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Evaluation of wound healing activity of the active fraction of stem extract of *Argemone mexicana*

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ABSTRACT

To assess the wound healing activity of active fraction of stem extract of *Argemone mexicana* Linn. The methanolic extract of *Argemone mexicana* was obtained by cold maceration extraction and then different fractionation was obtained. Different antioxidant methods were performed. Isolation of components from ethyl acetate fraction and characterization was done by using different instrumental analysis. Herbal gel was prepared using the extract of various ingredients in different proportion. The evaluation of gel was done using the various sorts of parameters viz., appearance, pH, spreadability. The evaluation of wound healing activity of active fraction of stem extract of plant was done on Wister albino mice using excision and incision wound models. Methanolic stem extract of *Argemone mexicana* Linn has shown profound presence of phytochemical constituents than other extracts. From the TLC it was found that the compounds isolated from ethyl acetate fraction show fluorescence at longer wavelength (360 nm). And from R_f value that is 0.33 and 0.66 value it was predicted that compound 1 (RK2A) and compound 2 (RK2B) may be berberine and sanguinarine or its analogue. Antioxidant activity of ethyl acetate fraction was found to be good. From the analgesic activity, anti-inflammatory activity it was found that ethyl acetate fraction having those properties which can be supportive study for wound healing. The ethyl acetate fraction of *Argemone mexicana* Linn treated wounds were found to be faster and rate of wound contraction was significantly increases as compared to control wounds ($p < 0.001$) while in incision wound model tensile strength was good. Results indicate that the *Argemone mexicana* Linn stem is source of wound healing treatment.

Keywords: *Argemone mexicana*, Excision wound, Incision wound, Gel, PTLC

INTRODUCTION

Wounds are injuries caused by physical chemical immunological insult to tissue and results in opening or breaking of skin. As wound infection is found majorly in developing country. More than 80% of population was depend on traditional medicine. The World Health Organization (WHO) has also recommended the evaluation of the effectiveness of plants in treatment where we lack safe modern drugs. Approximately one-third of all traditional medicines in use are for the treatment of wound and skin disorders [1]. Wound healing process has several steps viz., coagulation, inflammation, and granulation tissue formation, formation of matrix, connective tissue remodeling, collagenization and wound strength acquisition. Wound healing is an important biological process involving tissue repair and regeneration [2]. Mainly three phases are there inflammatory proliferative and remodeling phase also called 3R phase that is (Reactive phase, Regenerative phase and Remodeling phase) respectively [3]. The present study has been designed to determine the wound healing activity of the plant *Argemone mexicana* L. (Papaveraceae), which has been widely used in indigenous systems for this purpose, and to confirm that whether this plant has that much potency or not [4].

MATERIALS AND METHODS

Chemicals - All chemicals and solvents were of analytical grade (AR grade). Silica gel G (Merck, Darmstadt, Germany) was used for PTLC. Silica gel 60 F254 thickness of 0.2 mm a Merck product was precoated on to TLC plates wherein HPLC grade ethyl acetate and methanol was used Pet ether (60-80), chloroform solvents used for maceration was of CDH and Rankem. The DPPH used for anti-oxidant activity was of Sigma Aldrich Company.

Collection and Authentication of plant material - Stem of *Argemone mexicana* L. were collected from Birla Institute of technology, Mesra, Ranchi, Jharkhand. The plant was collected in month of August –September and identified by Dr S. Jha (Professor, Birla Institute of Technology, Mesra) authenticated from Central National Herbarium, Botanical Survey of India, Botanical Garden, Howrah [No. - CNH/Tech.II/2014/91/224]. The stem was dried in shade for 30 days. The powdered mass of stem was taken for phytochemical analysis and then extraction.

Screening of phytochemical: It was done to identify the presence of alkaloids, carbohydrates, flavonoids, anthraquinone glycosides, tannins and triterpenoids in Pet ether (60-80), ethyl acetate, chloroform, methanolic and aqueous extract.

Extraction: Successive extraction of powdered plant materials was done by Cold maceration method using petroleum ether (60-80), chloroform and methanol as solvents. The extract obtained was decanted and clarified by filtration through Wattman filter paper fraction was kept in a desiccators to avoid absorption of moisture [5]. Methanolic stem extract of *Argemone mexicana* Linn was subjected to further fractionation by separating funnel method using different solvents like aqueous, n-butanol, and ethyl acetate [6].

Antioxidant assay:

a) Free radical scavenging assay by DPPH (2, 2'-diphenylpicrylhydrazyl) method:

For each determination, the stock solution of ascorbic acid (1mg/mL) was diluted to a dilution series (100-500mcg/mL) with methanol. An aliquot of each dilution (1.5ml) was mixed with methanolic solution of DPPH (1.5mL, 0.06Mm). The mixtures were shaken vigorously and incubated at 37°C for duration of 30 min in the absence of light. At 517 nm of wavelength, the absorbance was determined against methanol as blank. The percentage of DPPH scavenging was calculated [7].

$$\text{Scavenging activity (\%)} = (\text{control absorbance} - \text{test absorbance}) / \text{control absorbance} \times 100$$

b) Hydrogen peroxide scavenging activity assay: Hydrogen peroxide (H₂O₂) scavenging activity was measured as described by Ruch, Cheng and Klaunig (1989) with slight modification. A Solution of hydrogen peroxide (40Mm) was prepared in phosphate buffer (pH 7.4). All fraction that is ethyl acetate, n-butanol and aqueous fraction (100-500mcg/ml) in distilled water were added to a hydrogen peroxide solution (0.6ml, 40Mm). Absorbance of hydrogen peroxide at 230nm was determined 10 min later against blank solution containing the phosphate buffer without hydrogen peroxide [8]. Formula for calculating percentage inhibition

$$\text{Scavenging activity (\%)} = (\text{control absorbance} - \text{test absorbance}) / \text{control absorbance} \times 100$$

Isolation of compound from fraction using PTLC

Preparative Thin layer chromatography (PTLC) - An aliquot of ethyl acetate active fraction was spotted manually on a preparative TLC glass plate (20 cm × 20 cm; 0.2mm thickness) with silica gel G binder (Merck, Darmstadt, Germany) and allowed to dry for a 30 minutes. After this, development of plate was done with ethyl acetate: formic acid: acetic acid: water (10:1.1:1.1:2.6, v/v/v/v) as mobile phase in a pre saturated glass chamber. The developed plate was dried under air and spots were visualized under UV-visible light. Four spots were developed. From that spots two spots were identified using mobile phase n-butanol: ethyl acetate: acetic acid: water (3:5:1:1 v/v/v/v) and n-propanol: water: formic acid (90:9:1 v/v/v) [9].

Characterization of isolated component by instrumental method of analysis

a) UV-visible spectrophotometry - The λ_{max} of isolated phytoconstituents were determined by using UV spectrophotometry range from 200 - 400nm.

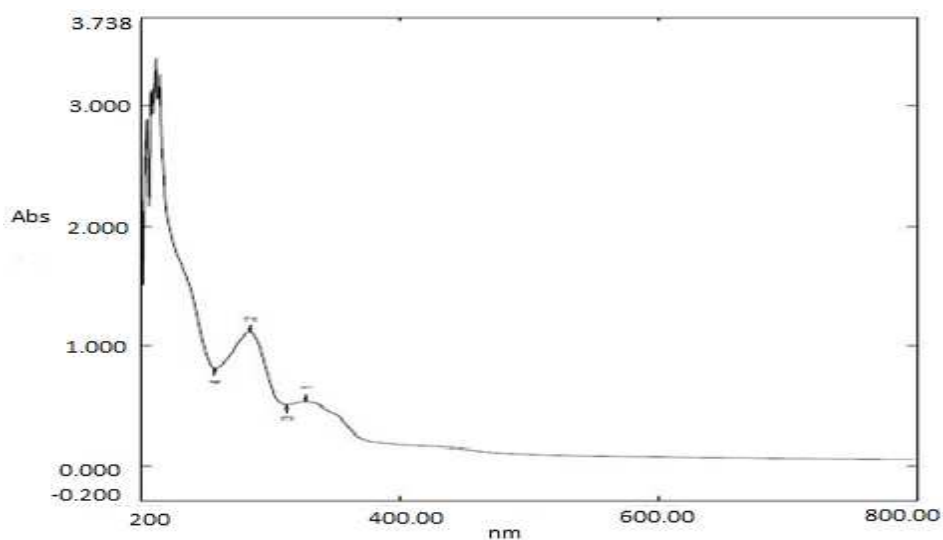


Fig 1(a): UV spectrum of compound1 (RK2A)

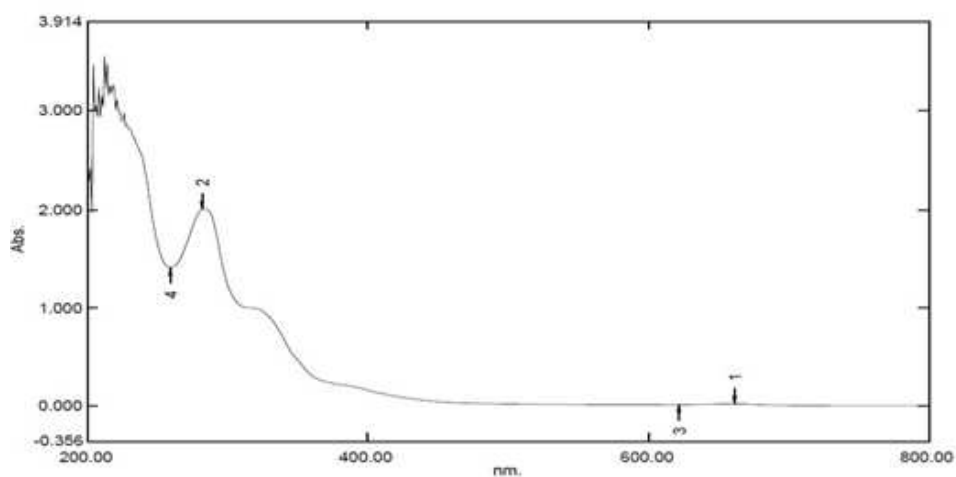


Fig 1(b): UV spectrum of compound 2 (RK2B)

b)IR spectrophotometry -The IR spectra of isolated phytoconstituents were taken by using KBR disc in the range of 4000 cm^{-1} to 400 cm^{-1} .

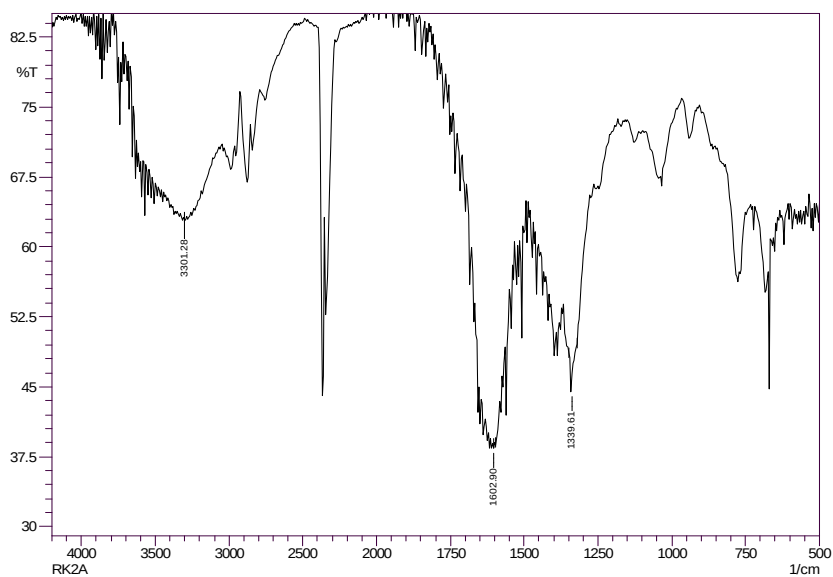


Fig 2(a): IR spectra of compound 1(RK2A)

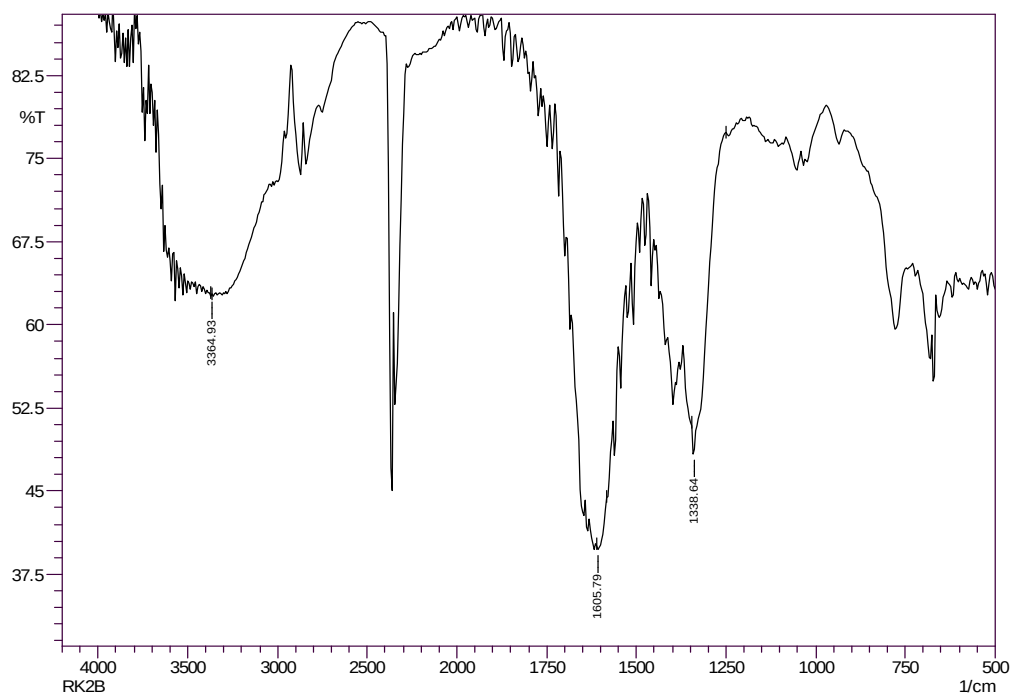


Fig 2(b): IR spectra of compound 2 (RK2B)

c) LC-MS analysis - The isolated compound were identified by LC-MS spectrum.

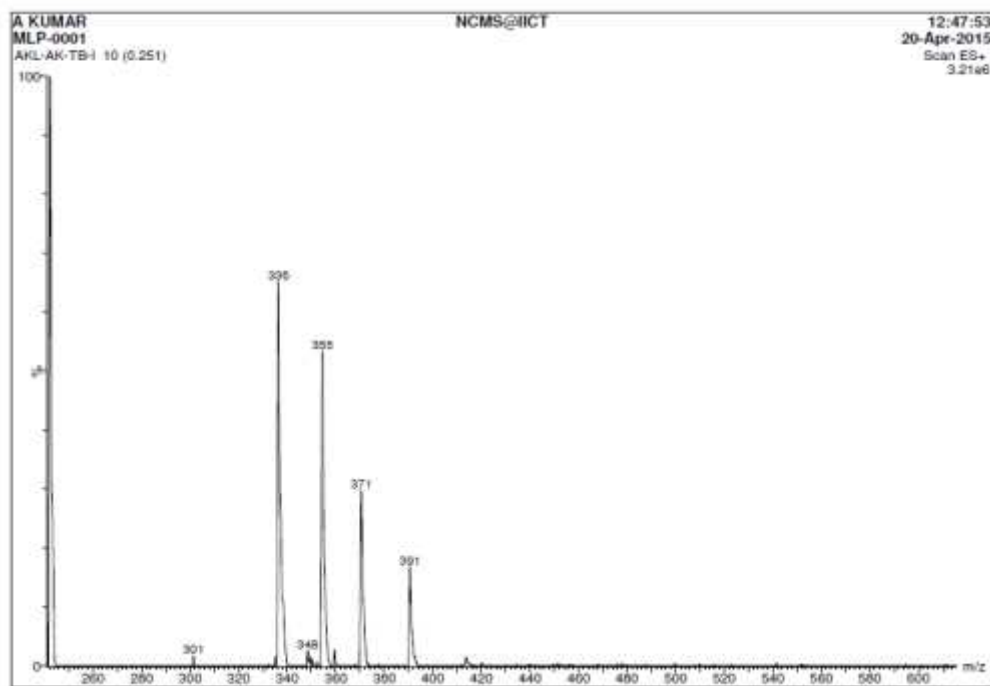


Figure 3 (a) LC-MS spectrum compound 1(RK2A)

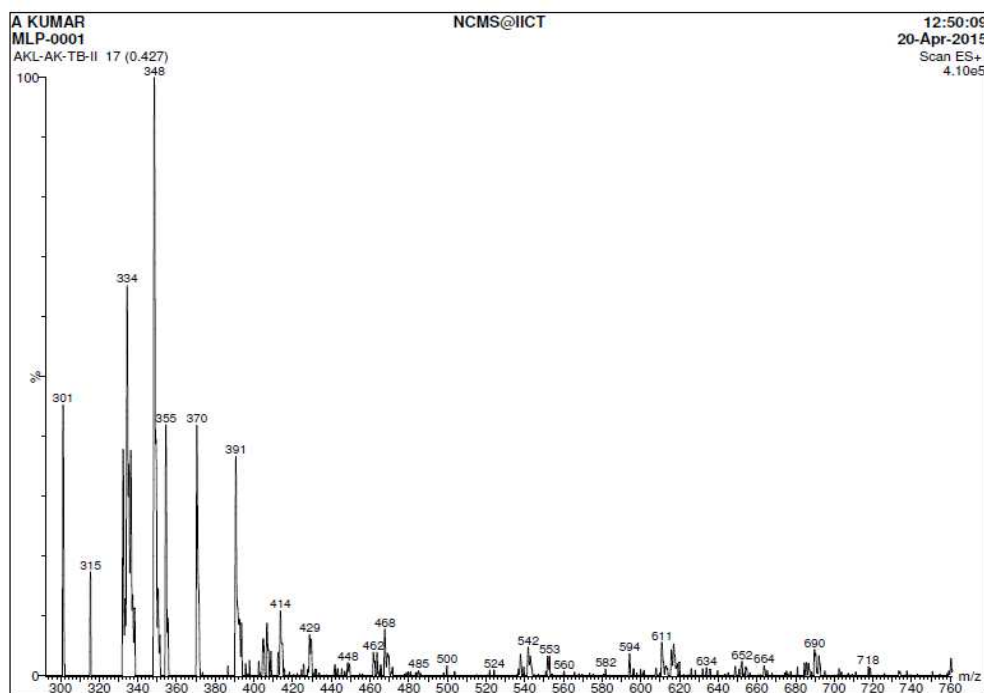


Figure 3 (b) LC-MS spectrum compound 2 (RK2B)

Formulation and evaluation of ethyl acetate fraction of *Argemone mexicana* Linn

a) Preparation of gel- Carbopol 934 was dispersed in 50ml of distilled water with continuous stirring. 2 ml of distilled water was mixed with methyl paraben and propyl paraben and dissolved by heating on water bath with continuous stirring. Solution was cooled and propylene glycol 400 was added gradually to form gel. Ethyl acetate fraction of methanolic extract was mixed distilled water was added to make up volume up to 100ml then small amount of triethanolamine was added to maintain the pH of gel formulation with continuous stirring [10].

b) Evaluation of plant extracts gel

Physical evaluation Physical parameter such as colour and appearance were checked.

Measurement of pH: About 1gm of gel weighed and allowed to dissolve by stirring with 100ml of distilled. pH of gel was measured by using pH meter.

Spreadability test: Spreadability was determined by apparatus which consists of glass slide. It is based on slip and drag characteristics of gels [11].

***In vivo* studies**

Acute toxicity study The acute oral toxicity study was performed to evaluate the acute toxicity effects and to determine minimum lethal dose (LD_{50}) of the extract. The albino mice male and female weighing 25-30g were used for the experiment. Extract was administered orally to different groups ($n=6$) of overnight fasted mice at dose of 20, 40, 60, 100, 250, 500 and 1000mg/kg body weight. After administration of the extract, all the animals were observed continuously for signs of the toxicity and mortality during 24 h, 48 h, and 72 h and beyond.

Analgesic study**a) Tail immersion method**

Animals: Swiss albino mice of (weighing between 20 and 25 g) were used for this method. Animals were procured from laboratory animal house of Department Of Pharmaceutical Sciences And Technology (Birla Institute of Technology, Mesra) (Protocol no PROV/BIT/ PH/ IAEC/ 25/ 2014). All animal experiments strictly complied with the approval of the institution and ethical committee

Chemicals: Standard Paracetamol (100mg/kg p.o.), Test - ethyl acetate fraction of methanolic stem extract of *Argemone mexicana* Linn. (250 mg/ kg post operative)

Experimental design: Animals were divided into three groups with three mice in each group.

Group 1: Standard treated with paracetamol + solution of 1% (v/v) Tween -80 in distilled water.

Group2: Control- Treated with solution of 1% (v/v) Tween -80 in distilled water.

Group3: Test1- treated with ethyl acetate fraction of methanolic stem extract of *Argemone mexicana* Linn. (250mg/kg) + solution of 1% (v/v) Tween -80 in distilled water

Experimental Method: One cm of the tail of mice was immersed in warm water kept constant at 50°C. Tail withdrawal is taken as end point. Cut off period is 10-12 sec. before injecting the drugs basal reaction time was taken for each animal at least three times with 5 minutes gap. After injection, reaction time was taken at 15, 30, 45 minutes and enhancement in reaction time was determined [12].

b) Acetic acid induced writhing method

Experimental Method: All the mice of test and standard groups were injected with respected drugs. After 15 minutes, acetic acid solution was administered to these animals. To the control group, only acetic acid solution was administered. The number of abdominal contractions, trunk twisting and extension of hind limbs were recorded during a period of 10 minutes and scores of average writhing were determined [12].

Anti-inflammatory study

Rats paw edema induced by carrageenan

Animal: Wister rats (weighing between 100 to 200 g) were used in this method. Animals were procured from laboratory animal house of Birla Institute of Technology, Mesra (Protocol no PROV/BIT/ PH/ IAEC/ 25/ 2014). All animal experiments strictly complied with the approval of the institution and ethical committee (IEC).

Chemicals: Standard– Indomethacin (10 mg/kg, s.c.), Test –Ethyl acetate fraction of methanolic stem extract of *Argemone mexicana* Linn. (250 mg/kg, i.p.), Inducer- Carrageenan (1% w/v, 0.1ml is injected underneath the plantar region)

Experimental design: Three groups consisting of three rats in each group.

Group 1: Standard- Treated with Indomethacin.

Group2: Control- No treatment, only carrageenan.

Group3: Test1- Treated with ethyl acetate fraction of methanolic stem extract of *Argemone mexicana* Linn (250mg/kg).

Experimental Method: To the standard and test groups respective drugs were injected. After 30 minutes 0.1 ml of 1 % (w/v) carrageenan in the plantar of the left paw. Control is treated only with carrageenan. Paw volume of both legs are noted at 15, 30, 60, 120, 180 minutes after carrageenan challenge. The percent inhibition in increase of edema volume for each animal group was calculated [12].

Wound healing models

a) Excision wound study

Animal: Swiss albino mice of (weighing between 20 and 25 g) were used for this method. Animals were procured from laboratory animal house of Birla Institute of Technology, Mesra, Ranchi, India (Protocol no PROV/BIT/ PH/ IAEC/ 25/ 2014). All animal experiments strictly compiled with the approval of the institution and ethical committee.

Drugs: Standard – Megaheal gel, Test - 2% gel of ethyl acetate fraction of methanolic stem extract of *Argemone mexicana* L., Control - Simple gel base without any drug.

Experimental design: Animals were divided into three groups with five mice in each group.

Group1: Standard- Treated with 0.2% of Megaheal gel.

Group2: Control- Simple gel base without any drug.

Group3: Test- Treated with 2% gel of ethyl acetate fraction of methanolic stem extract of *Argemone mexicana* Linn.

Experimental Method: Three groups with five animals in each group were anaesthetized by the open mask method with diethyl ether. Mices were taken and depilation was done on the back side with a wound inflicted by making an incision and cutting a circular area of thickness 7 mm radius on an already known area. Wound was kept in an open environment. Then the drugs i.e., simple gel, gel of ethyl acetate fraction (2% w/w), the Standard gel (0.2% w/w Megaheal gel) were administered topically till the wounds completely recovered [13].

Contractions, which contribute for closure of wound, were traced by the raw wound. Retracing of wound was done on a millimeter scale graph paper to calculate the wound area every alternate day. The percentage of wound healing was calculated with the help of below mentioned formula.

$$\% \text{ Wound contraction} = \frac{(\text{Area of wound on initial day} - \text{specific day wound area}) \times 100}{\text{Initial day wound area}}$$

b) Incision wound study

Experimental Method: Three groups with four animals in each group were anaesthetized using diethyl ether and one paravertebral-long incisions were made through the skin at a distance of about 1.5 cm from the midline of the depilated back of the mice. After the incision was made, the parted skin was kept together and stitched with surgical thread (No. 000) at 0.5cm intervals and a curved needle (No. 11) were used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. The wound was left undressed. Tensile strength was measured with a tensiometer on 10th day. The tensile strength increment indicates better wound healing stimulation by the applied drug [14].

$$\text{Tensile strength} = \text{Total breaking load (gm)} / \text{Cross-sectional area (mm}^2\text{)}$$

Histopathology of skin tissue - Histopathological study of regenerated skin was done to observe microscopically the growth of fibroblast, degree of epithelialisation, collagen deposition. The more of these indicate the healing of more potent wound.

Statistical analysis: All the data of in vivo studies were analyzed statistically using one way Analysis of Variance (ANOVA) followed by Dunnett's t test. The data are expressed as mean $\pm$ SEM. p- values less than 0.05 imply significance.

RESULTS

Preliminary Phytochemical screening - Methanolic stem extract of *Argemone mexicana* Linn has shown highly presence of phytochemical constituents than other extracts. Ethyl acetate extract showed presence of some phytochemical constituents like alkaloid, flavanoid, phenol, tannin. It was also noted that methanolic stem extract showed more positive results.

Preparative thin layer chromatography (PTLC) - Semi quantitative estimation and identification of compound of methanolic stem extract of ethyl acetate fraction was performed by PTLC method. PTLC separation of ethyl acetate fraction of the plant material present four different compounds as revealed by spots when visualized under UV light (254 nm and 364 nm).

Further isolation of two compound from that four bands of ethyl acetate fraction using solvent system n-butanol: ethyl acetate: acetic acid: water (3:5:1:1 v/v/v/v). An n-propanol: water: acetic acid (90:9:1 v/v/v) respectively. And isolated compound was conformed by using dragendorff's reagent (detecting reagent) using TLC plate.

Characterization of isolated compound by instrumental method analysis

UV instrumental analysis UV-Visible spectra of compound 1(RK2A) and Compound 2 (RK2B) are shown in figure 1 (a) and figure 1 (b) respectively. The UV spectrum of compound 1 of ethyl acetate fraction of methanolic extract showed absorption maxima at 257, 327 312 nm. And compound 2 (RK2B) showed absorption maxima at 282, 259, 324 nm.

b) IR spectroscopy analysis

The IR spectra of isolated phytoconstituents were taken in the range of 4000 cm⁻¹ to 400 cm⁻¹ [Figure 2 (a) and Figure 2 (b)].

c) LC-MS analysis - LC-MS analysis of compound 1 (RK2A) displayed base ion peak at m/z 336 ([M⁺]) that correspond to fragmentation at m/z 355 ([M+19]), m/z 371([M+35]), m/z 391([M+55]) of 5,6 - dihydrodibenzene moiety (Figure 3 (a)).

LC-MS analysis of compound 2 (RK2B) displayed base ion peak at m/z 334 ([M⁺]) that correspond to fragmentation at m/z 348 ([M+15]), m/z 355([M+23]), m/z 370([M+35]) of phenanthridine moiety (Figure 3 (b)).

From the TLC it was found that the compounds isolated from ethyl acetate fraction show fluorescence at longer wavelength (360 nm). And from R_f value i.e., 0.33 and 0.66, it was anticipated that compound 1 (RK2A) and

compound 2 (RK2B) may be berberine and sanguinarine or its analogue. Further UV spectroscopic analysis was done and relevant wavelength was found. IR spectroscopy analysis was also conducted and stretchings and vibration peak were found which very similar to established compound. LC-MS analysis was performed which conform similar mass of established compounds to that of isolated compound. Berberine and Sanguinarine compound which was isolated show positive test for alkaloid which show compound present in ethyl acetate fraction which can have some biological activity.

Table 1: PTLC of stem extracts and their R_f value

Solvent - Ethyl acetate :Acetic acid :Formic acid : Water (10:1.1:1.1:2.6 v/v/v/v)		
Material - Ethyl acetate fraction of <i>Argemone mexicana</i> Linn.		
Compound No.	R_f	Range
1	0.25	360 nm
2	0.33	254 nm, 360 nm
3	0.5	360 nm
4	0.66	360 nm

Table 2: TLC of isolated compound with their R_f value

Compound	R_f value	Visible colour	Dragendroff's reagent
1	0.33	Lemon yellow	Orange brown
2	0.66	Light orange	Orange brown

Antioxidant activity

a) DPPH scavenging activity

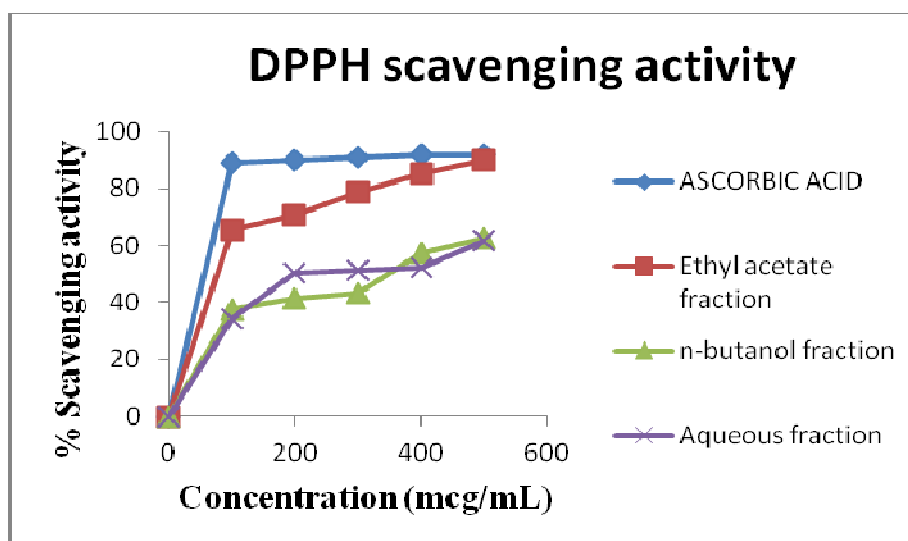


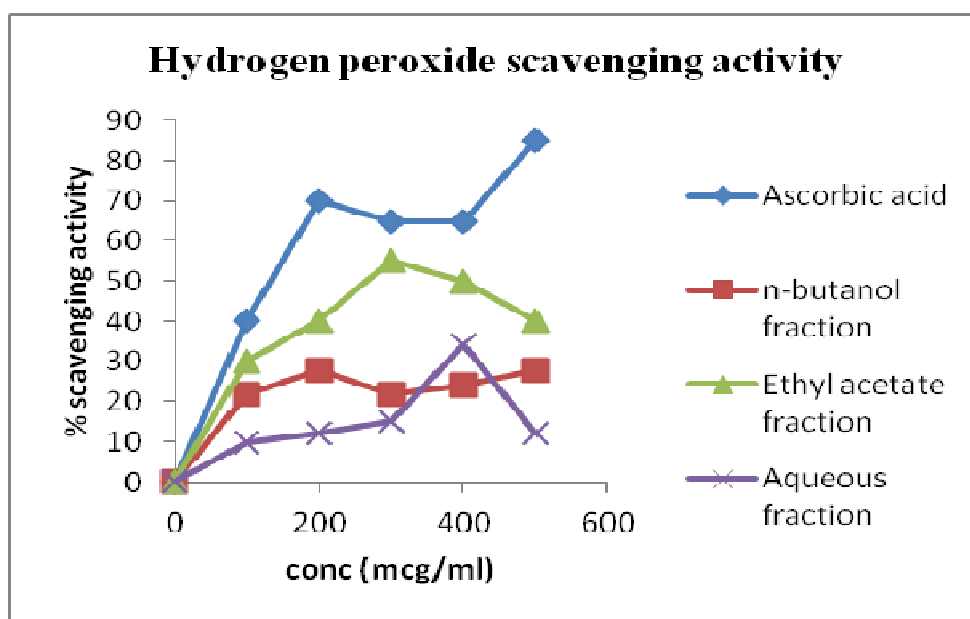
Fig 4: Various fractions showing DPPH scavenging activity

From the result (Table 3) it was found that ethyl acetate fraction was more potent having high percentage of activity than other fraction.

Table 3: For DPPH method IC 50 value of various fraction and standard

Sample	Value of IC ₅₀ (mcg/ml)
Ascorbic acid (standard)	56.34
Ethyl acetate fraction	130.6
n-butanol fraction	331.0
Aqueous fraction	340.0

b) Scavenging activity of hydrogen peroxide

Fig 5: Different fractions showing H₂O₂ scavenging activity

From the result (Table 4) it was found that it showing same result as in DPPH scavenging activity that ethyl acetate was potent than other fractions

Table 4: For H₂O₂ method IC₅₀ value of different fraction and standard

Sample	Value of IC ₅₀ (mcg/ml)
Ascorbic acid (standard)	222.0
Ethyl acetate fraction	430.0
n-butanol fraction	980.0
Aqueous fraction	1205

Formulation of gel

Ethyl acetate fraction gel was found to be of green colour appearance and showing good spreadability with neutral pH (See Table 5)

Table 5: Physical evaluation of fraction at time of gel formulation

Formulation	colour	Appearance	Spreadability (g-cm/sec)	pH
Control (gel base)	White	Clear and translucent	19.2	7.0
Test (Ethyl acetate fraction gel)	Green	Clear and translucent	21.58	7.4

In vivo studies**Acute toxicity study**

The acute oral toxicity study was performed and it was found it has shown no toxicity of drug extract at dose of 1000mg/kg p.o. No mortality was recorded in any group of mice after 72 hr of administration of extract to animals.

Analgesic activity**a) Tail immersion method**

Ethyl acetate fraction of methanolic stem extract was showing significant analgesic activity 84.20 and 82.54 respectively as compared to standard ($p < 0.01^{**}$). This result revealed that ethyl acetate fraction has central analgesic activity (Table 6). Maximum possible effect of different groups was shown (Table 7).

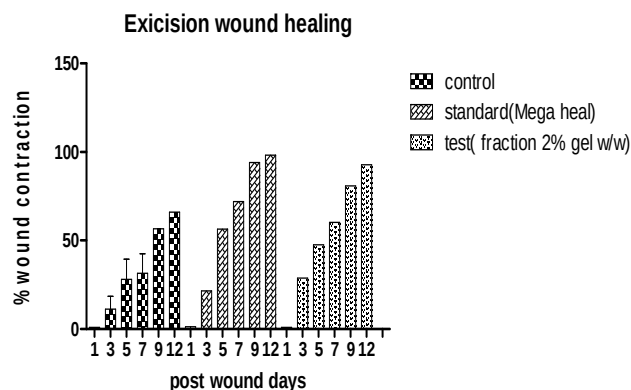


Fig 6: Percentage wound contraction in excision wound after treatment with standard and ethyl acetate fraction gel (2 w/w %)

Table 6: Analgesic activity of ethyl acetate fraction by tail immersion method

Groups	Basal time	30 min	60min	90min	120min
Control	3.54±0.17	3.32±0.31	3.50±0.31	3.88±0.20	3.44±0.22
Standard	3.62±0.34	7.20±0.90**	8.16±0.59**	7.16±0.57***	8.10±0.35***
Treated	3.30±0.2	5.64±0.70**	6.66±0.63**	6.90±0.49***	7.66±0.65**

Values are expressed as Mean ± SEM (n=5), **P<0.01, ***P<0.0001, when compared to control by one way ANOVA followed by Dunnet's t-test. Std-Paracetamol 100mg/kg, Treated- Ethyl acetate fraction (250mg/kg), control- Tween 80 in distilled water (5ml/kg)

Table 7: Percentage Maximum Possible Effects (%MPE) by tail immersion method

Groups	Percentage maximum possible effect (%MPE)			
	After 30 min	60 min	90min	120min
Control	—	—	—	—
Standard	94.60±1.720	94.20±1.241	92.80±1.254	91.20±1.254
Treated	82.20±1.958**	84.20±0.8602**	82.54±1.15***	81.25±1.54**

Std-paracetamol 100mg/kg, Treated- ethyl acetate fraction (250mg/kg), control-Tween 80 in distilled water (5ml/kg) Values are expressed as Mean ± SEM (n=5). **P<0.01, When compare to standard. One way ANOVA followed by Dunnet's t-test.

b) Acetic acid induced writhing method

Ethyl acetate fraction was showing significant inhibition of writhing responses compared to standard (p < 0.01**) and more potent peripheral analgesic activity as that of standard (Table 8).

When compare to control. One way ANOVA followed by Dunnet's t-test. Analgesic activity of treated group- 64% (ethyl acetate fraction 250mg/kg) compared to standard having 76%. It is highly significant ***-p<0.001

Table 8: Analgesic activity of ethyl acetate fraction by acetic acid induced writhing method

Groups	Time of onset (min)	Total no. of writhings	Percentage of inhibition
Control	3.80±0.37	10.80±1.24	—
Standard	11±0.44**	4.20±0.73***	76
Treated	9.60±0.92**	4.60±0.24***	64

Std-paracetamol 100mg/kg, Treated- ethyl acetate fraction(250mg/kg), control- acetic acid(1%) Values are expressed as Mean ± SEM (n=5), ***-P<0.0001, **-P<0.01

Table 9: Anti inflammatory effect and percentage inhibition of ethyl acetate fraction of *Argemone mexicana* stem extract on paw edema induced by carragennan

Groups	Volume (ml) of paw edema			
	30 min	60 min	90 min	120 min
Control	1.40±0.12	1.42±0.08	1.32±0.58	1.40±0.08
Standard	0.24±0.09*** (81.5%)	0.26±0.03*** (90.2%)	0.30±0.10** (89.36%)	0.29±0.10*** (90.05%)
Treated	0.63±0.06* (54.03%)	0.50±0.03** (64.42%)	0.43±0.06** (67.42%)	0.41±0.05** (71.63%)

Std- Indomethacin (10mg/kg), Treated- ethyl acetate fraction(250mg/kg), control- carragennan with 0.9% saline (1% v/v) Values are expressed as Mean ± SEM (n=5). *P<0.05, ***P<0.0001, **P<0.01 When compare to control and standard. Percentage of inhibition of paw edema activity shown in brackets One way ANOVA followed by Dunnet's t-test.

Anti-inflammatory activity

Fraction of ethyl acetate showing substantial decrease in paw edema volume at two hours and three hours in comparison of control group and also substantial increase in inhibition of paw edema value as time elapsed (30 mins, 1 hrs, 2 hrs, 3 hrs) as compared to control (Table 9).

From the analgesic activity, anti-inflammatory activity and angiogenesis study it was found that ethyl acetate fraction having those properties which can be supportive study for wound healing.

Wound model studies:

a) Excision wound model In excision method as ethyl acetate fraction of methanolic stem extract of *Argemone mexicana* Linn exhibited better wound healing activity when compared to that of control in both parameters measured i.e., wound contraction and period of Epithelization. This possesses potent healing activity when compared to standard (Megaheal gel) (Table 10).

Wound contraction exhibited by ethyl acetate fraction (92.87 ± 1.52) when compared with control (66.12 ± 1.25) and standard (98.16 ± 0.52) it is highly significant ****- $p < 0.0001$

Table 10: Wound contraction area in mice exhibited by ethyl acetate fraction gel of *Argemone mexicana* Linn in excision wound model

Post wound day	%Percentage wound contraction		
	Control	Standard	Treated
1	0.954 ± 0.32	1.25 ± 0.67	1.08 ± 1.25
3	5.69 ± 1.45	$21.55 \pm 0.64^*$	$20.82 \pm 2.36^*$
5	32.77 ± 0.66	$56.55 \pm 0.92^{**}$	$47.73 \pm 1.29^*$
7	48.17 ± 1.23	$72.08 \pm 0.79^{***}$	$60.32 \pm 1.22^{**}$
9	56.58 ± 1.55	$94.09 \pm 0.59^{***}$	$80.97 \pm 1.66^{**}$
12	66.12 ± 1.25	$98.16 \pm 0.52^{***}$	$92.87 \pm 1.52^{***}$

Std- Megaheal gel(2% w/w) Treated- ethyl acetate fraction(2% w/w), control- gel base(2%). Values are expressed as Mean $\pm$ SEM (n=5).

* $P < 0.05$, *** $P < 0.0001$, ** $P < 0.01$ When compare to control. One way ANOVA followed by Dunnet's t-test.

b) Incision wound model: In Incision method tensile strength was found to be 4.645 ± 0.1345 gm/mm² when compared to standard 5.022 ± 0.0522 gm/mm² and control 2.968 ± 0.0355 gm/mm² which indicates the presence of good collagen fibers formation.

Histological study

Histological study of incision wound healing skin of mice. MC: Mononuclear cells, F: Fibroblasts, C: Collagen, ET: Epithelial tongue, RE: Re-epithelization, G: Granulation, EE: Early Epithelization. In Control (simple base treated) mice's skin, presence of mononuclear cells, fibroblasts and collagen networks were not prominent. Some new blood vessels were there which indicating angiogenesis. Early epithelization showed in mice skin. Standard treated rats showing healed skin structures with well-synthesized, close to normal epidermis, restoration of adnexa, and profound fibrosis and collagen tissue within the dermis. In fraction treated mice's skin, presence of mononuclear cells and collagen net works were prominent, wound bridged by 2–3 layers of epithelial cells. Fibroblasts were also detected. Reepithilization of skin also has shown (Figure 7).

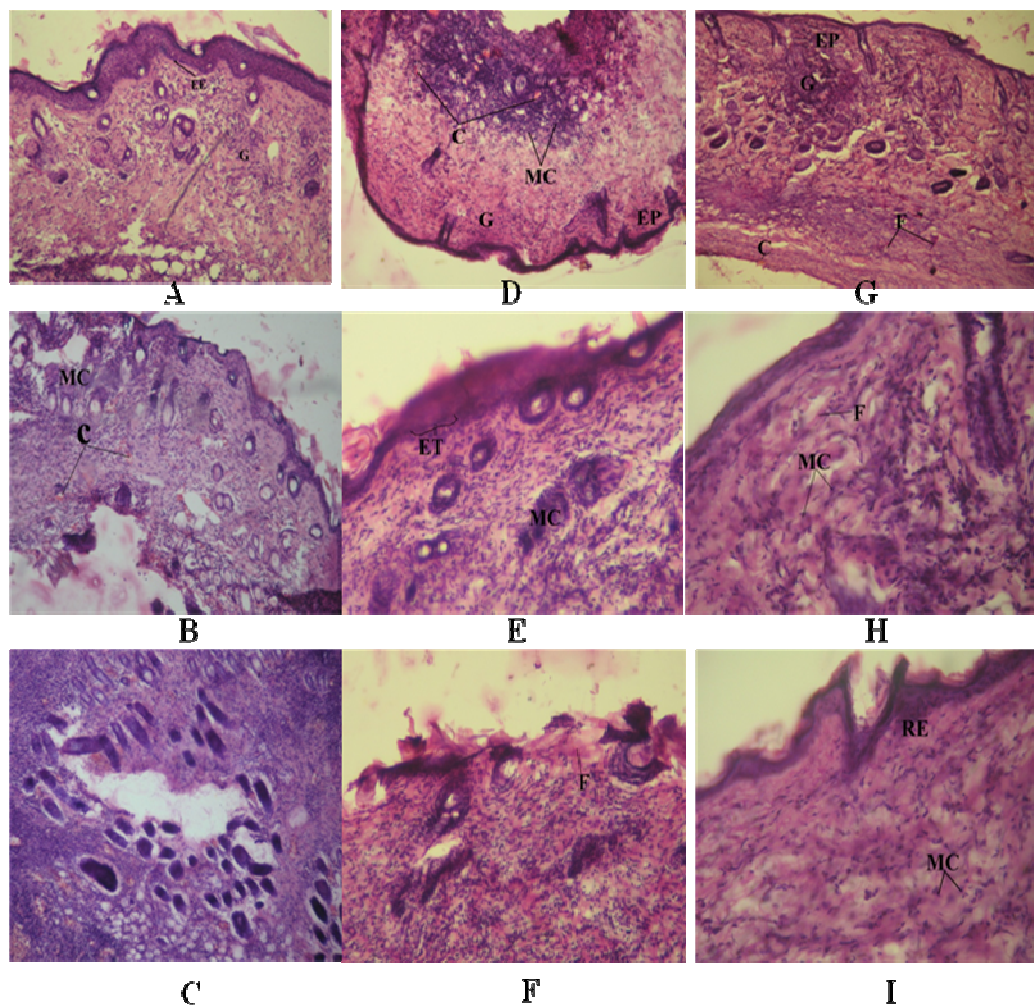


Fig 7: Histology of wound healing of mice's skin. Control (A-C), Standard (D-F), Test (G-I)

DISCUSSION

There are many plants used in the tribal areas for the treatment of wound, like *Plumeria alba* L., *Alstonia scholaris*, *Aloe vera*, *Mirabilis jalapa*, *Caesulia axillaris*, *Triumfetta rhomboidea*, *Urena lobata*. It has been found that through literature survey that *Argemone mexicana* Linn is one of the plant that is used in the treatment of wounds. Phytoconstituents present in the plant *Argemone mexicana* Linn which may be responsible for the wound healing activity which was revealed by phytochemical screening. Further isolation of compounds were studied and characterized tentatively with the help of modern analytical tools like UV spectrophotometry, FT-IR spectrophotometry, and LC-MS spectrophotometry. Based upon literature available on *Argemone mexicana* Linn. In the present study, fraction of ethyl acetate showed good activity in comparison with the n-butanol and aqueous fraction. The results obtained indicate that *Argemone mexicana* Linn stem extract exhibits free radical scavenging activity. Based upon these results, the ethyl acetate fraction was chosen for the in-vivo studies that is analgesic, anti-inflammatory activity. Further studied for wound healing activity it showed potent wound healing activity in comparison with the standard drug. From the result it was concluded that similar wound contraction was in standard and test drug which was significantly superior to the control at all the periods under study.

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