Original Article

A study on comparative anti microbial and wound healing efficacy of solvent extracts and succulent leaf extract of *Mikania scandens* (L.) Willd.

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ABSTRACT

Objective: A study for validation of reported wound healing property of fresh extract of succulent leaves of *Mikania scandens* (L.) Willd. was performed along with contemporary solvent extracts made from dry leaves of the plant, as the rural and ethnic people use the plant in this form.
Methods: The reported wound healing activity of the plant was studied in a novel way. For antimicrobial activity study, extract of succulent leaves was studied along with conventional methanolic and aqueous extracts of the dry leaves of the plant. The extracts were tested on *Escherichia coli*, *Salmonella enteritidis* and *Staphylococcus aureus* and the antimicrobial activity was tested both by conventional single disc diffusion method and a novel Spectrophotometric method. The wound healing and haemostatic activity were tested on induced punch wound of rabbit. Qualitative analysis for identification of phytochemicals present in the succulent leave extract was also performed.
Results: The succulent leave extract showed better antimicrobial activity against *Salmonella enteritidis* and *Escherichia coli* than methanolic extract, but showed lower activity against *Staphylococcus aureus* in disc diffusion and showed activity at 33% concentration against all the tested microorganisms in spectrophotometry. The methanolic extract of 5 mg/ml concentration failed to show any effect and the higher concentration of it as well as the fresh leave extract

above 33% concentration could not be evaluated due to presence of color materials. The succulent leave extract showed better wound healing potential than methanolic extract on rabbit wound. It took 25 seconds for haemostasis in 8 mm punch wound of rabbit. The fresh extract contains Alkaloid, Carbohydrate, Glycoside, Saponin, Protein and Amino Acid and also Phenolic Compound.

Conclusion: It was found that the fresh extract of the succulent leaves of *Mikania scandens* (L.) Willd. plant is having better antimicrobial activity and wound healing potential than methanolic extract on rabbit wound. The fresh extract can be used as haemostatic agent also.

Keywords: *Mikania scandens* (L.) Willd., Antimicrobial, Wound healing, Haemostatic, Punch wound, Rabbit.

INTRODUCTION

Natural products have historically been considered as an extremely productive source of new medicines in all cultures and continue to deliver a great variety of structural templates for drug discovery and development. Although products derived from natural sources may not necessarily represent active ingredients in their final form, the majority of all drugs in the market have their origin in nature[9,21]. In contemporary research, new drug discovery involves the identification of new chemical entities (NCEs), having the required characteristic of drug ability and medicinal chemistry. These NCEs can be sourced either through chemical synthesis or through isolation from natural products. Before the advent of high throughput screening and the post genomic era, more than 80% of drug substances were purely natural products or were inspired by the molecules derived from natural sources (including semi-synthetic analogs). An analysis into the sources of new drugs from 1981 to 2007 reveals that almost half of the drugs approved since 1994 were based on natural products[16].

In almost all the previous studies related with validation of reported medicinal property of plants, generally the plant parts were collected, dried and preserved. Then methanolic, ethanolic, acetone, aqueous etc. extracts of the preserved plant parts were made and stored at different manners. Then these were tested for their reported medicinal use *in vitro* or among *in vivo* animal models, either in that form or in semi-purified or purified form after identification of active principles[24]. Various modern methods are developed for extraction of phytochemicals from the solvent extracted plant materials[32], but fresh extracts collected from succulent plant parts were not considered as any item for validation of reported efficacy of plants. Generally, the ethnic and other rural people traditionally use the plants in their crude, fresh form in most of the time. It was argued that the concept of contemporary research have the limitation of loss of many aromatic and other phytochemicals present in the living plant, which may have very important role when used together[24].

Mikania scandens (L.) Willd. (Asteraceae) is known as Banchhalata/ Taralata in Bengali and Climbing hempvine in English. It is a common weed of plain land of India. The whole plant is used in Malaya for itches and in Java and South

Africa for wounds[2]. The leaves are applied in the form of poultice in wound[1]. The leaf paste and leaf juice is used in wound and bruises[20]. Juice of fresh leaves is used as haemostatic agent on fresh wounds in West Bengal, India[23]. Aqueous leaf extracts of this plant have been used in folk medicine to treat stomach ulcers[12].

The hydromethanol extract of the dry leaves of *Mikania scandens* has strong analgesic and antioxidant effects[25]. The ethanolic extract of the plant indicate the presence of alkaloid, glycoside, steroid, gums, reducing sugars, tannins etc. in chemical analysis[10]. The ethanolic extract of *Mikania scandens* showed antibacterial activity against *Bacillus subtilis* and *Bacillus megaterium*[10].

So, the present study was designed for validation of antimicrobial efficacy of methanolic and aqueous extracts of dry plant leaves as well as the fresh extract collected from the succulent leaves of the plant. The animal experiment was performed on rabbit by an ointment prepared from the hydro-methanolic extract obtained from the dry leaves as well as the fresh extract of the succulent leaves of the plant.

MATERIALS AND METHODS

A. Identification, extraction and preservation of plant materials

The taxonomic identification of plant was performed by plant taxonomist. The succulent leaves of the plant, *Mikania scandens* (L.) Willd., were collected during the month of September – October, 2013 from Ramchandrapur, Moyna, Purba Medinipur, West Bengal, India. After collection, the green, succulent leaves were washed with tap water, rubbed slightly individually with cotton soaked with distilled water to remove adhered particle, if any, and then were rinsed with distilled water and air dried.

Fresh extract preparation

A part of the washed, cleaned freshly collected plant leaves was taken and a pasty material was made from it with the help of pestle and mortar. Afterwards, the material was filtered by three layers of clean, white cotton cloth, centrifuged at 1000 rpm for 10 minutes and the supernatant was stored in airtight bottles at 4^0C for two hours and then at - 20^0C for further use.

Dry leaf preparation

Another part of the plant material was air dried in shade for seven days with changing of position twice daily and the dry leaves were stored in airtight nylon bottles at 4^0C for further use[11].

Aqueous and methanolic extract preparation

The methanolic and aqueous extracts were prepared from the dry leaves following conventional methods[11].

Preparation of semisolid methanolic extract

The liquid methanolic extract of the leaves was attached with Vacuum evaporator, where temperature was adjusted at 37^0C to get concentrated material. After evaporation of five hours, a portion of water was still present in the material. The material was then placed in some sterile petridishes inside the Ultraviolet ray sterilized Laminar flow for further evaporation of water. After evaporation, the pasty material was collected aseptically in sterile vials and kept at – 20^0C.

B. *In vitro* antibacterial study[4]

Microorganisms used

(a) *Escherichia coli.*
(b) *Salmonella enteritidis.*
(c) *Staphylococcus aureus*.

Source of bacteria

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Dilution of plant extracts for experiment

(i) Methanolic extract in water

Semisolid methanolic plant leaf extract (200 mg) was mixed with 1 ml of distilled water to obtain 200 mg/ml concentration.

(ii) Methanolic extract in methanol

Different quantities of semisolid methanolic plant leaf extract were mixed with 1 ml of 70 % methanol to obtain different concentrations.

(iii) Fresh extract

The fresh extract of the plant was used directly without any dilution or modification.

The fresh extract and the ointments were stored as aliquots and brought at 37^0C before application.

Procedure

The bacterial broth culture was adjusted to the bacterial concentration of 10^6/ml following the procedures described by Willey *et al*[26]. Then 100 µl of it was added in each plate and spread on the surface by a spreader. One sterile empty disc (Diameter of the disc is 7 ml, HiMedia) was taken each time to dip it for 30 seconds in the fresh plant extract/watery extract of dry plant leaves/ methanolic extract of plant diluted in water (200 mg/ml)/methanolic extract of plant diluted in methanol (100 or 200 mg/ml), allowed to dry for a few seconds and placed individually on the agar and pressed by the forceps slightly. The control discs were also placed on the agar. The plates were incubated for 37°C for 24 hours[3]. The process was repeated several times according to the requirement. The zone of inhibition of growth of bacteria was measured at the next day after bringing the plates from the incubator.

C. Study of antimicrobial efficacy and determination of minimum inhibitory concentration by spectrophotometry

The minimum inhibitory concentration (MIC) was evaluated by a novel type spectrophotometric method. The study was designed following partially the dilution method of four previous workers[5,13,14,26].

Only reduction of turbidity of the culture media than control was used to standardize the concentration of microorganisms in broth by some author[13,31], but spectrophotometric study was also performed by some others[8,26]. The wave lengths used were 405 nm[5] and 450 nm[8]. None of those studies were performed on fresh extract of any plant.

During the study, both 405 and 450 nm wavelengths were used, setting the plain bacterial broth media as control.

Procedure

Dilution of methanolic extract

The sterile test tubes were added with 3 milliliter of Broth media (HiMedia). Then the previously prepared methanolic plant extracts (200 mg/ml in 70% methanol) was added in different quantity to get different concentrations.

Dilution of fresh extract

The fresh extract was added with broth media at different quantity to get desired concentrations (Table 1).

Three sterile test tubes were added only with 3 ml of broth media for media control. Another three tubes having 3 ml of broth media (bacteria control) and all other test tubes having plant extracts were added with 100 µl of previously prepared bacterial

broth culture with bacterial concentration of 10^6/ml following the a standard procedure[26].

The media controls were kept at 4^0C and the other tubes of bacteria control and with plant materials were incubated for 24 hours at 37^0C. On the next day, the Optical Density (O.D.) of the materials was measured in UV VIS spectrophotometer of Systronics (India) model no.119 and the values were noted.

D. Study of efficacy on wound healing in rabbits

For this study, 10% ointment of methanolic extract was made[19] .The fresh extract was used as such.

Procedure

The rabbits were anesthetized by intramuscular injection of Xylazine @ 10 mg/kg body weight having name Xylaxin^R (Indian Immunologicals Limited, India) and Ketamin @ 40 mg/ kg body weight having name Kem^R (Unijules Life Science limited, India). The hair of the animals was removed carefully with blade.

Preparation of wound

The wound was prepared in anaesthetized clean animals. Punch wounds were prepared by an 8 mm. diameter skin biopsy punch [Acu Punch^R of Acuderm Inc (U.S.A.)] on either side of the depilated dorsum.

The test ointment, fresh extract and the control ointments were given immediately on the created wound in sufficient amount. The animals were placed in the cage with restricted movement with partial immobilization to protect the wound area from licking or causing damage by paw or teeth. The ointments/fresh extract were applied thrice daily.

Study of wound healing activities

1. Haemostatic efficacy study

Procedure

After creation of wound, 100 ul of fresh extract of leaves of *Mikania scandens* (L.) Willd. was added slowly on it with the help of micropipette. The wound was observed under convex lens (75 mm) to note the time interval of stoppage of bleeding and increase of total volume of liquid on the wound surface.

2. Physical studies

(a) Contraction of wound size

The wounds were measured by tracing the wound margin on a butter paper and then measured by a thread and the thread was measured by a scale. Then the length of the thread was considered as a boundary of a circle from there the area was measured. The healing of wound was considered on the day when encrustation was automatically removed and no measurable gap was found in the wound area. This was measured graphically on different days (3, 5, 7, 9 and 11 days post treatment wound), in mm. x mm. calculation.

(b) Wound healing index

Calculation of Wound Index was an arbitrary method of numerical expression of condition of the wound calculated[6] (Table 2).

3. Collection and staining of samples for histopathological study

Samples were harvested by punch biopsy from the wound site at regular intervals as per the protocol. After collection, the samples were preserved in 10% Neutral Buffer Formalin solution for routine histopathological study. The sections of five micron thickness were obtained from each sample and the slides were stained with routine Haematoxilin and Eosine method[29].

4. Biochemical study of wound tissue

The tissue material from the wound area was collected aseptically, labeled and stored at –20^{0}C. Then these were tested for quantification of Protein, DNA, RNA and Hydroxyprolin.

(i) Processing of wound tissue for lysate preparation

The tissue lysate was prepared as per standard protocol[15].

Wound tissue (100 mg) of each set was taken and chopped into pieces in ice cold condition. Then 1 ml of 10% Trichloric Acetic Acid (TCA) was added and the material was homogenized. The material was washed by adding another 4 ml. of 10% TCA. Then that was centrifuged at 2000 rpm for 10 minutes in cold centrifuge at 4^{0}C temperature. The precipitate was collected and the supernatant, which contained cell membrane, fat etc. was discarded. The precipitate was treated with 1 ml. of 0.1 M NAOH and was placed at 37^{0}C in the water bath for 1 hour. After that, it was added with 3 ml. of 20% TCA to dissolve. The mixture was then centrifuged at 2000 rpm for 10 minutes in cold (at 4^{0}C). The supernatant was collected which was having the protein part of the tissue dissolved in NAOH solution. The pellet part with DNA, RNA and some other nucleic acid matter was mixed with 400 µl. of 0.5M Perchloric Acid (PCA) and heated at 90^{0}C for 10 minutes in water bath. Then the material was centrifuged at 6000 rpm for 10 minutes at 4^{0}C temperature. The supernatant was divided into two parts for determination of DNA and RNA.

(ii) Estimation of protein

The total protein of the material was estimated following the procedure described by Lowry *et al*[17].

(iii) Estimation of tissue hydroxyproline

The tissue hydroxyproline was measured following the procedure described by Woessner[27].

(iv) Estimation of DNA was done by diphenylamine method[7]

(v) Estimation of RNA by Orcinol reaction[28]

E. Biochemical identification of phytochemicals present in the fresh extract of *Mikania scandens* Willd.

A preliminary qualitative analysis of the extract of the succulent leaves of *Mikania scandens* Willd was performed[30].

F. Toxicity study

The toxicity study of the methanolic and the fresh plant extract was performed as per the standard protocol[22].

Adult male Rats, weighing between 200-250 grams were taken for the study. Five rats were taken in Group I for study of methanolic ointment and another five rats were taken in Group II for study of fresh plant extract. They were kept 7 days in the animal house for acclimatization. The temperature of the animal house was kept between 22^{0}C to 25^{0}C. The animals were kept in light for 12 hours daily. Common rat diet of the animal house and unlimited supply of water was provided for the animals.

For testing of possible toxic effect of the methanolic extract, 10% ointment of methanolic extract was used. The fresh plant extract was used as such, undiluted.

Test Procedure: For restraining of the animals, injection of Ketamine @ 50 mg/Kg body weight and Xylazine @ 8 mg/kg body weight intramuscularly given before starting experiment.

The fur of the rats was removed by VEET^R [Rackitt Benckiser (India) Limited] ointment application before the experiment. The body parts were washed by distilled water thoroughly after removal of hairs. More

than 10% of the body part of the test animals was made hair free by that process.

The animals of Group 1 were treated with 10% ointment of the methanolic extract and animals of Group II were treated with fresh plant extracts of *Mikania scandens* (L.) Willd. on the skin surface and the ointment as well as the fresh extract was kept in close contact with the skin with a porous gauze dressing and non irritating tape for 24 hours. The ointment/fresh extract was changed daily. The experiment was repeated for 14 days.

RESULTS

See table 1-12 and figure 1.

DISCUSSION

The result of disc diffusion assay expressed that the methanolic and the fresh extract of *Mikania scandens* Willd. showed anti microbial effect on all the tested microorganisms. The methanolic extract showed better effects against *Staphylococcus aureus,* and the 200 mg/ml concentration showed the best effect. But against *Salmonella enteritidis* and *Escherichia coli*, the extract of the succulent leaves showed better activity than methanolic extracts of different concentrations (Table 3). The methanolic extract of 200 mg/ml concentration shows a better effect than 100 mg/ml concentration against all the tested microorganisms. Previous workers[13,18] took both 100 mg/ml and 200 mg/ml concentrations in other plants and observed better result in the higher concentration. The further higher concentrations were not taken as the plant extract remained at a thick state in higher concentrations (300 mg/ml or more) and remained adhered with discs. The methanolic extract failed to show its effect when diluted in water instead of methanol (Table 3). This may be due to ineffective dilution in separate media, in water in place of methanol. This is definitely a limitation of the methanolic extract. The aqueous extract made from the dry leaves of the plant failed to show any effect on any of the tested microorganisms.

A novel type of study was performed on the fresh extract to measure its effect on growth of micro organisms as well as for determination of MIC. The concept of measurement of antimicrobial efficacy through spectrophotometry was taken from the procedures adopted for determination of Minimum Inhibitory Concentration (MIC) of synthetic substances[26]. It was expected that the O.D value of the mixture of plant leaf extract with added bacteria should increase if the bacteria could grow and multiply. The O.D value should decrease if the plant extract was able to kill or inhibit the growth of bacteria. As the present extracts were having color material, the O.D values of extracts were always higher than the value of the broth with bacteria at post incubation.

The control of growth of bacteria was considered only at negative absorbance value in spectrophotometry of incubated samples. According to that idea, the fresh leave extract of *Mikania scandens* Willd. showed antimicrobial activity at 33% concentration against all the three microorganisms tested (Table 4, 5 and 6). The methanolic extract of 5 mg/ml concentration failed to show any antimicrobial effect against any of the tested microorganisms. Effect of the methanolic extract of higher concentrations and fresh extract having concentration above 33% could not be evaluated due to presence of deep color materials which caused an optical density on or above 2.00 (Table 4, 5 and 6).

The methods used in that experiment[14,26] were originally developed to test antibacterial substances, mainly of synthetic origin. There was no interference of color of those synthetic antibacterial chemicals. But both the methanolic as well as fresh extract of the plant were having some color, sometimes very deep. That caused the

limitation in the spectrophotometric study of different plant extracts.

Very few study reports are available on determination of antimicrobial activity of alcoholic extract of *Mikania scandens* Willd. As the study of extract of succulent leaves of *Mikania scandens* Willd. is reported for the first time in this article, so there was no possibility of getting any previous report on it. According to one report, the ethanolic extract of *Mikania scandens* showed antibacterial activity against *Bacillus subtilis* and *Bacillus megaterium* (7 mm ~ 28 mm)[10].

The *in vivo* study result of the fresh extract of the plant leaves on rabbit imply that the wound healing is better in the fresh extract treated wound as the wound healing index of 10% ointment of methanolic extract is higher (took more time to heal) than the fresh extract (1.57/1.42) and that of Povidone Iodine is 1.14. The control (base ointment) showed a value of 2.0 (Table10). Average reduction of wound size was also found better in the wounds treated with fresh extract than the 10% ointment of methanolic extract treated wound, though Povidone Iodine (5% w/w) showed further better result (Table 7). That corresponded with the wound healing day calculation i.e. 11 days in Povidone Iodine, 12 days in Fresh extract, 13 days in Methanolic extract and 14 days in Control ointment treated wounds (Table 9).

The fresh extract of succulent leaves of *Mikania scandens* (L) Willd. took 25 seconds on an average for control of bleeding from the created wound whereas Tr. Ferri. Per. Chlor took 13 seconds for that purpose (Table 8).

During analysis of the biochemical parameters of the wound tissues, a gradual increase in quantity of protein, Deoxyribonucleic Acid, Ribonucleic acid and Hydroxyproline was found with advancement of time during healing of wound. The increase was found comparatively better in the wounds treated with fresh extracts than the ointment made by methanolic extract of the plant leaves. The hydroxyproline level in day 3, 5, 7 and 9 was 3.77, 5.97, 9.90 and 11.67 in fresh extract treated wounds and 3.62, 5.92, 9.30 and 10.30 in methanolic extract treated wound which is comparable to 6.37, 10.90, 11.72, 13.67 in Povidone Iodine treated wounds. The figures were 3.62, 5.55, 9.37 and 9.97 in the base ointment treated wounds.

The Protein level of *Mikania scandens* fresh extract treated wound were 2.35, 4.92, 6.05 and 7.15 and it was 2.20, 2.85, 5.10 and 6.37 in case of methanolic extract ointment treated wounds in the corresponding days. The comparative figure were 4.35, 5.37, 7.25 and 8.47 in the Povidone Iodine treated wounds and 2.15, 2.65, 4.92 and 6.0 in the base ointment treated wounds in particular days.

The DNA level also differs among the wounds treated with different plant extracts. It was 1.67, 2.90, 5.47 and 6.17 against 1.20, 1.87, 3.27 and 4.87 in methanolic extract treated wounds. The corresponding figures in Povidone Iodine treated wounds were 1.80, 3.17, 6.00 and 6.77 and in base ointment treated wounds were 1.15, 1.75, 3.07 and 4.72 in the particular days (Table 11).

During analysis of the level of RNA extracted from the wound tissues, it was found that the quantity increased in the wound tissue with advancement of time. The fresh extract treated wounds showed better efficacy than methanolic extracts. These values were 0.70, 0.76, 1.13 and 1.38 for fresh extract and 0.62, 0.68, 1.00 and 1.21 for methanolic extract against 0.44, 0.51, 0.78, 0.91 figure of base ointment and 0.73, 0.86, 1.28 and1.53 in Povidone Iodine treated wounds in 3, 5, 7 and 9 days correspondingly (Table 11).

Histopathological views revealed that the rate of angiogenesis and collagenosis was better in the wounds treated with fresh leaf extract than those treated with methanolic extracts. The base ointment treated wounds

showed slowest recovery. The effect of Povidone Iodine was found better than all of these (Fig. 1).

During preliminary qualitative analysis of phytochemicals, it was found that the extract collected from the succulent leaves of *Mikania scandens* Willd. contain Alkaloid, Carbohydrate, Glycoside, Saponin, Protein and Amino Acid and also Phenolic Compounds. Some more specific quantitative analysis may determine the actual concentration of phytochemicals present in that extract (Table 12).

The result of the toxicity study reveal that the application of 10% ointment made from methanolic extract as well as the fresh plant extract on the skin of rat fail to produce any detrimental effect, either externally or internally.

CONCLUSIONS

It may be concluded that the fresh extract prepared from the succulent leaves of *Mikania scandens* (L.) Willd. plant showed an overall better antimicrobial efficacy than methanolic and aqueous extracts. The spectrophotometric evaluation of anti-microbial efficacy study may act as a new way of study of antimicrobial efficacy of plant, if the limitation made by the color materials can be removed. The fresh extract of succulent leaves is having better wound healing potential than methanolic extract on the punch wound of rabbit. It can be used as haemostatic agent also. The Fresh extract contain Alkaloid, Carbohydrate, Glycoside, Saponin, Protein and Amino Acids and also Phenolic Compounds. The fresh extract as well as the methanolic extract of that plant has no toxic effect on rat when used topically.

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Table 1. Dilution of fresh extract

Parameter	Quantity of materials added			
Fresh extract	1.5 ml	1.0 ml	0.5 ml	0.25 ml
Broth media	1.5 ml	2.0 ml	2.5 ml	2.75 ml
Concentration of Fresh Extract	50%	33%	16.50%	8.25%

Table 2. Parameters for calculation of wound index[6]

Serial	Wound condition	Wound index
a.	Complete healing of wound with scar formation	0
b.	Incomplete but healthy and satisfactory healing	1
c.	Healing process of wound is delayed but formation of granulation tissue observed	2
d.	Wound is healthy but healing process is yet to be started	3
e.	Formation of pus – evidence of tissue necrosis	4
	Total	10

Table 3. Average diameter of zone of inhibition of bacterial growth (mm) against extracts of *Mikania scandens* (L.) Willd. [n=6]

Parameters/Bacteria	*Escherichia coli*	*Salmonella enteritidis*	*Staphylococcus aureus*
Aqueous extract	--	--	--
Methanolic extract in water (200 mg/ml)	--	--	--
Methanolic extract in 70% methanol (100 mg/ml)	9	10	18
Methanolic extract in 70% methanol (200 mg/ml)	10	11	23
Fresh plant extract	12	13	15
Ceftriaxone (control)	24	28	25
Distilled water (control)	--	--	--
Methanol (70%) (control)	--	--	--

Table 4. Effect of *Mikania scandens* (L) Willd. on *Escherichia coli* in spectrophotometric study [n=3]

Plant: *Mikania scandens* (L) Willd. Bacteria: *Escherichia coli* (87O 157 strain) Set 0 = Only bacterial Media. Bacterial culture reading: At 405 nm: Pre inoculation 0.066; Post inoculation 1.083. At 450 nm: Pre inoculation 0.056; Post inoculation 1.049.					
Type of extract	Concentration	Pre 405	Post 405	Pre 450	Post 450
Methanolic extract	5 mg/ml	1.034	1.735	0.986	1.603
	10 mg/ml	≥2.00	≥2.00	≥2.00	≥2.00
	20 mg/ml	≥2.00	≥2.00	≥2.00	≥2.00
	50 mg/ml	≥2.00	≥2.00	≥2.00	≥2.00
Fresh extract	100%	≥2.00	≥2.00	≥2.00	≥2.00
	50%	≥2.00	≥2.00	≥2.00	≥2.00
	33%*	1.352	1.155	1.082	0.942
	16.5%	0.901	1.204	0.798	1.003
	8.25%	0.215	1.023	0.183	0.935

*Result can be considered as significant.

Table 5. Effect of *Mikania scandens* (L) Willd. on *Salmonella enteritidis* in spectrophotometric study [n=3]

Plant: *Mikania scandens* (L) Willd. Bacteria: *Salmonella enteritidis* Set 0 = Only bacterial Media. Bacterial culture reading: At 405 nm: Pre inoculation 0.186; Post inoculation 1.185. At 450 nm: Pre inoculation 0.144; Post inoculation 1.139.					
Type of Extract	Concentration	Pre 405	Post 405	Pre 450	Post 450
Methanolic extract	5 mg/ml	1.112	1.837	1.010	1.901
	10 mg/ml	≥2.00	≥2.00	≥2.00	≥2.00
	20 mg/ml	≥2.00	≥2.00	≥2.00	≥2.00
	50 mg/ml	≥2.00	≥2.00	≥2.00	≥2.00
Fresh Extract	100%	≥2.00	≥2.00	≥2.00	≥2.00
	50%	≥2.00	≥2.00	≥2.00	≥2.00
	33%*	1.210	1.142	1.124	0.981
	16.5%	0.877	1.205	0.715	1.019
	8.25%	0.288	1.481	0.204	1.215

*Result can be considered as significant.

Table 6. Effect of *Mikania scandens* (L) Willd. on *Staphylococcus aureus* in spectrophotometric study [n=3]

Plant: *Mikania scandens* (L) Willd. Bacteria: *Staphylococcus aureus* Set 0 = Only bacterial Media. Bacterial culture reading: At 405 nm: Pre inoculation 0.136; Post inoculation 1.157. At 450 nm: Pre inoculation 0.121; Post inoculation 1.145.					
Type of Extract	Concentration	Pre 405	Post 405	Pre 450	Post 450
Methanolic extract	5 mg/ml	1.152	1.698	1.114	1.582
	10 mg/ml	≥2.00	≥2.00	≥2.00	≥2.00
	20 mg/ml	≥2.00	≥2.00	≥2.00	≥2.00
	50 mg/ml	≥2.00	≥2.00	≥2.00	≥2.00
Fresh Extract	100%	≥2.00	≥2.00	≥2.00	≥2.00
	50%	≥2.00	≥2.00	≥2.00	≥2.00
	33%*	1.282	1.157	1.137	0.969
	16.5%	0.967	1.405	0.805	1.755
	8.25%	0.297	1.325	0.198	1.835

*Result can be considered as significant.

Table 7. Effect of plant extracts in reduction of wound size [n=6]

Day No.	*Mikania scandens* Willd.		Basic Ointment	Povidone Iodine
	Wound Size			
	Methanolic extract Ointment (MEO)	Fresh Extract (FE)		
Day3	32.89 (2.39)	25.79 (2.39)	36.77 (0.99)	23.45 (1.21)
Day5	16.29 (0.38)	13.79 (0.38)	17.88 (0.70)	11.82 (1.14)
Day7	12.09 (0.32)	8.22 (0.32)	13.12 (0.89)	6.95 (0.65)
Day9	4.69 (0.54)	2.27 (0.54)	9.67 (1.01)	1.18 (0.21)
Day11	1.50 (0.23)	0.79 (0.23)	2.73 (0.40)	0.00 (0.00)

Table 8. Haemostatic efficacy of the fresh extract of *Mikania scandens* (L) Willd. leaves [n=8]

Haemostatic agent	Bleeding time in rabbit after addition of fresh extract (seconds) [Average ±SE]
Fresh Extract	25± 1.87
Control (open Wound)	120± 2.91
Tr. Ferri. Per Chlor.	13± 2.54

Table 9. Calculation of wound healing day [n=6]

Name of the plant	Type of ointment /Extract used	Complete closure of wound surface (Days) in Punch Wound											
		3	4	5	6	7	8	9	10	11	12	13	14
Mikania scandens (L) Willd.	MEO											√	
	FE										√		
Simple ointment base	BO												√
Povidone Iodine	PIO									√			

Table 10. Calculation of wound index [n=6]

Name of the plant	Type of ointment/Extract used	Wound Index of Punch Wound in rabbit							Average Wound Index at 13th day
		1st day	3rd day	5th day	7th day	9th day	11th day	13th day	
Mikania scandens(L) Willd.	MEO	3	2	2	2	1	1	0	1.57
	FE	3	2	2	1	1	1	*	1.42
Simple ointment base	BO	3	3	2	2	2	1	1	2.0
Povidone Iodine	PIO	3	2	1	1	1	0	--	1.14

* As wound was cured on 12 th. day, so no number was allotted. The number "0" was allotted for the day of wound healing.

Table 11. Quantitative analysis of Protein, DNA, RNA and Hydroxyproline of wound tissue of rabbit treated with various extracts of *Mikania scandens* Willd. [n=6]

Plant number and date of collection	Protein (mg/g wet tissue)		DNA (mg/g wet tissue)		RNA (mg/g wet tissue)		Hydroxyproline (mg/g)	
	MEO	FE	MEO	FE	MEO	FE	MEO	FE
	Average (SEM)	Average (SEM)	Average (SEM)	Average (SEM)	Average (SEM)	Average (SEM)	Average (SEM)	Average (SEM)
Pl. 3D	2.20 (.050)	2.35 (.100)	1.20 (.050)	1.67 (.075)	0.62 (.010)	0.70 (.010)	3.62 (.025)	3.77 (.025)
Pl. 5D	2.85 (.050)	4.92 (.025)	1.87 (.075)	2.90 (.050)	0.68 (.025)	0.76 (.015)	5.92 (.025)	5.97 (.125)
Pl. 7D	5.10 (.150)	6.05 (.200)	3.27 (.025)	5.47 (.175)	1.00 (.050)	1.13 (.015)	9.30 (.050)	9.90 (.050)
Pl. 9D	6.37 (.125)	7.15 (.050)	4.87 (.025)	6.17 (.075)	1.21 (.025)	1.38 (.030)	10.30 (.050)	11.67 (.175)
BO 3D	2.15 (.050)	2.15 (.050)	1.15 (.050)	1.15 (.050)	0.44 (.030)	0.44 (.030)	3.62 (.275)	3.62 (.275)
BO 5D	2.65 (.100)	2.65 (.100)	1.75 (.100)	1.75 (.100)	0.51 (.010)	0.51 (.010)	5.55 (.050)	5.55 (.050)
BO 7D	4.92 (.025)	4.92 (.025)	3.07 (.125)	3.07 (.125)	0.78 (.010)	0.78 (.010)	9.37 (.325)	9.37 (.325)
BO 9D	6.00 (.100)	6.00 (.100)	4.70 (.150)	4.70 (.150)	0.91 (.020)	0.91 (.020)	9.97 (.175)	9.97 (.175)
PIO 3D	4.35 (.100)	4.35 (.100)	1.80 (.050)	1.80 (.050)	0.73 (.045)	0.73 (.045)	6.37 (.175)	6.37 (.175)
PIO 5D	5.27 (.175)	5.27 (.175)	3.17 (.075)	3.17 (.075)	0.86 (.040)	0.86 (.040)	10.90 (.200)	10.90 (.200)
PIO 7D	7.25 (.300)	7.25 (.300)	6.00 (.100)	6.00 (.100)	1.28 (.035)	1.28 (.035)	11.72 (.475)	11.72 (.475)
PIO 9D	8.47 (.325)	8.47 (.325)	6.77 (.075)	6.77 (.075)	1.53 (.035)	1.53 (.035)	13.67 (.175)	13.67 (.175)

Table 12. Qualitative biochemical analysis of succulent leaf extract of *Mikania scandens* Willd. [n=3]

S. No.	Phytochemical	Test	Result
1.	Alkaloids	Hager's Test	+
2.	Carbohydrates	Molish's Test	+
3.		Benedict's Test	+
4.	Glycosides	Borntrager's Test	+
5.	Saponins	Shaking test	+
6.	Proteins and Amino Acids	Millon's Test	+
7.	Phenolic Compounds and Tannins	Ferric Chloride Test	+

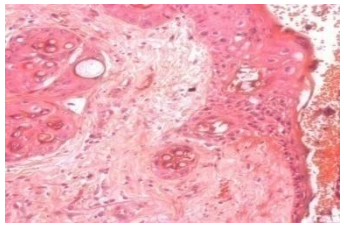
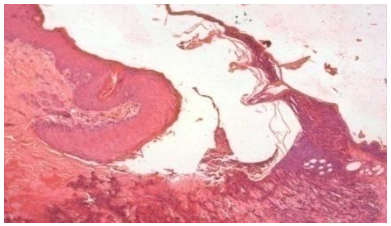
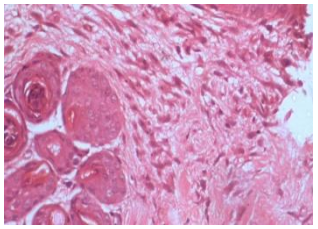
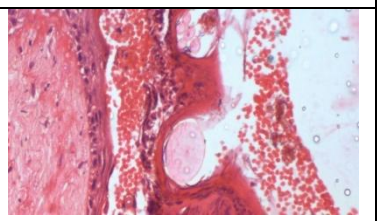
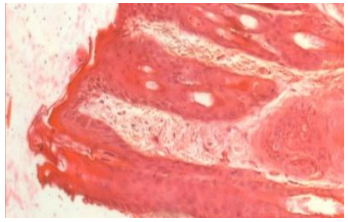
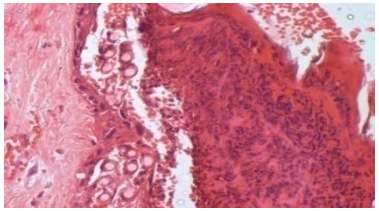
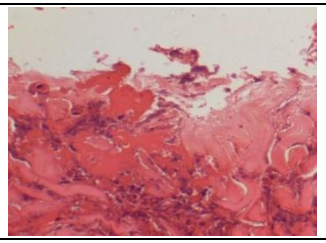
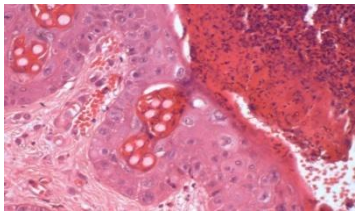
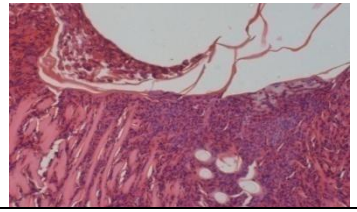
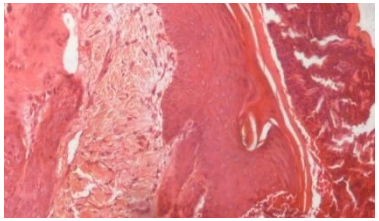
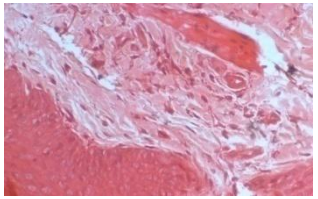
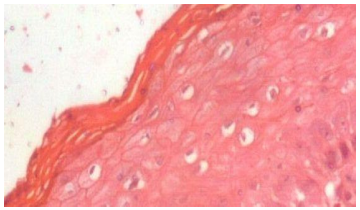
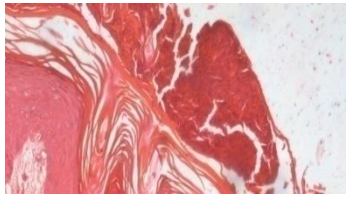
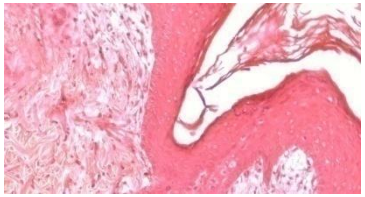
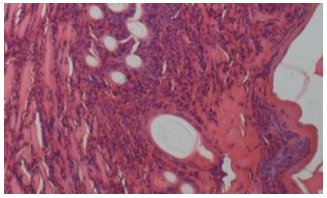
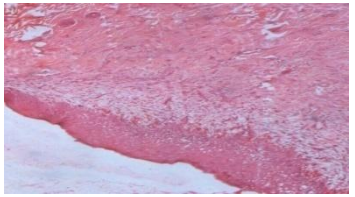
Day of sample collection	MEO treated wound	FE treated wound	BO treated wound	PIO treated wound.
D3				
D5				
D7				
D9				

Figure 1. Effect of *Mikania scandens* Willd. on punch wound of rabbit