Pharmacological Screening of *Ampelocissus Latifolia* Leaves for Impaired Wound Healing and Antimicrobial Potential

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Abstract— Microbial infection and Wound infections are most common in developing countries because of poor hygienic conditions. Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium spp, Escherichia coli, and Pseudomonas aeruginosa are some important organisms causing wound infection. Infected wounds heal more slowly and have an increased incidence of scarring. P. aeruginosa is the predominant organism, which causes air born infection and its frequency of infection is more in burn patients. A wide range of antibiotics is available for treatment of wound infections, but they are now proved to have adverse effects in the human body, also these pathogens developed resistance to the antibiotics targeted against them. Researchers paid much attention on extracts of biologically active compounds isolated from plant species used in herbal medicine. A number of researchers worked on various parts of Ampelocissus latifolia species for a number of activities. This study focused on evaluation of methanolic extract of Ampelocissus latifolia leaves on antimicrobial and wound healing activity. The crude extraction was carried out using methanol. Antimicrobial activity of the extract was carried out against gram negative bacteria (Escherichia coli) and gram positive bacteria (Staphylococcus aureus) by disc diffusion assay. The crude extract was prepared in 5% (w/w) and 10% (w/w) ointment and evaluated for wound healing activity using excision wound models in rats. The methanolic extracts of Ampelocissus latifolia were found to possess significant wound-healing activity, which was evidenced by increase in the rate of wound contraction. The present study has demonstrated that the methanolic extract of A. latifolia have properties that render them capable of promoting accelerated wound-healing activity compared with placebo control.

Indexed Terms— Wounds healing, Ampelocisus latifolia, Antimicrobial, Microbial infection, Wound infections

I. INTRODUCTION

Over the past 50 years, there has been a great diversity of new drugs developed using high-throughput screening methods and combinatorial chemistry; however, natural products and their derived compounds have continued to be highly important components in pharmacopoeias. Therefore, there is great potential for future discoveries from plants and other natural products which, thus, offer huge potential in deriving useful information about novel chemical structures and their new types of action related to new drug development. Conditions required for wound healing Systemic factors. These include good nutritional status and general health. Infection, impaired immunity, poor blood supply and systemic conditions, e.g. diabetes mellitus and cancer, reduce the rate of wound healing. Local factors that facilitate wound healing include a good blood supply to provide oxygen and nutrients and remove waste products, and freedom from contamination by, e.g., microbes, foreign bodies or toxic chemicals.

All studies to be found antimicrobial and wound healing activity of Ampelocissusla tifolia leaves extract by using excision wound model on experimental animal Albino wistar rats total of 24 animals were selected and divided into four groups. Where as a control , blank ointment and as a standard 1 % w/w soframycin used. A number of researchers worked on various parts of *Ampelocissus latifolia* species for a number of activities. The literature study showed that *Ampelocissus latifolia* species is used for various aliment including antimicrobial and wound healing activity. various part of this plant have been explored for antimicrobial and wound healing activity but there was a gap on the study of various extracts of leave of *Ampelocissus latifolia* species as a part of active material. The present study is

an attempt to explore the antimicrobial and wound healing activity of *Ampelocissus latifolia* leaves extracts.

II. MATERIAL AND METHOD

Table 1: list of reagent and chemicals

S.No.	Reagents and chemicals	Company Name
1.	Glacial Acetic Acid	Clorofilt ind
2.	Petroleum ether	Clorofilt ind
3.	Conc. H_2SO_4	Clorofilt ind.
4.	Ethanol	Clorofilt ind
5.	Nitroprusside	Fisher scientific
6.	Sodium Hydroxide	Merk
7.	Ammonia	Merk
8.	95% Alcohol	Clorofilt ind
9.	Conc. HCl	Clorofilt ind
10.	Magnesium	Himedia
11.	Chloroform	Clorofilt ind.
12.	1% Copper Sulphate Solution	Rankeem

Procurement of plant material *Ampelocissus latifolia* Leaves

Depending upon the literature survey report and traditional claim in various communities, the plant *Ampelocissus latifolia leaves* was chosen for current work. Plant materials were collected from the local area of Bhopal (M.P.) India.

- Extraction of plant material by various methods

In present study, plant material was extracted by continuous hot percolation method using Soxhlet apparatus. Powdered material of *Ampelocissus latifolia leaves* was placed in thimble of soxhlet apparatus. Soxhlation was performed at 60°C using petroleum ether as non-polar solvent. Exhausted plant material (marc) was dried and afterward re-extracted with methanol solvent. Soxhlation was continued till visual colour change was observed in siphon tube and completion of extraction was confirmed by absence of any residual solvent, when evaporated. Obtained extracts was evaporated using rotary vacuum evaporator (Buchitype) at 40°C. Dried extract was weighed and percentage yield for each extract was determined using formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract Weight}}{\text{of Plant Material used}} \times 100$$

- Qualitative Phytochemical Estimation of Extracts

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in *Ampelocissus latifolia* extract by using standard procedures**.** The extracts prepared in Petroleum ether,Ethyl acetate and Methanol were subjected to testing.

- Quantitative Phytochemical Estimation

- TPC

Folin-Ciocalteu method was used for the quantitative estimation of Total Phenolic Content (TPC). As a reference standard Gallic acid was used in different concentrations (20-100 g/ml) in ethanol.

- TFC

By using colorimetric assay, quantitative estimation of Total Flavonoid Content (TFC) was determined. The reference standard used for the estimation and plotting of calibration curve is Rutin. Different concentrations of rutin (20-100g/ml) were prepared in ethanol.

In-vitro antimicrobial activity

Preparation of Nutrient media

- 28 g of nutrient agar powder was dissolved in 1 litre of distilled water.
- pH of media was checked before sterilization.
- Media was sterilized in autoclave at 121°C at 15 lbs pressure for 15 minutes
- After sterilization, media was allowed to cool but not solidify
- Poured nutrient media into plate and placed them in the laminar air flow until the agarwas solidified.

Formulation of herbal ointment

Simple ointment bases were prepared using hard paraffin, cetostearyl alcohol, white soft paraffin, and wool fat according to the British Pharmacopoeia. All the ingredients were melted together and stirred until

it was cold. Twotypes of ointment formulations were prepared from theextract: 5% w/w and 10% w/w.

In-vivo wound healing activity

Animals were selected randomly from animal house of Pinnacle Biomedical Research Institute (PBRI), Bhopal, India and further divided into various treatment groups randomly and kept in propylene cage with sterile husk as bedding. Relative humidity of 30.7 % at 22±2°C and 12:12 light and dark cycle was maintained in the animal house and fedwith standard pellets (Golden Feeds, New Delhi, India) and water was available *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. Separate group (n=6) of rats was used foreach set of experiments. Animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal.

Wound creation (Excision wound model)

Total of 24 animals were selected and divided into four groups each containing six animals. They were anaesthetized with slight vapour inhalation of anaesthetic ether in anaesthesia chamber. Thedorsal surface of animals was shaved and full skin thickness was excised from the sterile dorsal marked area to get a wound measuring about 1 cm diameter. The animals were placed singly in individual cages. The wound was remained open environment. Wounds were left open and the ointment was applied topically twice a day (once in the morning and evening) onto each rat for 15 days . The contraction of wound was expressed as percentage of the reduction in wound size.Percentage of wound contraction was measured using the formula given below

$$\text{Percentage of wound contraction} = \frac{\text{Initial wound area} - \text{Specific day wound area}}{\text{Initial wound area}} \times 100$$

Table 2: Design of experiment

S.No.	Groups	Number of animals	Dose
1	Control	6	Blank ointment
2	Standard	6	1% w/w Soframycin
3	*Ampelocissus latifolia* methanolic extract	6	ıtment of A.L. extract
4	*Ampelocissus latifolia* methanolic extract	6	ıtment of A. L. extract

III. RESULT AND DISCUSSION

Table 3: Percentage yield of *Ampelocissus latifolia* extract:

S. No.	Solvent	Colour ofextract	Weight of Plant material (gms)	Weight of extract (gms)	% yield
1.	Petroleu mether	Light yellow	100.20	0.425	0.424
2.	Methano l	Dark yellow	97	4.66	4.804

Qualitative Phytochemical Screening of *Ampelocissus latifolia*

Table 4: Phytochemical evaluations

No.	periment	Results	
		Pet Ether Extract	Methanolic extract
	Test for Carbohydrates		
1	Molisch's Test	-ve	+ve
2	Fehling's Test	-ve	+ve
3	Benedict's Test	-ve	+ve
	Test for Protein & Amino acids		
4	Biuret's Test	-ve	+ve
5	Ninhydrin Test	-ve	+ve
	Test for Glycosides		
6	BorntragerTes t	-ve	+ve
7	Killer killaniTest	-ve	+ve
	Test for Alkaloids		
8	Mayer's Test	-ve	+ve
9	Hager's Test	-ve	+ve

10	Wagner's Test	-ve	+ve
	Test for Saponins		
11	Froth Test	-ve	-ve
	Test for Flavonoids		
12	Lead acetate	-ve	+ve
13	Alkaline reagent test	-ve	+ve
	Test for Triterpenoids and Steroids		
14	Libermann-Burchard Test	+ve	+ve
15	Salkowski Test	+ve	+ve
	Test for Tannin and Phenolic Compounds		
16	Ferric Chloride Test	-ve	+ve
17	Gelatin Test	-ve	+ve
18	Lead Acetate Test	-ve	+ve

On phytochemical screening of methanolic extract of *Ampelocissus latifolia leaves* showed the presence of alkaloid, flavonoid, steroids and glycoside.

- Quantitative Analysis

Preliminary phytochemical testing of crude extracts confirmed the presence of phenolics and flavonoids in plant material. To estimate their amount total phenolic (TPC) and total flavonoid content (TFC) assays were performed.

Total Phenolic content (TPC) estimation Table 5 Standard table for Gallic acid

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.166
2.	40	0.245
3.	60	0.375
4.	80	0.468
5.	100	0.537

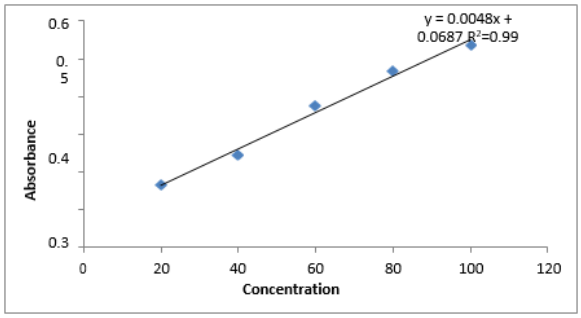


Figure 1: Graph represent standard curve of Gallic acid

Table 6: Total Phenolic Content in extract

S.No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.174	24 mg/gm
2	0.157	
3	0.163	

Table 7-Total Phenolic Content of extract *Ampelocissus latifolia*

Extracts	Total Phenolic content (mg/gm equivalent ofGallic acid)
Methanol	24

Total Flavonoids content (TFC) estimation Table 8Standard table for Rutin

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.137
2.	40	0.158
3.	60	0.216
4.	80	0.365
5.	100	0.476

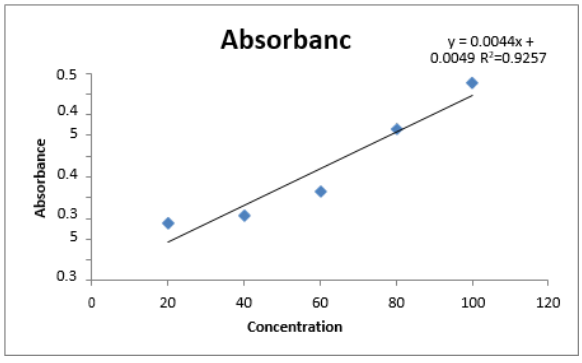


Figure 2: Graph represent standard curve of Rutin

Total Flavonoid Content in extract Table 9:Total Flavonoid Content

S.No	Absorbance	TFC in (mg/gm) equivalent of Rutin
1	0.245	
2	0.265	
3	0.248	62 mg/gm

Table 10: Total Flavonoid Content of extract *Ampelocissus latifolia*

Extracts	Total Flavonoid content (mg/gm equivalent ofGallic acid)
Methanol	62.00

In-vitro antimicrobial activity

Table 11: *In-vitro* antimicrobial activity of *Ampelocissus latifolia leaves*extract against gramnegative bacteria (*Escherichia coli*) and gram positive bacteria (*Staphylococcus aureus*)

Bacterial strain	Different concentrations of *Ampelocissus latifolia* extract			
	25µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Escherichia coli (Gram negative)	0mm	7mm	8mm	12mm
Staphylococcus aureus (gram positive)	7mm	9mm	10mm	12mm

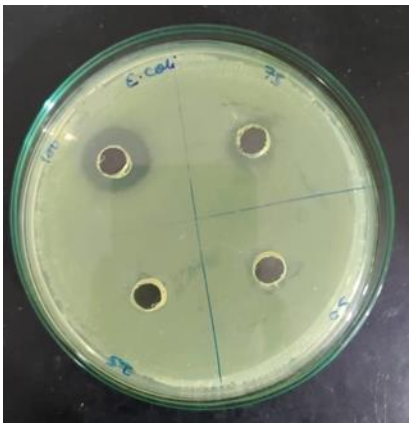


Figure 3: Zone of inhibition of *Ampelocissus latifolia* extract against gram negative bacteria

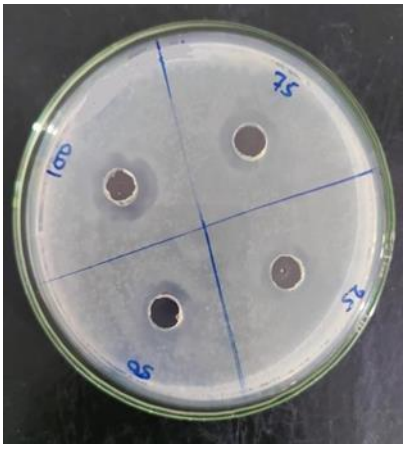


Figure 4: Zone of inhibition of Ampelocissus latifolia extract against gram positive bacteria

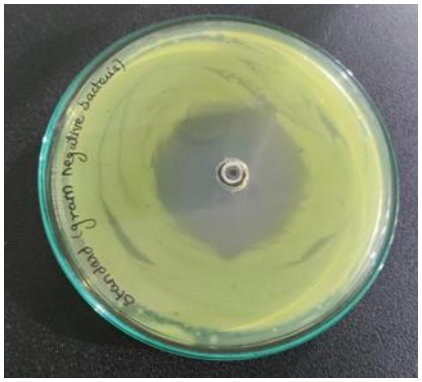


Figure 5: Zone of inhibition of standard (Gentamycin) against gram negative bacteria

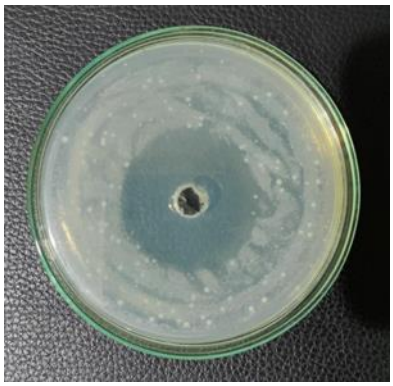


Figure 6: Zone of inhibition of standard (Ofloxacin) againstgram positive bacteria

Wound contraction studies for *Ampelocissus latifolia*

Circular excision wound (1 cm diameter) was surgically created on the dorsal side of rat. *Ampelocissus latifolia* extract samples were topically applied on the wound twice daily. The progression of healing wasmonitored by measuring the wound area. Results are expressed as percentage of wound closure.

Table 12: Percentage wound closure in various treatment groups

Groups	% Wound closure				
	Day 3	Day 6	Day 9	Day 12	Day 15
Control	12.05±1.5	32.75±1.46	68.49±2.6	80.95±2.4	83.24±1.8
Standard (soframycin)	58.46±2.8	68.24±1.4	84.28±2.9	92.85±2.7	99.07±1.6
ALE (5 % ointment)	35.85±2.2	60.85±3.0	81.85±2.8	88.49±1.8	93.85±3.2
ALE (10% ointment)	54.22±1.2	62.75±1.6	82.46±3.2	89.76±1.5	94.28±1.9

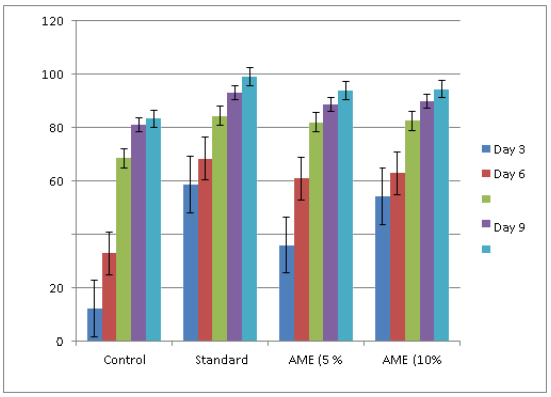
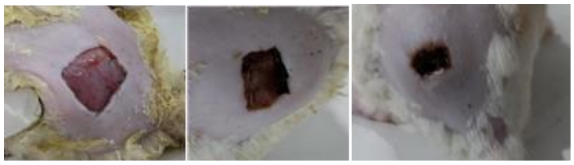
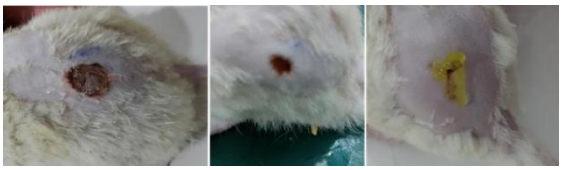


Figure 7: Excision wound healing activity of methanolic extract of *Ampelocissus latifolia*

Images of wound closure



(a) (b) (c)



(d) (e) (f)

Figure 8: Images showing wound closure after applying 10% ALE extract (a) Day 1 (b)Day 3 (c) Day 6 (d) Day 9 (e) Day 12 (f) Day 15

SUMMARY AND CONCLUSION

Antimicrobial activity of AM extract determined that the extract inhibited the growth of microbes against the gram positive (*Staphylococcus aureus*) and gram negative strains (*Escherichia coli*). A significant zone of inhibition was recorded against two selected pathogenic bacterial strains.

Further we were performed the wound healing studies like contraction of wound model for 15 days. For wound healing activity, the extract was loaded in the ointment with the use of hard paraffin, cetostearyl alcohol, white soft paraffin, and wool fat. The development wound curing activity by excision model was evaluated by wound shrinkage of the excision wound of different groups viz treated with blank ointment base control group, Standard group treated with 10% (w/w) soframycin ointment act as a references group and also the analysis of treated group with the methonolic extract of *Ampelocissus latifolia* 5 and 10% ointment.

At the lowest concentration of 5% w/w extract ointment, significant contraction similar to that ofsoframycin (standard) was started on the 4th day. Better contraction similar with the standard was

recorded at higher concentrations of the extract ointment (10% w/w). Excision wound contraction was measured and expressed as percentage of reduction in wound size. Application of ALE ointment on marked area exhibited statistically symbolic contraction of wound analysis when compared to control simple ointment base group. Rats showed normal healing process withsigns of improvement at weekly intervals and this was determined by their contraction rate. A dose dependent effect was observed in rats treated with ointment

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