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"PHYTOCHEMICAL INVESTIGATION & PHARMACOLOGICAL EVALUATION OF ANTIMICROBIAL AND WOUND HEALING ACTIVITY OF *BARLERIA PRIONITIS* LEAVES EXTRACTS"

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ABSTRACT

Barleria prionitis is a famous perennial plant commonly known as porcupine flower or Vajradanti. It is a shrub with yellow flowers, inhabit most parts of India. Various parts of the plant such as leaves, roots, aerial parts, flowers, and stems are used in the traditional system of medicine. The objective of the study was undertaken to investigate the antimicrobial and wound healing activity of Barleria prionitis leaves extract . The leaves of Barleria prionitis were collected, processed & standardized as per official methods. The extracts of Barleria prionitis leaves were extracted and studied to detect the chemical compounds present by defattying with Petroleum-ether60-800C then successive extraction was done by Ethylacetate & Methanol. Qualitative phytochemical screening revealed the presence of carbohydrates, proteins, flavonoids, tannins, alkaloids & steroids. Total polyphenol content & total flavonoid content was determined using UV-Visible spectrophotometer. DPPH scavenging assay were performed to evaluate the antioxidant capacity of all extracts & they showed maximum antioxidant activity. Then these extracts used to evaluate acute oral toxicity, antioxidant, antimicrobial and wound healing activity. The evaluation of in-vitro antimicrobial and in vivo wound healing activity in rodents was done using various experimental models. Excision model methods were performed to evaluate the wound healing activity by using 1% as well as 2% methanolic extracts ointments and 1% as well as 2% ethyl acetate extracts ointments , in both the extract ointments the rats showed the decreased epithelial size of wound on post wounding days for both 1% as well as 2% methanolic extract and 1% as well as 2% ethyl acetate leaves extract compared to standard group. Along with this the antimicrobial activity was done by using both extracts. In the evaluation of antimicrobial activity , it was found that the Barleria prionitis leaves extracts exhibit significant Antibacterial activity on microorganisms like Bacillus substillus, Staphylococcus aureus & E.coli. The present study shows that Barleria prionitis leaves extract have better wound healing and antimicrobial activity.

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INTRODUCTION

Barleria prionitis Linn (Acanthaceae) is widely distributed throughout Africa, India, Sri Lanka and tropical Asia. It is commonly known as Vajradanti, the juice of the leaf is used in cataract and fever. The dried bark is used in cough treatment and the leaves chewed to relieve toothache. The paste of the root is applied to disperse boils and glandular swellings. It exhibits several medicinal properties. The leaves are chewed to relieve toothache. Juice of the leaves is used in ulcer and fever. Paste of the roots is applied to disperse boils and glandular swellings. Leaves area used by some tribal communities for the treatment of piles and to control irritation. Plant is also used in stiffness of limbs, enlargement of scrotum and sciatica. In recent years multiple drug resistance has developed due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious diseases. *Barleria prionitis,* a perennial, acanthaceous, barbed, bushy medicinal plant, including in Barleria genus containing 300 species is famous for its medicinal value from ancient time.

MATERIALS& METHODS

Collection, Identification & Authentication-

Collection, authentication, Identification, processing and storage has been done according to standard procedure for the plant material

Processing of Crude Drug

The collected dried leaves of plant was segregated and pulverized by mechanical grinder and the powder was passed through appropriate sieve and subjected to extraction. Powdered drug is stored in air tight container for further use.

Pharmacognostic evaluation of plant material

Microscopic Characters of leaves

Color : Green
Odor : Slightly aromatic
Taste :Mucilaginous
leaves : Greenish to yellow in colour
flower: Yellow in colour

Photo no.1: Leaves of *Barleria prionitis* Linn.

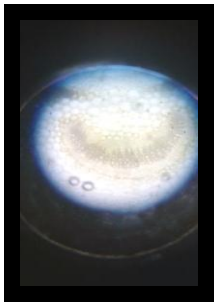


Photo no. .2 : T. S. of *Baleria prionitis* Linn. Leaves

Physicochemical Parameters:

The powdered drug was evaluated for its physicochemical parameters like total ash values, acid insoluble ash, water soluble ash &loss on drying. All the results are tabulated in following table.

Determination of Total Ash Content:

2 gm of the air dried crude drug weighed in a tarred silica dish and incinerated at a temperature not exceeding 450^{0}C until free from carbon. After incineration the material was cooled and weighed. The percentage of ash value was calculated with reference to air dried drug. The physiological parameters obtained from plant is shown in **Table no.1.**

Determination of Acid Insoluble Ash Value:

2 gm of the air dried crude drug weighed in a tarred silica dish and incinerated at a temperature not exceeding 450^{0}C until free from carbon. After incineration the material was cooled and boiled with 25 ml of 2 M hydrochloric acid for 5 minutes. The insoluble matter was collected in a Gooch crucible or on ash less filter paper. The collected insoluble matter was washed with hot water, ignited and cooled in a desiccator and weighed. The percentage of acid-insoluble ash value was calculated with reference to air dried drug.

Determination of Water Soluble Ash Value:

2 gm of the air dried crude drug weighed in a tared silica dish and incinerated at a temperature not exceeding 450^{0}C until free from carbon. After incineration the material was cooled and boiled with 25 ml of water for 5 minutes. The insoluble matter was collected in a Gooch crucible or on ash less filter paper. The collected insoluble matter was washed with hot water, ignited and cooled in a desiccator and weighed. The percentage of water soluble and water-insoluble ash value was calculated with reference to air dried drug.

Extraction of plant material

Selection of Solvent:

As per literature review &the nature of phytochemicals present in drug as well as on the basis of their polarity, the solvents were selected for the extraction of the leaves of *Barleria prionitis*Linn. Like Petroleum-ether (60-80^{0}C), ethyl acetate, methanol. Extraction of the crude drug is shown in **Photo no.3.**

Selection of Extraction method:

According to the literature survey & nature of phytochemicals present in drug, the extraction method was selected. The extraction method selected for extraction from the leaves of *Barleria prionitis* Linn. was continuous hot extraction method using soxhlet apparatus. The method was selected for its efficiency (U, Ahmed F, Khanojia P, Kukreja K, Kumari S, Bhat RA.2016)

Material used:

Soxhlet apparatus, Heating mental, Powdered drug, Petroleum-ether (60-80^{0}C), ethyl acetate, methanol.

Procedure:

Extraction of *Barleria prionitis* Linn.leaves was carried out by continuous hot extraction method in Soxhlet extractor. Firstly 1000 gm of course powder of BPL was extracted with 3000 ml of Petroleum-ether (60-80^{0}C) according to the standard method till colorless solution was observed in siphon tube.After completion of extraction, extract was collected, cooled & solvent was allowed to evaporate in order to get ethyl acetate extract. Percentage yield of all the extracts were calculated by using following formula and it is represented in **Table no.2**

$$\% \text{ yield} = \frac{\text{Weight of extract obtained after extraction}}{\text{Weight of powder drug used for extraction}} \times 100$$

Phytochemical Screening of Extracts

Phytochemical Qualitative analysis

Qualitative chemical tests were carried out for all four extracts to identify the presence of various chemical constituents and presented them in **Table No.3.**

Development of TLC fingerprint:

Development of TLC fingerprints profile of the extracts:

All the extracts of selected plant material were subjected to TLC studies using various solvent systems to determine the presence of various phytoconstituents. R_f value of the separated compounds were recorded and given in **Table No.4.**and some images of TLC are given in **Photo no.3**

Total Polyphenolic Content

Total phenol content of the extracts was determined by using the Folin-Ciocalteu method. 4 ml of Folin Ciocalteu reagent was mixed with 1 ml of extract solution, this solution mixture was kept on standing for 5 min & then 5 ml of sodium carbonate was added to it. The absorbance of reaction mixture was measured against blank (without extract) at 765 nm using UV-Visible spectrophotometer. Gallic acid was used as standard for determination of total polyphenol content of extract. The calibration curve was drawn using various concentrations of gallic acid (50, 100, 150, 200, 250 µg/ml).it is shown in **Photo no.6.** The total polyphenol content was expressed as gallic acid equivalent in mg/g of the extract & was calculated by using following equation obtained from standard gallic acid graph **(r^2 = 0.9918)** Results are given in **Table no.5 and table no.6.** **{Absorbance (y) = mx + c}**

Total Flavonoid Content

Principle:

Aluminium trichloride colorimetric method was used for the determination of total flavonoid content of extracts which showed positive test for flavonoids.This method is based on the nitration of any aromatic ring bearing a catechol group.1 ml of extract solution was mixed with 4 ml of distilled water & 0.3 ml of $NaNo_2$. After 5 min 0.3 ml of $AlCl_3$& 2 ml of NaOH was added, at last total volume was made up to 10 ml with distilled water. The solution was mixed well & absorbance of the solution mixture was measured at 510 nm against prepared blank (without extract). Rutin was used as standard for determination of total flavonoid content of extracts. The calibration curve was drawn using various concentrations of rutin in **Photo no.7.** (100, 200, 300, 400, 500 µg/ml). The total flavonoid content was expressed as rutin equivalent in mg/g of the extract & was calculated by using following equation obtained from standard rutin graph **(r^2 = 0.9964)**Results are given in **Table no.7 and Table no.8.** **{Absorbance (y) = mx + c}**

Pharmacological Screening of Plant Extracts

ANTI-OXIDANT SCREENING

Determination of DPPH Scavenging Activity

In DPPH scavenging activity, all the four extracts showed decrease in absorbance & increase in % inhibition as the concentration of extract was increased. All three extracts showed better activity at 50µg/ml& Ethyl acetate extract of BPL has the maximum anti-oxidant activity compared to ME-BPL shown in **chart no.1.**

EVALUATION OF WOUND HEALING ACTIVITY

IAEC Approval:

All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) following the guidelines of CPCSEA.The studies were performed with the approval of Institutional Animal Ethics Committee (IAEC) Protocol Approval No. R-5: X dated 23/12/2016

Weight:

Rats of 180-250 gm

Route of administration:

Standard- topical route of administration

Test –topical route of administration

Housing Condition:

Animals were housed in a group of six animals in separate cages under controlled conditions of temperature (22±2^{0}C - 25±2^{0}C) & 12 h light-dark cycle. All animals were provided with free access to food & water.

EXCISION MODEL

Principle:

This method has been described by Mortan and malon*et al.* (1972) as a facile mean for evaluating potential of wound healing.

Animal Grouping:

The animals were randomly divided into seven groups of six animals, each namely-

1) Group I (Positive Control)
2) Group II (Negative control)
3) Group III (Standard)
4) Group IV (Methanol extract 1%)
5) Group V (Methanol extract 2%)
6) Group VI (Ethyl acetate extract 1%)
7) Group VII (Ethyl acetate extract 1%)

Positive Control: Simple ointment base
Negative control: without base
Standard: Framycetin sulphate cream
Test Dose-I: MT-BPE 1%
Test Dose-II: MT-BPE 2%
Test Dose-III: EA-BPE 1%
Test Dose-IV: EA-BPE 2%

Procedure:

- Rats were anesthetized with ketamine (30 mg/kg, ip) or ether and an area of about ≈500 mm2 was marked on the back of the rat by a standard ring.
- Full thickness of the marked skin was then cut carefully.
- Wounds were traced on 1 mm2 graph paper on the day of wounding and subsequently at a gap period of 3^{rd,} 6th ,9th ,12th ,15th days till 18th, then on the alternate days until healing was completeChanges in wound area were measured. Significance in wound healing of the test groups is derived by comparing healed wound area on respective days with healed wound area of control group The wound closure was calculated.

Evaluation:

Changes in wound area were measured And it is represented in Photo no. 6. Significance in wound healing of the test groups is derived by comparing healed wound area on respective days with healed wound area of control group The wound closure was calculated and Average results of all groups of *Barleria prionitis* linn leaves extract treated or non treated on post wounding days on rats represented in Table no.11.and it was compared with control.

On 3rd day, the group of animal treated with standard ointment i.e. soframycin showed significant decrease in wound area when compared with positive control group. The test groups i.e. BPL-EA 1%, BPL-EA2%, BPL-M1% and BPL-M2% showed highly significant decrease in wound area when compared with positive control group. Effect of extract of *Barleria prionitis*; Linn.leaves on 3rd day of wound healing activity represented in Chart no.2.

On 6th day, the group of animal treated with standard ointment i.e. soframycin showed significant decrease in wound area when compared with positive control group.The test groups i.e. BPL-EA 1%, BPL-EA2%, BPL-M1% and BPL-M2% showed highly significant decrease in wound area when compared compared with the standard except BPL-EA 1% showed no significant decrease in wound area .Effect of extract of *Barleria prionitis*; Linn.leaves on 6th day of wound healing activity represented in Chart no.3.

On 9th day, the group of animal treated with standard ointment i.e. soframycin showed significant decrease in wound area when compared with positive control group. The test groups i.e. BPL-EA 1%, BPL-EA2%, BPL-M1% and BPL-M2% showed highly significant decrease in wound area when compared with positive control group. . The test groups i.e. BPL-EA 1%, BPL-M1% and BPL-M2% showed no significant decrease in wound area when compared compared with the standard except BPL-EA 2% it showed significant decrease in wound area . Effect of extract of *Barleria prionitis*; Linn.leaves on 9th day of wound healing activity represented in Chart no.4.

On 12th day, the group of animal treated with standard ointment i.e. soframycin showed significant decrease in wound area when compared with positive control group. The test groups i.e. BPL-EA 1%, BPL-EA2%, BPL-M1% and BPL-M2% showed highly significant decrease in wound area when compared with positive control group.

The test groups i.e. BPL-EA 1%, BPL-EA2% and BPL-M2% showed highly significant decrease in wound area when compared compared with the standard except BPL-M1% it showed significant decrease in wound area . Effect of extract of *Barleria prionitis*; Linn.leaves on 12th day of wound healing activity represented in Chart no.5.

On 15th day, the group of animal treated with standard ointment i.e. soframycin showed significant decrease in wound area when compared with positive control group. The test groups i.e. BPL-EA 1%, BPL-EA2%, BPL-M1% and BPL-M2% showed highly significant decrease in wound area when compared with positive control group. . The test groups i.e. BPL-EA 1%, BPL-EA2% ,BPL-M1%and BPL-M2% showed highly significant decrease in wound area when compared compared with the standard. Effect of extract of *Barleria prionitis*; Linn.leaves on 15th day of wound healing activity represented in Chart no.6.

On 18th day, the group of animal treated with standard ointment i.e. soframycin showed significant decrease in wound area when compared with positive control group. The test groups i.e. BPL-EA 1%, BPL-EA2%, BPL-M1% and BPL-M2% showed highly significant decrease in wound area when compared with positive control group. . The test groups i.e. BPL-EA2% and BPL-M2% showed highly significant decrease in wound area when compared compared with the standard except BPL-EA 1%, BPL-M1% it showed significant decrease in wound area . Effect of extract of *Barleria prionitis*; Linn.leaves on 18th day of wound healing activity represented in Chart no.7.

From above observation it was found that the *Barleria prionitis leaves* extracts exhibit significant wound healing activity.

STATISTICAL ANALYSIS:

The results were subjected to statistical analysis by using ANOVA followed by Dunnett's test to calculate the significance difference if among the groups. The values of $P<0.001$ were considered statistically highly significant represented in Chart no.2, Chart no.3, Chart no.4, Chart no.5, Chart no.6, Chart no. 7.

ANTIBACTERIAL ACTIVITY

Bacterial strains

Escherchia coli ,*Staphyllococcus aureus* , *Bacillus subitilus*,t hese were strains used for anti bacterial activity. The Bacteria were incubated on a nutrient agar-slant for 48 hours at 37 degree Celsius.

Evaluation for Antibacterial Activity

A loopful of bacteria was taken from the stock culture and dissolved in O.1ml of saline. All the tests were done by well diffusion method.Various crude solvent extracts are impregnated with discs and placed on Nutrient Agar plates. These plates are already inoculated with 20 ml of Nutrient broth medium with Gram positive and Gram negative bacteria. Respective solvent without plant extracts served as negative control. Standard antibiotics were used as reference or positive control. Plates were incubated at 37 degree Celsius for 24 hours. After the incubation period, the diameter of the inhibition zone around the leaf extracts were measured and also compared with the diameter of inhibition zone of commercial standard antibiotics. Antibacterial activity of *Barleria prionitis* Linn.leaves extract against bacterial strains represented in Photo no.7 and percentage of zone inhibition is shown in Table no 12 and Table no.13. From above observation it was found that the *Barleria prionitis leaves* extracts exhibit significant Antibacterial activity. In the Ethyl acetate extract having better antibacterial activity on *Bacillus subs illus* than *Staphylococcus aurous* & *E.coli.* In the Methanol extract having better antibacterial activity against *Bacillus subs illus* & *Staphylococcus aurous* than *Ecolab.*

OBSERVATIONS & RESULTS

Pharmacognostic Evaluation

:

Table No.1: Physicochemical Parameters Of *Barleria Prionitis;* Linn. Leaves

Sr. No.	Standardization parameters	Results
1	Total Ash	6.2 %
2	Acid insoluble Ash	0.90%
3	water soluble Ash	0.90%
4	LOD	10%

Extraction of *Barleria prionitis;* Leaves

Table No. 2: Physical Properties Of *Barleria Prionitis;* Leaves Extracts.

Sr	Extracts	Colour	Consistency	Net Weight	%y
1	PE-BPL	Dark Green	Sticky	8.32 gm	5.54 w/w
2	EA-BPL	Yellowish Brown	Sticky	5.46 gm	3.64 w/w
3	MT-BPL	Brownish black	Sticky	4.73 gm	3.15 w/w

Phytochemical Screening

Table No.3:Phytochemical Analysis Of Extracts

Sr. No.	Phytochemicals	PE-BPL	EA-BPL	MT-BPL
1	**Alkaloids**			
	a) Dragendoff's test	-	-	-
	b) Mayer's test	+	+	+
	c) Wagner's test	-	+	+
2	**Carbohydrates**			
	a) Molisch's test	+	+	+
	b) Barfoed's test	-	+	-
3	**Fixed Oils**			
	a) Sudan Red III	-	-	-
	b) Solubility Test	-	-	-
4	**Flavonoids**			
	a) Shinoda test:	+	+	+
	›) Sulphuric acid test	+	+	+
5	**Glycosides**			
	ı) Keller-killiani test	-	+	-
	Modified Borntrager's	-	+	-
6	**Proteins**			
	a) Biuret test	-	+	-
	b) Hydrolysis test	-	+	-
7	**Saponins**			
	a) Foam test	-	-	-
	b) Heamolytic test	-	-	-
8	**Steroids**			
	Libermann-Burchard test	-	-	-
	b) Salkowski test	-	-	-

TLC Finger printing

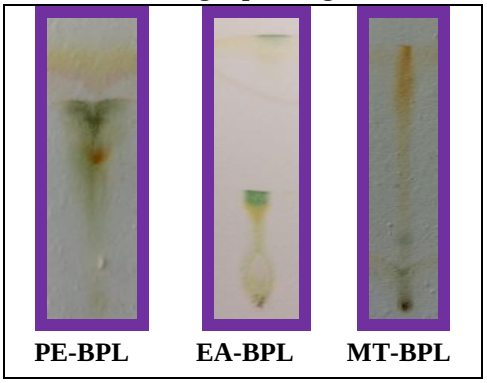


Photo No.3:TLC Finger printing of *Barleria prionitis;* Linn. Leaves extracts.

Table No.4:TLC fingerprinting of *Barleria prionitis*; Linn leaves extract with its solvent system, proportions, spraying reagents used and R_f Values.

Sr.no.	Extracts	Solvent systems	Proportions	Spraying Reagent	R_f	Color
1	PE BPL	Hexane:Chloroform:Acetic acid:Methanol	(1:2:0.1:1)	Sulphuric acid	0.52	Green
2	PE BPL	Hexane:Chloroform:Acetic acid	(1:2:0.1)	Sulphuric acid	0.66	Pink & green
3	EA BPL	Hexane:Chloroform:Acetic acid:Methanol	(1:2:0.1:1)	Sulphuric acid	0.83	Brown
4	EA BPL	Hexane:Chloroform:Acetic acid:Methanol	(1:2:0.1)	Sulphuric acid	0.46	Yellow & Green
5	MT BPL	Hexane:Chloroform:Acetic acid:Methanol	(1:2:0.1:1)	Sulphuric acid	0.76	Reddish
6	MT BPL	Hexane:Chloroform:Acetic acid:Methanol	(1:2:0.1:1)	Sulphuric acid	0.42	Brown& yellow

Total Polyphenolic content

Table No.5: Calibration Curve of Gallic acid.

Sr. No.	Concentration (µg/ml)	Absorbance
1	50	0.417
2	100	0.850
3	150	1.447
4	200	1.913
5	250	2.312

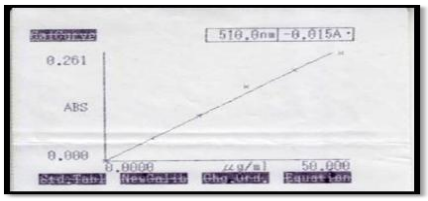


Photo No.4: Calibration Curve of Gallic acid.

Table No.6:Total Polyphenolic content of *Barleria prionitis;* Linn.leaves extracts.

Sr. No.	Extracts	Concentration (µg/ml)	Absorbance	TPC mg/g of GAE
1	PE-BPL	100	0.488	**52.47**
2	EA-BPL	100	0.439	**47.20**
3	MT-BPL	100	0.362	**38.92**

Total Flavonoid content

Table No.7: Calibration Curve of Rutin.

Sr. No.	Concentration (µg/ml)	Absorbance
1	100	0.171
2	200	0.156
3	300	0.180
4	400	0.220
5	500	0.261

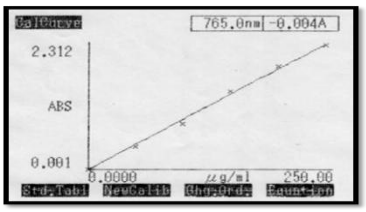


Photo No.5: Calibration Curve Rutin.

Table No.8:Total Flavonoid Content Of *Barleria Prionitis;* Linn. Leaves Extracts.

Sr. No.	Extracts	Conc (µg/ml)	Absorbance	tfc mg/g
1	PE-BPL	100	0.02933	**5.33**
2	EA-BPL	100	0.02536	**5.45**
3	MT-BPL	100	0.03000	**4.61**

Pharmacological Screening

Antioxidant Activity

Table No.9: DPPH scavenging activity of Ascorbic acid.

Sr. No.	Standard	Concentration (µg/ml)	% Inhibition
1	Ascorbic acid	10	**95.66**
2	Ascorbic acid	20	**96.32**
3	Ascorbic acid	30	**97.86**
4	Ascorbic acid	40	**98.58**
5	Ascorbic acid	50	**99.12**

Values are expressed as mean ± SEM (n = 3)

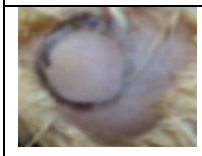
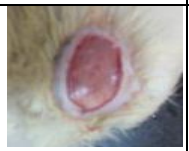
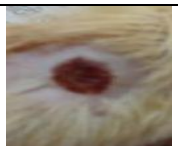
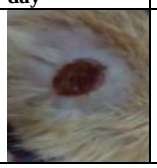
Table No.10: DPPH scavenging Activity Of *Barleria Prionitis;* Linn. Leaves Extracts.

Sr. No.	Extracts	Conc(µg/ml)	% Inhibition
1	PE-BPL	10	**24.52**
2	PE-BPL	20	**72.56**
3	PE-BPL	30	**88.97**
4	PE-BPL	40	**91.52**
5	PE-BPL	50	**96.16**
6	EA-BPL	10	**81.20**
7	EA-BPL	20	**81.84**
8	EA-BPL	30	**87.85**
9	EA-BPL	40	**92.67**
10	EA-BPL	50	**94.52**
11	MT-BPL	10	**48.77**
12	MT-BPL	20	**80.41**
13	MT-BPL	30	**83.15**
14	MT-BPL	40	**88.24**
15	MT-BPL	50	**91.10**

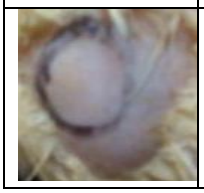
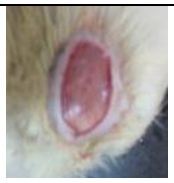
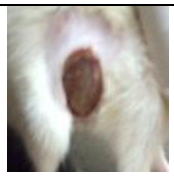
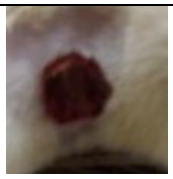
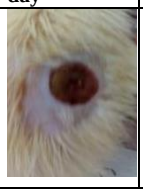
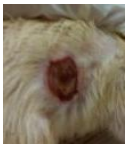
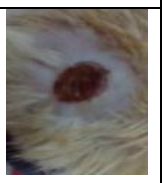
In DPPH scavenging activity, all the four extracts showed decrease in absorbance & increase in % inhibition as the concentration of extract was increased. All three extracts showed better activity at 50µg/ml& Ethyl acetate extract of BPL has the maximum anti-oxidant activity compared to ME-BPL.

OBSERVATION OF PHARMACOLOGICAL ACTIVITY OF DIFFERENT MODEL

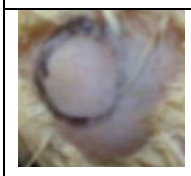
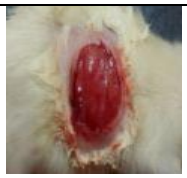
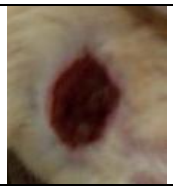
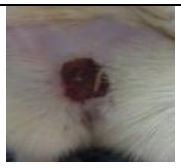
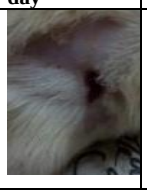
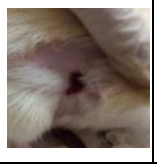
Control base treated

0 day	1st day	3rd day	6th day	9th day	12th day	15th day	18th day
							

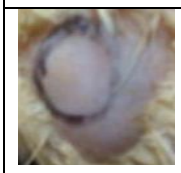
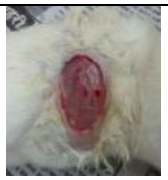
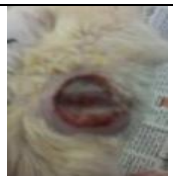
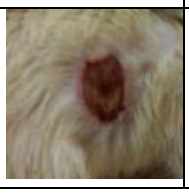
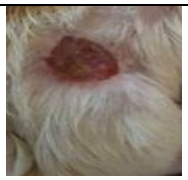
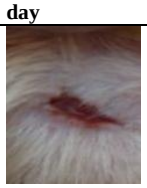
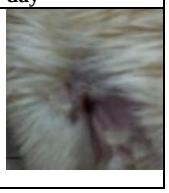
Control group without base treatment

0 day	1st day	3rd day	6th day	9th day	12th day	15th day	18th
							

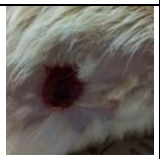
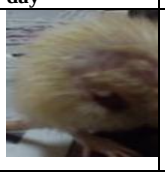
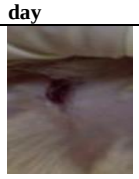
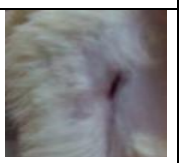
Standard drug treated

0 day	1st day	3rd day	6th day	9th day	12th day	15th day	18th day
							

Test group 1% Methanol

0 day	1st day	3rd day	6th day	9th day	12th day	15th day	18th day
							

Test group 2% Methanol

0 day	1st day	3rd day	6th day	9th day	12th day	15th day	18th day
							

Test group 1% Ethyl acetate

0 day	1st day	3rd day	6th day	9th	12th day	15th day	18th day

Test group 2% ethyl acetate

0 day	1st day	3rd day	6th day	9th day	12th day	15th day	18th day

Photo No.6: PHOTOS OF EXCISION MODEL OF WOUND HEALING.

Evaluation of wound healing activity

EXCISION MODEL

Table No.11 Average Results Of All Groups Of *Barleria Prionitis* Linn Leaves Extract Treated Or Non Treated On Post

	Post wounding days						
Rats	**0**	**3**	**6**	**9**	**12**	**15**	**18**
1.Positive control	2±0.0	1.82±0.01	1.7±0.02	1.5±0.02	1.3±0.01	1.21±0.00	1±0.01
2.Negative control	2±0.0	1.9±0.01	1.8±0.01	1.5±0.01	1.32±0.01	1.15±0.00	1±0.00
3.Standard	2±0.0	1.7±0.03#	1.3±0.04*	1±0.04**	0.94±0.00**	0.68±0.00**	0.06±0.02**
4.BPL-EA 1%	2±0.0	1.8±0.02#, #	1.5±0.02#,#	1.2±0.02**,#	0.91±0.00**,#	0.7±0.00**, #	0.1±0.03**, #
5.BPL –EA 2%	2±0.0	1.7±0.03#, #	1.1±0.10**,#,▫	1±0.04**,#,▫	0.90±0.00**,#,▫	0.1±0.08**,**,▫	0.05±0.03**, #,▫
6.BPL-ME 1%	2±0.0	1.8±0.02#,#	1.3±0.03*,#	1.2±0.03**,*	1±0.00**,#	0.49±0.00**,*,▫	0.1±0.00**, #
7.BPL-ME 2%	2±0.0	1.6±0.04#,#, ▫	1.2±0.09**,#,▫	1.1±0.02**,#	0.64±0.05**,**,▫	0.28±0.01**,**,▫	0.06±0.00**,#,▫

Wounding Days On Rats.
n=6
BPL-EA= *Barleria prionitis* leaves Ethyl acetate extract, BPL-ME= *Barleria prionitis* leaves Methanol extract.
* Significant difference when standard and test compared with positive control (P<0.05)
** Highly Significant difference when test compared with positive control (P< 0.001)
# No significant difference when test compared with standard
▫Significant difference when test compared with standard

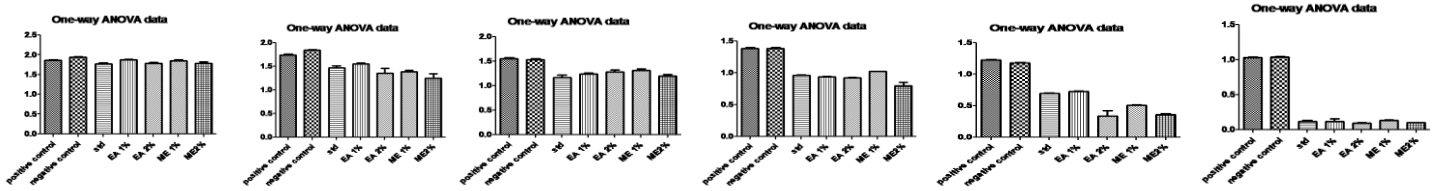


Chart No.2: Effect of extract on 3rd day of wound healing activity, Chart No.3: Effect of extract on 6th day of wound healing activity, Chart No.4: Effect of extract on 9th day of wound healing activity, Chart No.5: Effect of extract on 12th day of wound healing activity,Chart No.6: Effect of extract on 15th day of wound healing activity, Chart No.7: Effect of extract on 18th day of wound healing activity.

ANTIBACTERIAL ACTIVITY

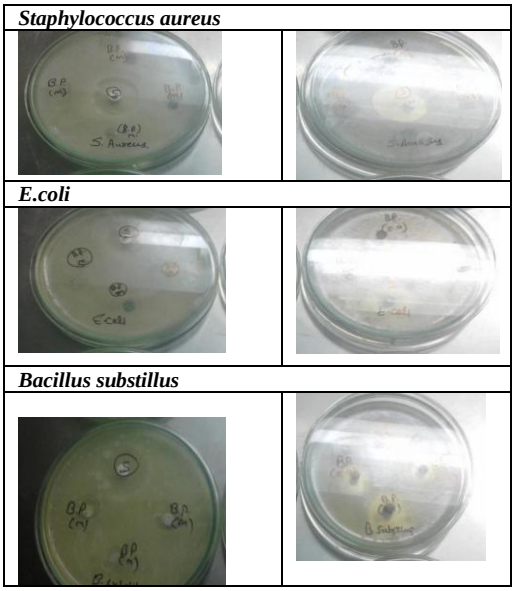


Photo No.7: Antibacterial activity *of Barleria prionitis* Linn. Leaves extract against bacterial strains.

Table no.13:Antibacterial activity of Methanolic extract of *Barleria prionitis* linn leaves.

Table no.12. Antibacterial activity of Ethyl acetate extract of *Barleria prionitis* Linn leaves.			
Sr. No.	**Tested microorganism**	**Zone of inhibition(mm)**	
		Ethyl acetate extract of *Barleria prionitis*	**Streptomycin**
1.	*Staphylococcus aureus*	12mm	20mm
2.	*E.coli*	12mm	25mm
3.	*Bacillus subs illus*	14.3mm	22mm
Sr. No.	**Tested microorganism**	**Zone of inhibition(mm)**	**Zone of inhibition(mm)**
		Methanolic extract of *Barleria prionitis*	**Methanolic extract of *Barleria prionitis***
1.	*Staphylococcus aureus*	10.3mm	25mm
2.	*E.coli*	10mm	19mm
3.	*Bacillus subs illus*	14.3mm	26mm

CONCLUSION

Barleria prionitis, a perennial, acanthaceous, barbed, bushy medicinal plant, including in *Barleria* genus containing 300 species is famous for its medicinal value from ancient time. The leaves of *Barleria prionitis* were collected ,From the continuous hot extraction using Soxhelt apparatus four extracts namely Petroleum ether (60-80^{0}C), Ethyl acetate & Methanolic were obtained. The percentage yield of each extract was calculated & they were stored for further use. After subjecting to phytochemical screening of extracts showed the presence of carbohydrates, proteins, flavonoids, alkaloids, tannins & saponins. The TLC finger printing of extracts using various solvent proportions showed different colored spots. The R_f values of these spots were measured. The extracts were also studied to determine their total polyphenol & flavonoids contents. Folin Ciocaltue reagent method was used for total polyphenolic contents; the calibration curve was obtained from gallic acid in concentrations of (50, 100, 150, 200, 250 µg/ml) & the total polyphenol content was expressed as gallic acid equivalent in mg/g of the extract. Aluminiumtrichloride complexation method was used for determination of total flavonoid content, the calibration curve was drawn using various concentrations of rutin (100, 200, 300, 400, 500 µg/ml). The evaluation of antioxidant activity of *Barleria prionitis* leaves extracts was done by using the DPPH scavenging activity & % RRI activity at concentrations of 50, 100, 150 µg/ml. All the four extracts showed maximum antioxidant activity at 150µg/ml.In DPPH method, percentage scavenging activity as percent inhibition was calculated and compared with the standard .The evaluation of antioxidant activity of *Barleria prionitis* leaves extracts was done by using the DPPH scavenging activity at concentrations of 50, 100, 150 µg/ml. All the extracts showed maximum antioxidant activity at 150µg/ml. In DPPH method, percentage scavenging activity as percent inhibition was calculated and compared with the standard (Ascorbic acid). In DPPH scavenging activity, all the extracts showed decrease in absorbance & increase in % inhibition as the concentration of extract was increased. All three extracts showed better activity at 150µg/ml & methanolic extract of BPL has the maximum anti-oxidant activity compared to EA-BPL..For studying *in-vivo* wound healing activity , four animal models were used. 60 rats were required, animals were divided in six groups; control, base treated, standard& two test groups (1% & 2%) each group containing six animals. Animals housed in separate cages under controlled temperature condition (22 ± 2°C) with a free access to food & water.Evaluation of excision model, Animals are divided into six groups, each group contain six animals. Group 1 is control (simple ointment base), group 2 is standard (treating with 0.1% w/w framycetin ointment), group 3 is treating with 1% w/w Methanolic extract and in group 4 it is treating with 2% Methanolic extract of plant part and group 5 and 6 are treating with 1% and 2% Ethyl acetate plant extract. A circular wound of about 2.5cm is to made on the depilated thoracic region under light ether anesthesia. The observation of % wound closure is done on 4th, 8th , 12th ,16th post wounding days. No of days required for falling of scar without any residual raw wound gives the period of epithelisation. The ointment of plant extract ,reference standard & simple ointment base is applied twice daily until the recovery. In the evaluation of antimicrobial activity, it was found that the *Barleria prionitis leaves* extracts exhibit significant Antibacterial activity on microorganisms like *Bacillus subs illus*, *Staphylococcus aurous* & *Ecolab.* The present study shows that *Barleria prionitis leaves extract* have better wound healing and antimicrobial activity.

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