upon herbal remedies.

Utilization of herbs and plants for restorative uses has a long practice. In India and China, these foundations go back a huge number of years. When thought of as "traditional medication" utilized by local or old societies, herbal medication has risen as a well-known other option or supplement to current medication. As indicated by the World Health Organization, 4 billion individuals, just

about 70 % of the total populace, utilize herbal medication for some part of essential human services (Abramov V, 1996). It is assessed that in the United States alone, herbal dietary enhancements surpass $3 billion every year (The U.S. Food and Drug Administration, 1999).

Ayurveda, the ancient curative system of India, succeeded in the Vedic Era in India. As per historical evidences, the classical texts of Ayurveda, Charaka Samhita and Sushruta Samhita were composed around 1000 B.C. The Ayurvedic Materia Medica includes 600 herbal plants along with therapeutics. Herbs like turmeric, fenugreek, ginger, garlic and holy basil are essential portion of Ayurvedic preparations. These Ayurvedic preparations formulations include one herb or more than one herb (poly-herbal preparation).

Medicinal herb is a collection of various phytochemicals like alkaloids, saponins, glycosides, sesquiterpene lactones, resins, oleoresins and oils (essential and fixed). Now a day there is rising in attention to produce formulations make up of plant based. Researchers are regularly identifying chemical constituents from herbs, isolated them and detecting their pharmacological actions.

The World Health Organization has currently defined traditional medicine (involving herbal drugs) as contain therapeutic practices that have been in existent, for hundreds of years, before the development and spread of modern medicine and still in use today [9]. In India, around 20,000 medicinal plants have been recorded although traditional communities are using only 7,000 – 7,500 plants for the treatment of several function diseases [10]. The art of herbal medicine is to dissect pharmacologically and therapeutically active herbal drugs from harmful and toxic plants and to develop combinations of several medicinal plants compounds as safe and effective herbal remedies. Standardization and strict control measures are necessary to monitor viable high quality of herbal products and to prohibit contaminations that imperfectly affect patients consuming herbal [11, 12].

2. Material and Methods:

Collection and Extraction Method

The matured and healthy leaves of Martynia annua L. were harvested from the wild and shade dried for 10 days. The shade dried leaves powdered using mechanical grinders. The 50 g of leaves of powder of M. annua successively extracted with 300 mL of petroleum ether, acetone, chloroform, ethanol by using the Soxhlet extractor for 8 hours at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whattman filter paper (No.1) and then concentrated in vacuum at 40ºC using Rotary evaporator. The residues obtained were stored in a freezer -20º C until further tests.

Percentage yield of Extract

Accurately about 500gm of powdered leaves was subjected to the extraction as per the methodology described and percentage yield was calculated.

3. Preformulation study

Determination of λmax

Accurately weighed 10 mg of Martynia annua Leaves extract was dissolved in 10 ml of methanol and volume make up to 100 ml, this concentration of 100µg/ml. Martynia annua Leaves was scanned over the wavelength of 200-800 nm in UV spectrophotometer to determine the wavelength of maximum absorbance.

Plotting of calibration curve of Martynia annua Leaves extract

From the standard stock solution 1, 2, 3, 4 and 5 ml were withdrawn and volume was made up to 10 ml with methanol to give a concentration of 10, 20, 30, 40, and 50 µg/ml. Absorbance of these solutions was measured against a blank methanol at 408 nm for Martynia annua Leaves extract and the calibration curve was plotted.

4. Phytochemical Study

The concentrated extracts were used for preliminary screening of various phytoconstituents like. Carbohydrates, alkaloid, flavonoid, tannins, sterols, terpenoid, steroid, phenol, cardiac glycosides, anthraquinone glycosides, saponin glycosides, coumarin Glycosides and amino acid were detected by the standard method described in literature.

5. Characterization by Chromatographic Studies:

HPTLC:

Basic steps involved in HPTLC:

Extracts used: petroleum ether, acetone, methanol.

Application mode: CAMAG Linomet V.

Development mode: CAMAG Twin trough chamber

Sample Application:

The samples were dissolved in same solvent and 10µl quantity of sample was applied on the HPTLC silica merk 60F 254 graded plate sized 6cm x 10cm as narrow bands using CAMAG Linomat 5 injector.

Chromatogram Development:

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analysed. After elution, plates were taken out of the chamber and dried.

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Scanning:
Plates were scanned under UV flow at 408 nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each extract and Rf values were tabulated.

Mobile Phase:
Mobile phase was selected on the basis of the chromatogram of phyto constituent detected through TLC of each extract. Among all the solvent systems the best solvent system was solvent system- Toluene: Chloroform: Acetone: Petroleum ether: methanol (4:3.5:2.5:0.5:0.5) which showed good separation.

Mangiferonic Acid
It is a bioactive compound, against lifestyle related disorders. It possesses several health endorsing properties such as antioxidant, antimicrobial, antidiabetic, antiallergic, anticancer, hypocholesterolemic, and immunomodulatory. It protects against different human cancers, including lung, colon, breast and neuronal cancers, through the suppression of tumornectosis factor α expression.
The isolation and purification for further studies was done by Thin Layer Chromatography Method (TLC). The isolation method was found for the Mangiferonic Acid.

Method:
a) Mobile Phase used for development was n-hexane: Ethyl Acetate (90:1)
b) Samples was applied to the pre-coated plates of silica gel 60 plates with application of micropipette and kept for air drying at room temperature for 5 minutes.
c) The plates were put into the chamber containing mobile phase for 45 minutes.
d) After TLC separation, the plates was removed from the chamber and dried at room temperature for 3 minutes.
e) The bands were visualized by UV light chamber.
f) The visualized bands were scraped off from the plate and scrapped powder was dissolved in methanol.
g) The solution was centrifuged at 12000rpm for 15 minutes.
h) Supernatant fluid was collected and filtered from 0.22 micron filter and dried under reduced pressure.
i) The purified compound was separated and stored for further analysis.

6. Evaluation of Anti-convulsant Activity:

Drugs and Chemicals:
Diazepam (D.Z.) & Pentylenetetrazole (P.T.Z.) acquired as of Sigmas (St.LouisMO-USA). Phenytoin sodium (PS) & Picrotoxin (PCT) were conventional like contribution example as of PiramalHealth Care limited, H.P, India. Thiopental sodium was acquired as of market. The standard BA was purchase as of Yucca Enterprises, Mumbai. Every one drug solution was newly ready through dissolve/ suspend in usual saline intended in intra peritoneal (ip) administered.

Animals:
The Wistar albino rats (200-250 g) as well as Swissalbino mices (25.00 –30.00 gms) were obtain as of middle animals houses for institute and used for study. Animal was house on space hotness (25.00 ± 1.00 ^{0} C) by 50.00-55.00 % family member dampness plus known normal laboratories foodstuff in addition to waters ads libitum. Study is conduct following obtain principles group permission as of the Institutionals Animals Ethics Committees.
For this study, animal exist randomiz keen on 06 groups of eight animals every plus allowable to get used to for single week previous to the experiment.

7. Preformulation of herbal tablet:

Angle of repose:
It was determined by help of funnel method. Add accurately weighed granules in a funnel. The granules were allowed to flow through the funnel freely onto the surface. Measure the diameter and height of the powder cone and angle of repose was calculated from the following formula.

Tan Ɵ= h/r

Where Ɵ= angle of repose,

H= height of powder cone formed,

R=radius of powder cone formed

Loose bulk density
LBD is determined by adding a weighed amount of granules into a graduated cylinder and measuring the volume and mass.

LBD= Weight of the powder/ volume occupy in cylinder

Tapped bulk density (TBD)
For determination of TBD, known amount of granules into a graduated cylinder and measuring the volume and mass.
LBD= Weight of the powder/ volume occupy in cylinder

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Hausner ratio

It is the measurement of frictional resistance to the drug.

It is determined by using the following formula:

Hausner ratio= TBD/LBD

Compressibility index

It was determined by the Carr's compressibility index.

Compressibility index (%) = (TBD-LBD) X 100/TBD

Loss on drying

A glass stoppered bottle was taken to determine LOD. Accurately weighed 1 gram of granules was added to it. The bottles were placed in the drying chamber. The stopper was removed from the bottle and contents were dried for a specified time to achieve a constant weight. (Lachman et al., 1987)

Loss on drying (%) = [(Initial weight- Final weight)/ (Initial weight)] X100

Table: Formulation of tablet

Sr. No	Ingredients	F1	F2	F3	F4	F5	F6
1	Plant extract	500	500	500	500	500	500
2	Lactose	10	20	30	10	20	30
3	Starch	30	20	10	-	-	-
4	Gum Acacia	-	-	-	30	20	10
5	Magnesium stearate	5	5	5	5	5	5
6	Talc	5	5	5	5	5	5
Tablet weight 550 mg		550	550	550	550	550	550

Evaluation of herbal tablet

Uniformity of Weight:

Randomly selected 20 tablets of each formulation were individually weighed. The average value was calculated and compared to individual tablet weights.

General appearance:

While considering the general appearance, the colour, odour and texture of the tablet were observed.

Hardness test:

Tablet requires a certain amount of strength or hardness and resistance friability to withstand mechanical shocks of handling in all processes. The hardness of randomly selected 20 tablets of each formulation was determined by the Monsanto hardness tester.

Percentage friability test:

The friability of tablets was determined by Roche friabilator. Percentage of weight loss of 20 tablets randomly selected from each batch tumbled in friability apparatus. After 4 minutes of rotating at 25 rpm, the dust of tablets was removed and the percentage of weight loss was calculated.

Disintegration test:

The disintegration time of tablets was determined using the digital microprocessor based disintegration test apparatus. One tablet was introduced into each tube and added a disc. The assembly was suspended in a 1000 ml beaker filled in with water. The volume of water was such that the wires mesh at its highest point (at least 25 mm) below the surface of the water, and at its lower point (at least 25 mm) above the bottom of the beaker. The apparatus was operated and maintained at 37 ± 2^0C. The time requires to all tablets to disintegrate and pass through wire mesh was noted.

Stability Studies:

The stability parameters of a drug dosage form can be influenced by environmental conditions of storage, i.e. temperature, light, air and humidity, as well as the package components. All the formulations were subjected for stability study at long term testing for 12 months and accelerated temperature conditions, i.e. 25 ± 2^0C/60% RH$\pm5^0$C and 40^0C/75%RH$\pm5^0$C for six months. The different parameters such as colour, odour and texture of the tablets, average weight, hardness, and friability and disintegration time were studied at accelerated temperature conditions.

Results

Preformulation Study

Determination of λ_{max}: An absorption maximum was found to be at 408nm.Hence 408nm was selected as λ_{max} for further studies.

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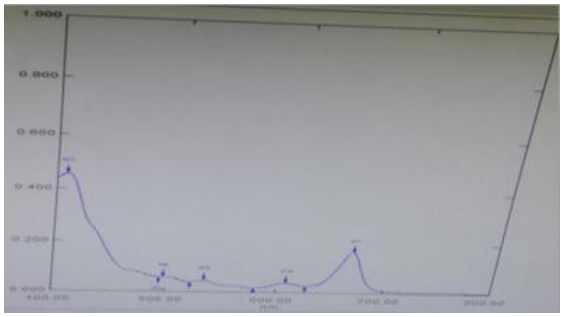

Fig: UV visible spectroscopy of Martynia annua Leaves

Calibration curve

Absorbance of these solutions was measured against a blank (methanol) at 408 nm for Martynia annua leaves extract and the calibration curve was plotted.

Table: Data of concentration vs absorbance

Sr.no.	Concentration (mg/ml)	Abs. (nm)
1	0	0
2	10	0.042
3	20	0.099
4	30	0.140
5	40	0.196
6	50	0.246

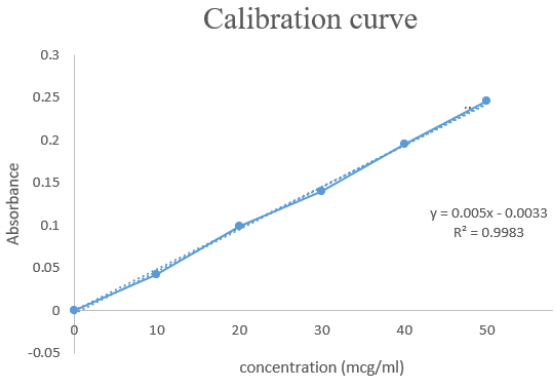

Fig: Calibration curve of Martynia annua L. Extract

Phytochemical Study

In this study the extracts of plants were subjected to phytochemical screening study and the results are represented in Table-1. Alkaloids, Carbohydrates, Flavonoids, Saponins, Steroids, Mucilages were present in the plant extracts. Only the glycosides are absent. Protein and fixed oil are absent in the extracts. Presence of Alkaloids, Flavonoids, amino acids, saponins, carbohydrate, Tannins, etc are commonly present in the extracts which may be responsible for anticonvulsant activity.

Table: Phytochemical study

Sr.no.	Plant constituents	Test of identification	Results
1	Alkaloids	Wagers test Hager's test Mayer's test Dragendroff's test	- + - -
2	Glycosides	Borntrager's test Keller killiani test	- -
3	Carbohydrates	Molisch test Benedict's test Barfoed's test Fehling's test	+ - + +
4	Phenolic compounds and tannins	Lead acetate test Ferric chloride test Ellagic acid test	+ + -
5	Saponins	Foam test	+
6	Flavonoids	Shinoda test Ferric chloride test Alkaline reagent test	+ - -
7	Mucilage & gums	With 90% alcohol	+
8	Protein and amino acid test	Biuret test Xanthoprotein test Millions test Ninhydrin test	- - + -

Characterization by Chromatographic Studies:

HPTLC:

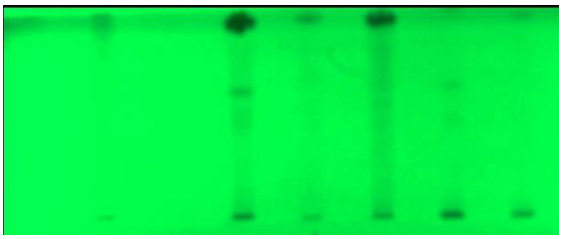

Figure: HPTLC screening at 408nm

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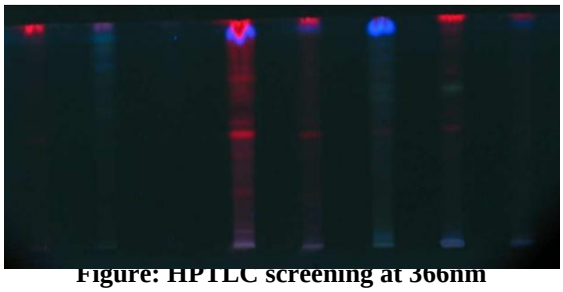
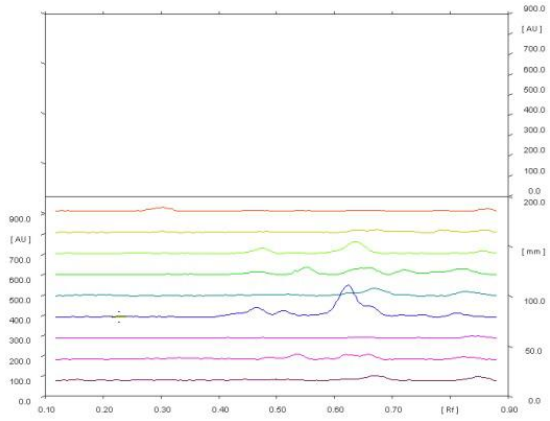


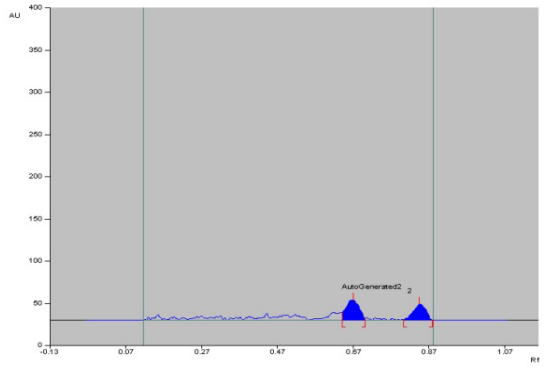
Figure: HPTLC screening at 366nm

HPTLC analysis, at short UV 408nm and after derivatization different spots were found in extracts i.e. methanol extract, acetone extract and petroleum ether extract which indicates the presence of different phytocomponents.

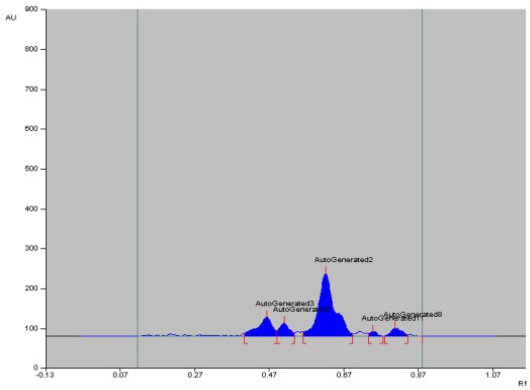
Plant Parts	Extracts	No. Of Peaks	Rf values	% Area
Leaf	Pet. Ether	2	0.64,0.80	57.06,42.94
	Acetone	5	0.40,0.49,0.56, 0.74,0.78	18.17,8.23, 65.63,2.21, 5.76
	Methanol	2	0.43,0.58	22.66,73.34



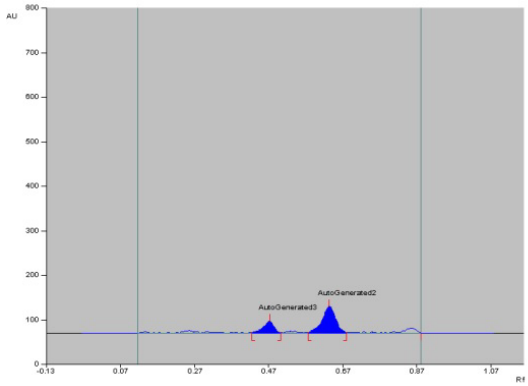
Graph: 2D Spectra of HPTLC of *Martynia annua L.*



Graph: Track-1 Petroleum ether leaf extract



Graph: Track-2 Acetone leaf extract



Graph: Track-3 Methanol leaf extract

HPTLC fingerprint patterns have been evolved for different extracts of Martynia annua L. Plant leaves. There were total three extracts of leaves, which applied accurately on HPTLC precoated aluminium plates with mobile phase Toluene: Chloroform: Acetone: Petroleum ether: methanol (4:3.5:2.5:0.5:0.5 v/v).

HPTLC analysis of leaves of *Martynia annua* L.

The petroleum ether extract showed 2 peak area covering 57.06 and 42.94% cover area with Rf value 0.64 and 0.80. while acetone extract showed 5 peak area covering 18.17, 8.23, 65.63, 2.21 and 5.76% cover area with Rf value 0.40, 0.49, 0.56,

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0.74 and 0.78. The methanolic extract showed 2 peak area covering 57.06 and 42.94% cover area with Rf value 0.43 and 0.58.

Observations:

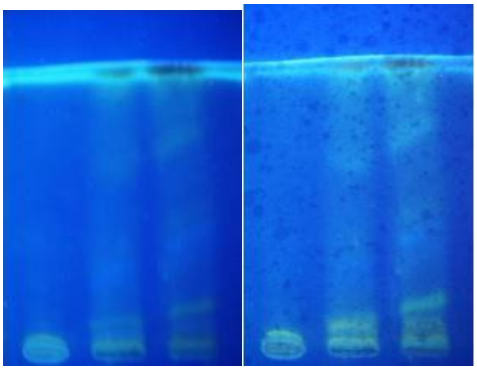
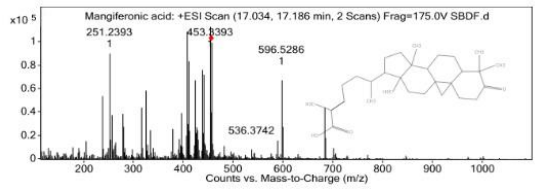


Figure: Images of developed TLC plates after development in mobile phase

Mass Spectra:

The mass spectrum shows a molecular ion peak at m/z 453 $(M-1)^-$ which is in good agreement for the proposed structure of known Mangiferonic acid. The mass spectra shown in fig. The mass spectrum shows a molecular ion peak at m/z 457.3711 (M+) which is in good agreement for the proposed structure of known Oleanolic acid. The mass spectra is shown in figure.



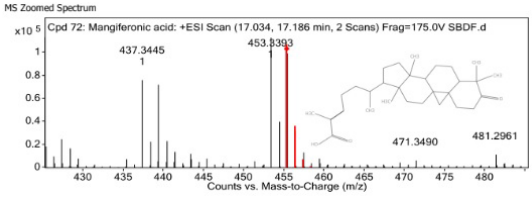
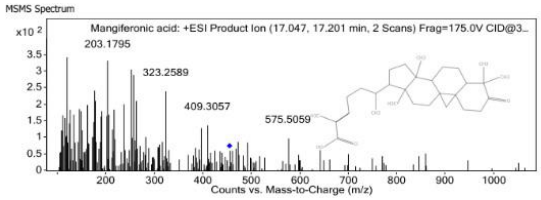


Figure: The mass spectra of Compound 1 (Mangiferonic acid)

MS Spectrum Peak List

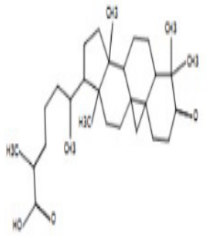
m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
251.2393			1	90295.18		
407.2979			1	109155.39		
409.3129			1	84088.98		
437.3445			1	76460.91		
439.3598			1	72203.13		
453.3393			1	113035.33		
455.3547	455.352	-6	1	99591.67	C30 H46 O3	(M+H)+
456.358	456.3554	-5.84	1	32091.59	C30 H46 O3	(M+H)+
457.3669	457.3585	-18.26	1	13417.69	C30 H46 O3	(M+H)+
458.3673	458.3615	-12.73	1	3973.35	C30 H46 O3	(M+H)+



MS/MS Spectrum Peak List

m/z	z	Abund
119.0876		343.82
161.1329		200.11
175.1529	1	241.92
177.1679		212.22
203.1795		332.14
237.2236	1	200.56
251.2396	1	306.55
256.2675		290.06
261.1871		212.37
323.2589		240.61

Compound Structure



Evaluation of anticonvulsant activity:

Acute Toxicity Study

In acute toxicity study, *Martynia annua* L. leaves extract (2g/kg) produced no toxicity inside rat as well as mices in excess of 72 hours. Within sub-acute toxicities (four week) as well as chronics toxicities (06 month) within as well as monkey, *Martynia annua* L. leaves extract prosuced no undesirable effect (Singh, 2008).

MES-induced seizures

The anticonvulsant activity of *Martynia annua* L. leaves extract was determined using electrically induced (MES) convulsion in rats. The *Martynia annua* L. leaves extract showed protective effect by significantly increasing latency of HLTE as compared with controls ($p<0.001$). *Martynia annua* L. leaves extract within the dose of 50 & 100 mg/kg show moderately important effects on latency of HLTE, duration of HLTE & duration of convulsion ($p<0.01$) while 200 mg/kg dose of extract showed a extremely protecting effect ($p<0.001$). The *Martynia annua* L. leaves extract significantly reduced duration of HLTE ($p<0.001$) in dose dependent manner. The duration of convulsion was also significantly reduced by *Martynia annua* L. leaves extract in all three doses ($p<0.001$). PS was completely protecting the animals by inhibiting the HLTE ($p<0.001$).

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PCT-induced seizures

The *Martynia annua* L. leaves extract at any doses did not show a significant effect against seizures induced by PCT.

PTZ-induced seizures

In PTZ-induced seizures, the administration of *Martynia annua* L. leaves extract, in a dose of 200 mg/kg, 30 min. prior to the injection of PTZ, significantly ($p<0.001$) delayed the onset of HLTE. DZ in a dose 4 mg/kg totally abolished the episodes of convulsions ($p<0.001$). there was less significant effect of the *Martynia annua* L. leaves extract at dose of 100 mg/kg on onset of HLTE ($p<0.01$) as compared to control. There was no significant effect of *Martynia annua* L. leaves extract at dose of 50 mg/kg on onset of HLTE.

Thiopental sodium-induced sleeping time

Complete values for sleep latencies (onset of sleep) as well as sleep time are obtained in table . No alteration was observed on onset of sleeping at any doses of *Martynia annua* L. leaves extract as compared to control. However, the duration of sleeping was significantly increased ($p<0.001$) in *Martynia annua* L. leaves extract (200 mg/kg) as compared to control. Similarly, animals treated with DZ (4 mg/kg, i.p.), as expected, an increase in duration of sleeping ($p<0.001$) and did show significant effects onset of sleeping ($p<0.001$). There was no significant effect observed on *Martynia annua* L. leaves extract in doses 50 & 100 mg/kg, on onset of sleeping as well as on duration of sleeping.

Table: Effect of Martynia annua L. leaves extract on maximal electroshock-induced seizures in rats

Group	Dose (mg/kg, i.p.)	Latency of HLTE (s)	Duration of HLTE (s)	Duration of convulsion (s)
Control	Vehicle	1.17±0.17	14.83±0.65	333.83±8.19
PS	25	00.00±0.00***	00.00±0.00***	127.17±8.57***
Extract	50	2.67±0.21**	7.50±0.43**	151.67±1.45**
Extract	100	2.67±0.21**	7.50±0.43**	147.50±5.79**
Extract	200	4.00±0.37***	5.00±1.30***	112.33±9.02***

Significance (Value) expresses by mean plus or minus S.E.M., N=6.00, Oneway Analysis of Variances (A.N.O.V.A.) follow through multiples compare Dunnet's tests,**p<0.01, ***p<0.001 vs. Control.

Table: Effect of Martynia annua L. leaves extract on picrotoxin-induced convulsion in mice

Group	Dose (mg/kg, i.p.)	Onset of Convulsion (min)	Onset of HLTE (min)
Control	Vehicle	3.67±0.46	16.57±1.46
DZ	4	6.51±0.33**	0.0±0.0
Extract	50	3.82±0.19	17.90±2.88
Extract	100	3.90±0.16	18.48±1.20
Extract	200	4.36±0.19	21.78±0.99

Significance (Value) expresses by mean plus or minus S.E.M., n=6.00, Oneway Analysis of Variances (A.N.O.V.A.) follow through multiples compare Dunnet's tests, **p<0.001 vs. Control

Table: Effect of Martynia annua L. leaves extract on pentylene (PTZ)- induced seizures in mice

Group	Dose (mg/kg, i.p.)	Onset of HLTE (min)	Convulsion (%)	Protection (%)
Control	Vehicle	3.59±0.28	100.00	0.00
DZ	4	00.00±0.00***	0.00	100.00

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Extract	50	4.25±0.35	100.00	0.00
Extract	100	5.52±0.34**	83.33	33.33
Extract	200	10.08±0.50***	50.00	50.00

Significance (Value) expresses by mean plus or minus S.E.M., n=6.00, Oneway Analysis of Variances (A.N.O.V.A.) follow through multiples compare Dunnet's tests, **p<0.01, ***p<0.001 vs. Control.

Table: Effect of Martynia annua L. leaves extract on Thiopental sodium-induced sleeping time

Group	Dose (mg/kg, i.p.)	Onset of sleep (min)	Duration of sleep (min)
Control	Vehicle	3.14±0.11	8.32±0.347
DZ	4	1.97±0.20***	40.42±1.86***
Extract	50	3.17±0.10	8.60±0.59
Extract	100	3.59±0.18	9.892±0.34
Extract	200	2.53±0.23	29.57±1.56***

Significance (Value) expresses by mean plus or inus S.E.M., n-6.00, Oneway Analysis of Variances (A.N.O.V.A) follow through multiples compare Dunnet's tests, ***p<0.001 vs. Control

Invitro Studies:

Preformulation study of powder blends

Angle of repose of different granules indicate excellent flow properties ranging from 25-29^0. The results of loose bulk density, tapped bulk density, Hausner ratio and compressibility index shows good flow characteristics mention in Table No

Table No. Preformulation parameters of powder blends of tablets of *Martynia annua* Leaves extract

Parameters	F1	F2	F3	F4	F5	F6
Angle of repose	27.12±1.13	29.12±1.14	25.51±1.70	27.02±1.01	30.87±1.58	26.85±1.01
Loose bulk density (g/cm^3)	0.783±0.015	0.787±0.012	0.775±0.012	0.756±0.020	0.779±0.019	0.769±0.010
Tapped bulk density (g/cm^3)	0.901±0.021	0.906±0.025	0.898±0.016	0.883±0.037	0.909±0.029	0.896±0.026
Hausner ratio	1.1±0.029	1.151±0.024	1.159±0.031	1.17±0.035	1.167±0.014	1.164±0.031
Compressibility index (%)	13.04±2.23	13.12±1.83	13.67±2.35	14.36±2.63	14.29±1.03	14.10±2.33
Loss on drying (%)	0.982±0.003	0.986±0.005	0.983±0.002	0.981±0.004	0.980±0.003	0.983±0.001

Data represented as mean ±SD, n=3

IR spectra of Plants, excipients and formulations

The IR spectroscopy of Plants, excipients and formulations were performed using Shimadzu-8400S.

IR spectra of Plant extract, Excipients and Formulations containing Starch and Gum Acacia as polymer.

Extract (Drug) Excipient compatibility study:

Interaction studies were performed by FTIR Spectroscopy. IR spectra of plant extract and

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polymers blend exhibited no additional peaks. The typical peaks of the extract were also seemed in the spectra of all the extract-polymer blends.

Evaluation of herbal tablets:

All the tablets of different extracts were evaluated by using various parameters and the results found were in the Pharmacopoeia limit. Uniformity of weight of tablets were found in the limits of 5% criteria, whereas hardness of tablets lies in between 6.98-7.02 which shows a considerable hardness that will ease to disintegrate the tablets. Friability of tablets found to be in the range of 0.36-0.47 shows mechanical stability of tablets. Time taken by tablets to disintegrate was 12-13 minutes which was in the range of Pharmacopoeia. All the results of evaluation parameters were shown in Table no.

Table No. Evaluation parameters of tablets of *Martynia annua* Leaves extract

Parameters	F1	F2	F3	F4	F5	F6
Uniformity of weight	1.03±0.51	**1.45±0.68**	1.49±0.85	1.79±0.77	1.50±0.75	1.60±0.62
Colour	Dark brown	**Dark brown**	Dark brown	Dark brown	Dark brown	Dark brown
Odour	Characteristic	**Characteristic**	Characteristic	Characteristic	Characteristic	Characteristic
Texture	Smooth	**Smooth**	Smooth	Smooth	Smooth	Smooth
Hardness (kg/cm^2)	7.05±0.123	**6.98±0.142**	6.99±0.159	7.02±0.148	6.94±0.154	7.01±0.146
Friability (%)	0.68±0.02	**0.71±0.02**	0.75±0.04	0.68±0.02	0.72±0.02	0.75±0.01
Disintegration time(minutes)	14.09±0.80	**11.51±0.41**	13.09±0.65	13.54±0.75	12.00±0.52	13.44±0.66

Data represented as mean ±SD. n=3

Stability studies

F2 formulation was selected for stability studies for three months shows no noticeable derivations in colour, odour, texture, uniformity of weight, hardness, friability and disintegration time were observed in all the formulations, results shown in table no.

Table No. Stability studies for *Martynia annua* L. Tablets (F2 formulation)

Parameters		Long term				Accelerated	
	Initial	**3 month**	**6 month**	**9 month**	**12month**	**3 month**	**6 month**
Uniformity of weight	1.45±0.68	1.41±0.71	1.44±0.67	1.42±0.67	1.39±0.70	1.63±0.97	1.39±1.14
Colour	Dark brown	No change	No change	No change	No change	No change	Brown
Odour	Characteristic	No change	No change	No change	No change	No change	No change
Texture	Smooth	No change	No change	No change	No change	No change	No change
Hardness (kg/cm2)	6.98±0.142	6.96±0.153	6.955±0.147	6.955±0.135	6.955±0.147	6.94±0.11	6.92±0.14
Friability (%)	0.71±0.02	0.72±0.02	0.73±0.01	0.74±0.01	0.74±0.01	0.75±0.02	0.77±0.002
Disintegration time (minutes)	11.51±0.41	11.46±0.45	11.40±0.31	11.38±0.39	11.31±0.43	10.51±0.73	09.48±0.45

Data represented as mean ±SD, n=3

Conclusion:

From the extract of Martynia annua L. leaves, several phytochemicals were identified. One of these compounds were isolated by preparative HPTLC method and the compound was characterized by spectroscopy methods and identified as Mangiferonic Acid. The in-vivo studies revealed that plant extract result possessed potential anti-convulsant in the animal's model investigates as well as these provide a rationale in favour of its uses inside traditionally drug in favour of organization of convulsion. The present results suggested that Martynia annua L. leaves extract containing marketed formulation is also being useful for the management of epilepsy.

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