PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *VIGNA RADIATA* STEM BARK EXTRACTS FOR ITS WOUND HEALING ACTIVITY

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***Abstract*: The present study reports physicochemical characterization, antimicrobial and Wound Healing activity of extracts from *Vigna Radiata* stem bark collected from local region of Nanded, Maharashtra, India. Different physical parameters like ash values, extractive value, Loss on drying, solubility etc were evaluated for powdered drug. The extracts were obtained from Soxhlet method by using ethyl acetate and ethanol as solvents for extraction and subjected for preliminary physicochemical evaluation. Total phenolic and flavonoids content were also analyzed. The presence of primary and secondary metabolites such as carbohydrate, proteins, alkaloids, phenolic compounds, saponins was confirmed through preliminary phyto-chemical analysis. Antimicrobial activity showed strong antibacterial and antifungal activities with increase in concentration of ethyl acetate and ethanol leaf extracts. The *In-Vivo* wound Healing activity of *Vigna Radiata* stem bark was evaluated by excision wound model in rats using Soframycin as a standard. Both the extracts showed significant reduction in wound size. The result suggest that *Vigna Radiata* stem bark extracts possess wound healing activity and this might be due to flavonids. Phenolic compound, steroid and proteins present in extract.**

***Keywords*: *Vigna Radiata,* Ethyl Acetate and Ethanolic extract, Phytochemical screening, Antimicrobial effect, Wound Healing activity.**

1. Introduction

A wound may be described in many ways; by its aetiology, anatomical location, by whether it is acute or chronic, by the method of closure, by its presenting symptoms or indeed by the appearance of the predominant tissue types in the wound bed. All definitions serve a critical purpose in the assessment and appropriate management of the wound through to symptom resolution if viable, healing

Types of Wound

1. Open wound
   E.g. Incised wound, tear wound.
2. Closed wound
   E.g. Blood tumor, crush injury.
3. Acute wound
   E.g. Surgical incisions.
4. Chronic wound
   E.g. Hypoxia, diabetes mellitus

Vigna radiata stem bark :

Mung bean (Vigna radiata L.) is a food source of vitamins, minerals, and essential amino acids and has a high nutrient value comparable to that of soybean (Glycine max L. Merr.) and kidney bean.

Mung bean is traditionally known as a functional food, and its functional components have been identified over decades with the development of analytical techniques. In recent years, the physiological functionality of mung bean has received attention, particularly with respect to the content of anti-angiotensin I-converting enzyme and to antitumor, antioxidant, anti-diabetic, and anti-melanocyte effects. Mung bean starch is also considered to be the most suitable raw material for starch noodle-making, as it contains resistant starch that can escape digestion in the small intestine. Starches that are fermented in the gut are generally recognized as components that can improve the gut environment. found in the mung bean Most flavonoids have polyhydroxy substitutions and can be classified as polyphenols with obvious antioxidant activity. Vitexin (apigenin-8- C-β-glucopyranoside) and isovitexin (apigenin-6-C-β-glucopyranoside) have been reported to be present in mung bean seeds at about 51.1 and 51.7 mg g −1 In starch granules, amylose and amylopectin are densely packed in a semicrystalline state with inter- and intramolecular bonds. Amylose is insoluble in cold water and is resistant to chemicals and enzymes. Mung bean is also an excellent source of protein with an ideal essential amino acid profile. It contains a variety of essential amino acids and is rich in lysine. The intake of mung bean protein may improve the plasma lipid profile by normalizing insulin sensitivity. Mung bean also contains fatty acids such as linoleic acid and linolenic acid that promote the growth and health of organisms. The physical and chemical properties of triglycerides and their applications depend on the fatty acid constituents in molecules. Pigmented grain contains many secondary metabolites such as phenolic acids and flavonoids. Phenolic acids represent the most common form of phenolic compounds and make up one of the major and most complex groups of phytochemicals in grain. Flavonoids have many health-related functions, such as antineoplastic activity, inoxidizability, and radioresistance. Both phenolic acids and flavonoids contribute to the antioxidant activity of mung bean.

2. MATERIAL AND METHODS

1.Collection, Identification and Authentication of Plant Material:

The fresh Leaf of *Trigonella Foenum-graecum* was collected from local region of Nanded i.e. from local market and authenticated by **Dr. Shrirang S. Bodke,** Head, Department of Botany& Horticulture, Yeshwant Mahavidyalaya, Nanded**.**

2.Processing of plant material:

Shade drying of the leaves up to complete removal of moisture was done. (Took around 15 days)Dried leaves were powdered by hand crushing and sieved through sieve number 30.

3. Prepration of Extract

Three extracts of whole plant of *vigna radiata* was prepared

- Petroleum ether extract by continuous hot extraction method
- Ethyal acetate extract by continuous hot extraction method
- Ethanol extract by continuous hot extraction method

The extract obtained and the dried mass was weighed and recorded. The percentage of yield was calculated.

$$\text{(\%) yield} = \frac{\text{Wt. of extract}}{\text{Wt. of powdered drug}} \times 100$$

Preparation of Petroleum ether extract (A):

Dried powdered plant was successfully extracted with petroleum ether by Soxhlet extractor apparatus according to the standard method till colorless solution was observed in siphon tube. 300 gm of the powdered plant and 1000 ml petroleum ether was used for extraction. After completion of extraction extract was cooled and dried. The extract was stored in air tight container and kept in decicator till use. Percentage yield of extract was calculated.

Preparation of Ethyl acetate extract (B):

Dried powdered plant was successfully extracted with Ethyal acetate by Soxhlet extractor apparatus according to the standard method till colorless solution was observed in siphon tube. 250 gm of the powdered plant and 1000 ml chloroform was used for extraction. After completion of extraction extract was cooled and dried. The extract was stored in air tight container and kept in decicator till use. Percentage yield of extract was calculated.

3. Phytochemical Evaluation :

1.Total Phenolic Content

Total Phenolic Content was determined by using the **Folin-Ciocalteu assay**. An aliquot (1ml) of extract or standard solution of Gallic acid [2, 4, 6, 8, 10µg/ml] was added to 10ml of volumetric flask, containing 9ml of distilled water. A blank reagent using distilled water was prepared. 0.5 ml of **Folin-Ciocalteu** phenol reagent was added to the mixture and shaken. After 5 minutes 2 ml of 2% $NaHCO_3$ solution was added to the mixture. The volume was then made up to the mark. After incubation for 120 minutes at room temperature, the absorbance against the reagent blank was determined at 746 nm with an UV-Visible spectrophotometer.

2. Total Flavonoids Content

Total Flavonoid Content was measured by the aluminum trichloride colorimetric assay. An aliquot (1ml) of extracts or standard solutions of Rutin (50, 100, 150, 200 and 250µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the flask was added 0.3 ml 5% $NaNO_2$, after five minutes 0.3 ml 10 % $AlCl_3$ was added. After five minutes, 2 ml 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 258 nm.

4. ***In-vitro*** **Antioxidant activity:**

DPPH radical scavenging assay:

The measurement of radical scavenging activity of any antioxidant is commonly associated with the using of DPPH method because it is quick, reliable and reproducible method.

The free radical scavenging activity of the compound was measured by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay as per standard reference.

Principle:

The assay is based upon the theory that H^+ is antioxidant. The antioxidant effect is proportional to the disappearance of DPPH· in test sample due to which purple solution changes to yellow.

The degree of color change from purple to yellow at different concentrations was spectrophotometrically measured at 516 nm. The degree of discoloration indicated the scavenging potential of the antioxidant compounds in the term of hydrogen donating ability.

Procedure:

10ug/ml to 50ug/ml of both test i.e. rutin as well as standard i.e. ascorbic acid were prepared in methanol along with that 100ug/ml of DPPH. 10ug/ml to 50ug/ml of both test i.e. rutin as well as standard i.e. ascorbic acid were prepared in methanol along with that 100ug/ml of DPPH. An equal amount of methanol and DPPH served as control. After 30 min of incubation at room temperature in the dark, the absorbance was recorded at 516 nm.

DPPH radical scavenging activity = 1 - [$A_{sample}/A_{control}$] * 100

Where,
A_{sample} and $A_{control}$ are absorbance of sample and control

5. *In vivo* Wound Healing activity:
Principle:
The rats were inflicted with excision wounds as described by Morton and Malone (1972) using Ketamine.

Animal used:
For the study *Wistar rats* of either sex, of weight 150-200gm were selected.

Test group:
For the study nine groups of animals were made. Each group having six rat.

Route of administration: Topical administration.

Animal Grouping and drug administration:

Wistar rats of either sex weighing 150-200gm., obtained from animal house of college. The Animals were randomly divided into seven groups of six animals in each group namely

1) Positive Control: Treated with plain ointment base
2) Negative Control: Non treated
3) Standard: Treated with standard drug, i.e., Soframycin
4) Test Dose-1: Treated with 2% ethyl acetate extract (VGREA 2%)
5) Test Dose-2: Treated with 5% ethyl acetate extract (VRGEA 5%)
6) Test Dose-3: Treated with 2% ethanolic extract (VGRE 2%)
7) Test Dose-4: Treated with 5% ethanolic extract (VGRE 5%)

Procedure:

- The animals were divided into three groups with six in each were anaesthetized by open mask method with Ketamine before wound creation.
- The particular skin area was shaved 1 day prior to the experiment. An excision wound was inflicted by cutting away a 300 mm^2 full thickness of skin from a predetermined shaved area.
- The wound was left undressed to the open environment. The ointment base, standard drug ointment and extract of plant ointment (2%, w/w) & (5%, w/w) was applied topically to the control group, standard group and treated group respectively, till the wound get completely healed.
- In this model, wound contraction was monitored.
- Wound contraction was measured as percent contraction in each 2 days after wound formation.
- From the healed wound, a specimen sample of tissue was collected from each rat for histopathological examination.

Evaluation

An excision wound margin will be traced by following the progressive changes in wound area planimetrically, excluding the day of wounding. The size of wound will be traced on a transparent paper in every 2 days, throughout the monitoring period. The tracing will be then shifted to graph paper, from which the wound surface area will be evaluated. The evaluated surface area will be then employed to calculate the percentage of wound contraction, taking initial size of wound, 300 mm^2, as 100%, by using the following formula as:

$$\% \text{ wound contraction} = \frac{\text{initial wound size} - \text{specific day wound size}}{\text{Initial wound size}} \times 100$$

6. RESULTS

Table no.1 - Observations for Phytochemical qualitative analysis

Test for	Petroleum Ether	Chloroform	Ethanol
Carbohydrate			
a) Molisch's test:	+	+	+
b) Barfoed's test:	+	+	+
c) Fehling's test:	+	+	+
d) Benedict's test:	+	+	+
Proteins			
a) Biuret test:	+	+	+
b) Millons test:	-	+	+
Alkaloids			
a) Dragendoff's test:	-	+	+
b) Mayer's test:	+	-	-
c) Wagner's test:	-	-	-
Glycosides			
a) Borntrager's test:	-	-	+
b) Modified Borntrager's test:	+	+	+
Flavonoids			
a) Shinoda test:	-	+	+
b) Zinc hydrochloride test:	-	-	+
c) Alkaline reagent test:	-	+	+
Tannins			
a) Lead Acetate Test	-	-	-
b) $Fecl_3$ test	-	-	-
Amino acids:			
a) Million's test	-	+	+
b) Ninhydrine test	+	+	+

Table no.2-Total phenolic content of *Vigna radiata* stem bark extracts

Sr. No.	Conc. µg/ml	Extracts	Absorbance	Phenolic content (mg GAE/g DW)
1	100	Petroleum ether	0.090	26.77 ± 0.18
2	100	Ethyl acetate	0.107	33.42 ± 0.37
3	100	Ethanol	0.196	58.39 ± 0.21

(N=3) Note: GAE/g DW denotes Gallic Acid Equivalent per gram dry weight.

Above observation table reveals that Petroleum ether, Ethyl acetate and Ethanol have Phenolic content as 26.77 (mg

GAE/g DW), 33.42 (mg GAE/g DW), 58.39 (mg GAE/g DW) respectively. Ethanol extract shows more phenolic content than Petroleum ether and Aqueous as per comparative evaluation of phenolic content of extracts.

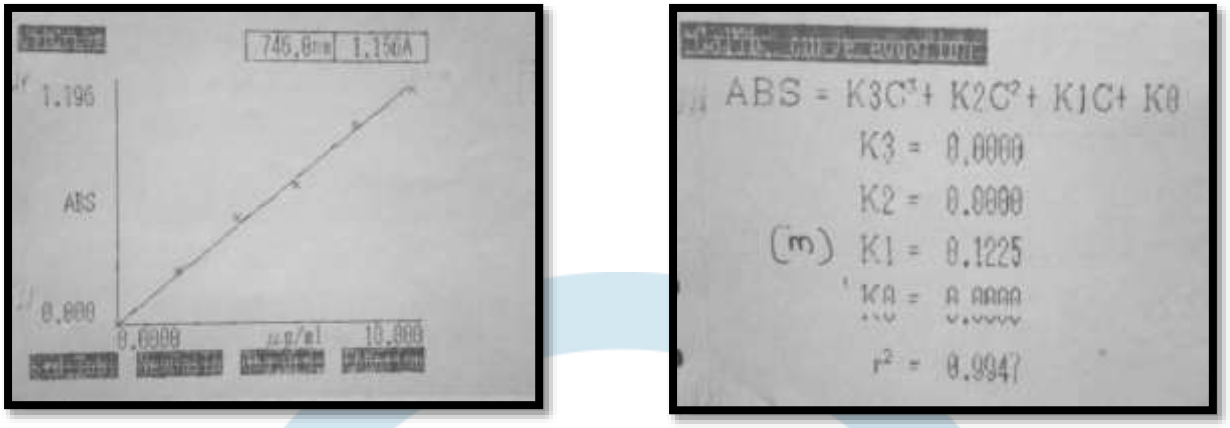

Fig no.1-Calibration Curve of Gallic acid

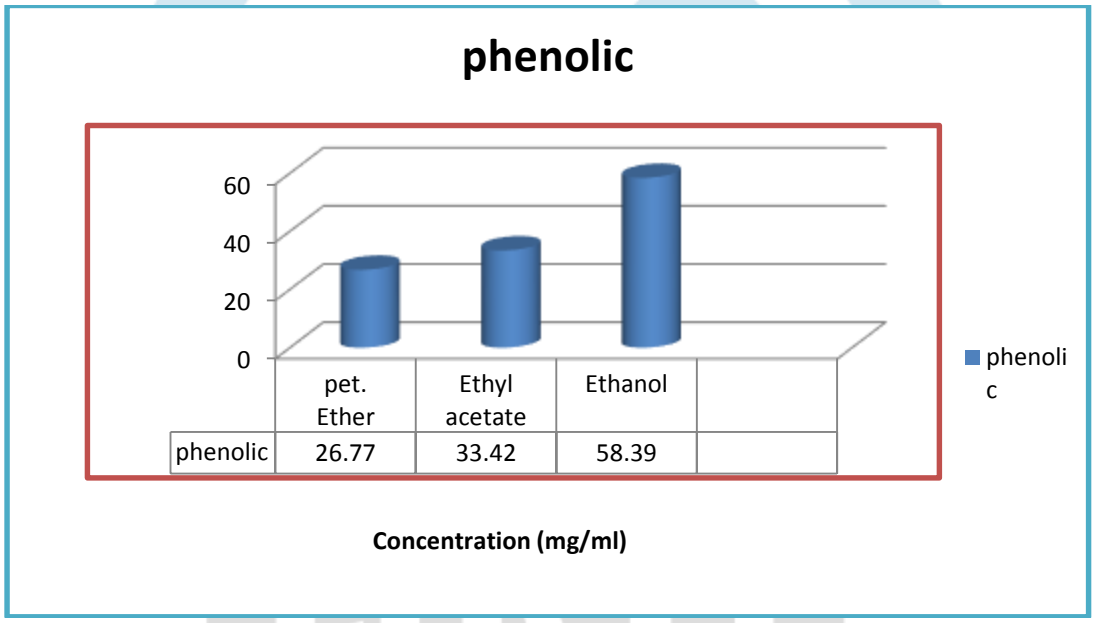

Chart 1: Effect of Phenolic content of extracts

The concentration absorbance calibration curve for sequentially and separately prepared stock solution of standards of Gallic acid solution was taken. The absorbance measured at 765 nm for 20, 40, 60, 80, 100 µg/ml concentration Gallic acid solution are in a range of 0.067 to 0.331 within the range of concentrations, the calibration curve of Gallic acid has clearly exhibited linearity. Above table indicate that the Ethanolic extract contain more phenolic content (58.39 mg GAE/g DW) than Ethyl acetate and Petroleum ether extract (33.42 mg GAE/g DW, 26.77 mg GAE/g DW) respectively equivalent to Gallic acid.

Table no.3-Estimation of Total Flavonoid Content

Sr. No.	Conc. µg/ml	Extracts	Flavonoid content (mg Ru/g DW)
1	100	Petroleum ether	39.00 ± 0.19
2	100	Ethyl acetate	51.44 ± 0.16
3	100	Ethanol	61.83 ± 0.17

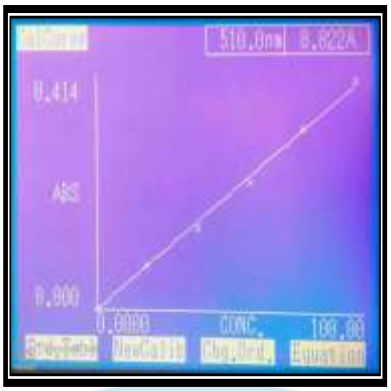

Fig no.2-Calibration Curve of Rutin

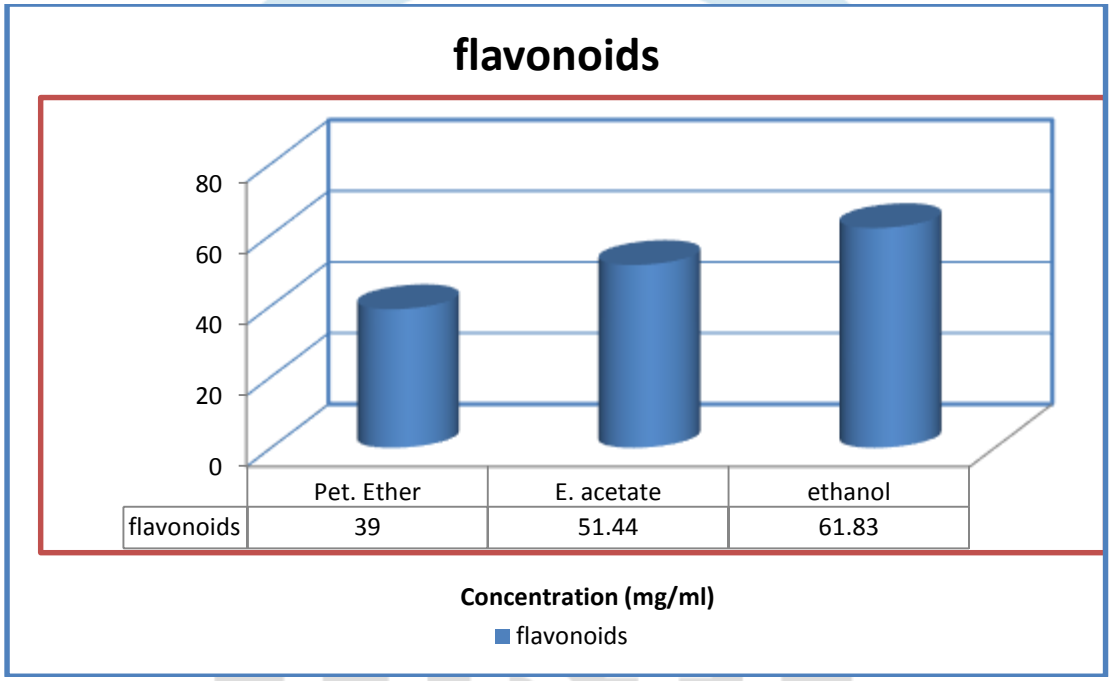

Chart 2: Effect of flavonoid content of extracts

1. Confirmation of phytoconstituents with Thin Layer Chromatography

Sr. No.	Conc. µg/ml	Absorbance of Ascorbic acid	Absorbance of Gallic acid	Absorbance of rutine
1	25	0.182	0.287	0.285
2	50	0.192	0.224	0.236
3	75	0.088	0.116	0.124
4	100	0.059	0.093	0.102
5	125	0.024	0.043	0.032

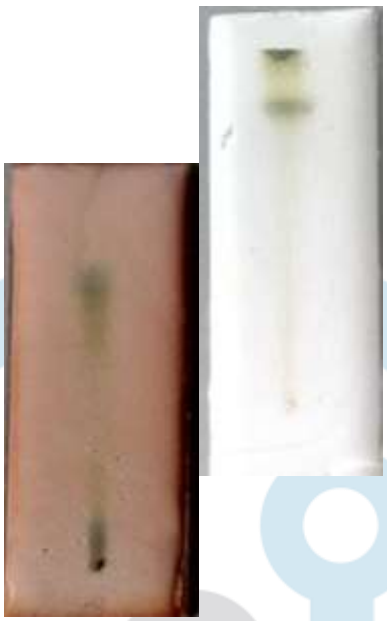

Fig no.3 **Fig no.4**

Toluene : Ethyl acetate **Toluene : Ethyl acetate : Formic acid**
9 : 1 **2 : 8 : 1**
Ethyl acetate extract **Ethanolic extract**

Table No.6.-Total Antioxidant Content of Standard

Table No. 4- Rf values chloroform extract

Pigment / solvent band	R_f Value
Pheophytin - a (green)	0.5
Chlorophyll *a* (blue green)	0.37
Chlorophyll *b* (Green)	0.3
Xanthophylls (yellow)	1.1

Table No.5- Rf values of Ethanol extract

Pigment / solvent band	R_f Value
Pheophytin - a (green)	0.57
Chlorophyll *a* (blue green)	0.61
Xanthophylls (yellow)	0.5

Table no. 7- Effect of % Inhibitions of Standard

Sr. No.	Conc. µg/ml	Ascorbic acid % inhibition	Gallic acid % inhibition	Rutin % inhibition
1	25	62.62	41.06	41.47
2	50	73.51	54.00	51.54
3	75	81.93	76.18	74.53
4	100	87.88	80.90	79.05
5	125	95.07	91.17	93.42

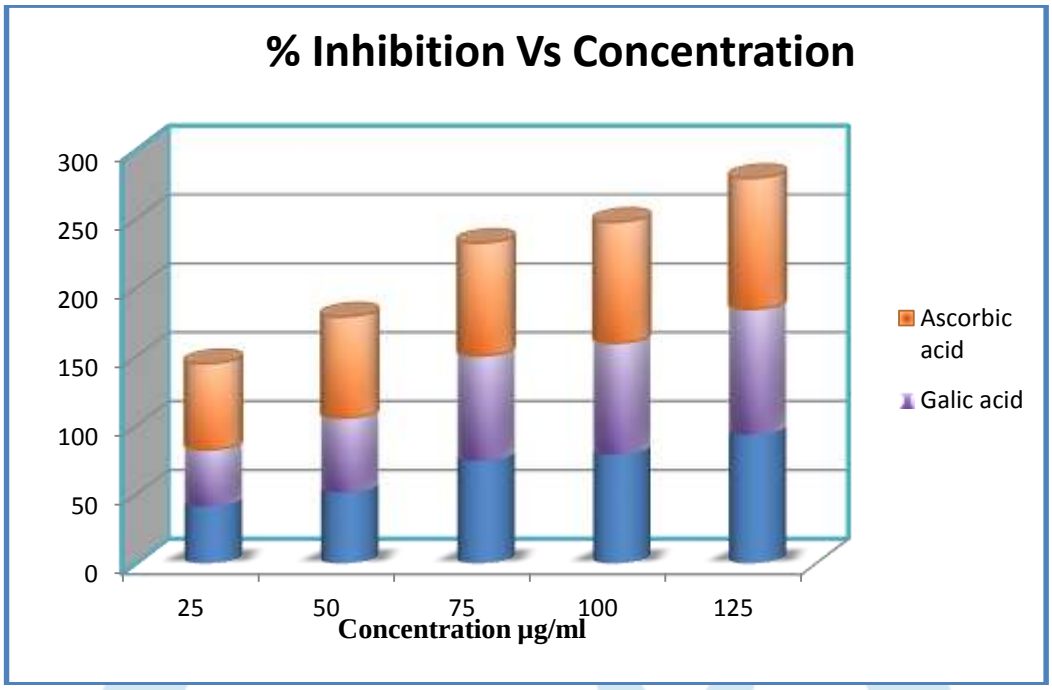

Chart no. 3. Effect of % Inhibitions of Standard

Table no.8- Comparative DPPH Scavenging assay method of *Vigna radiata* (Pet. Ether, Ethyl acetate and Ethanolic) stem extracts

Sr. no.	Conc. µg/ml	Petroleum ether % inhibition	Ethyl acetate % inhibition	Ethanol % inhibition	Ascorbic acid % inhibition
1	25	53.62	56.11	58.34	62.62
2	50	60.93	59.89	69.84	73.51
3	75	68.84	76.31	79.08	81.93
4	100	74.05	82.88	86.26	87.88
5	125	78.01	88.90	90.89	95.07

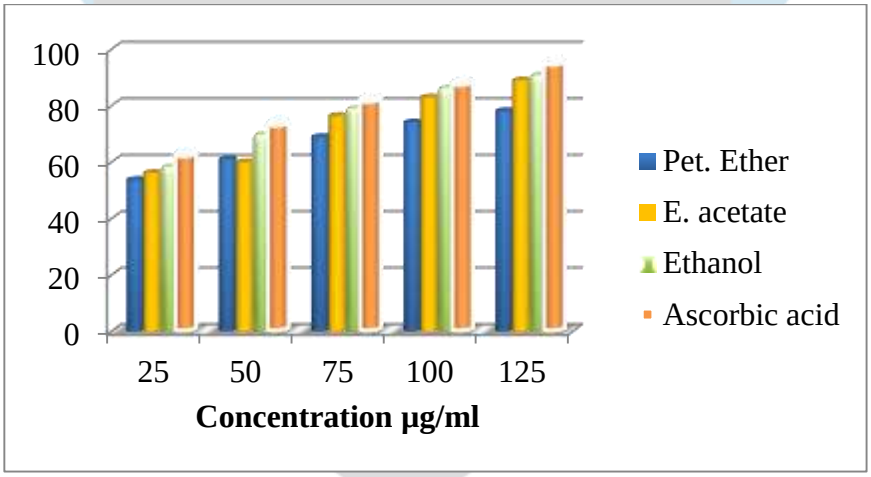

Chart 4. Effect of % Inhibition of Extracts

Fig no.4- Zone of inhibition of reference standard (Penicillin)

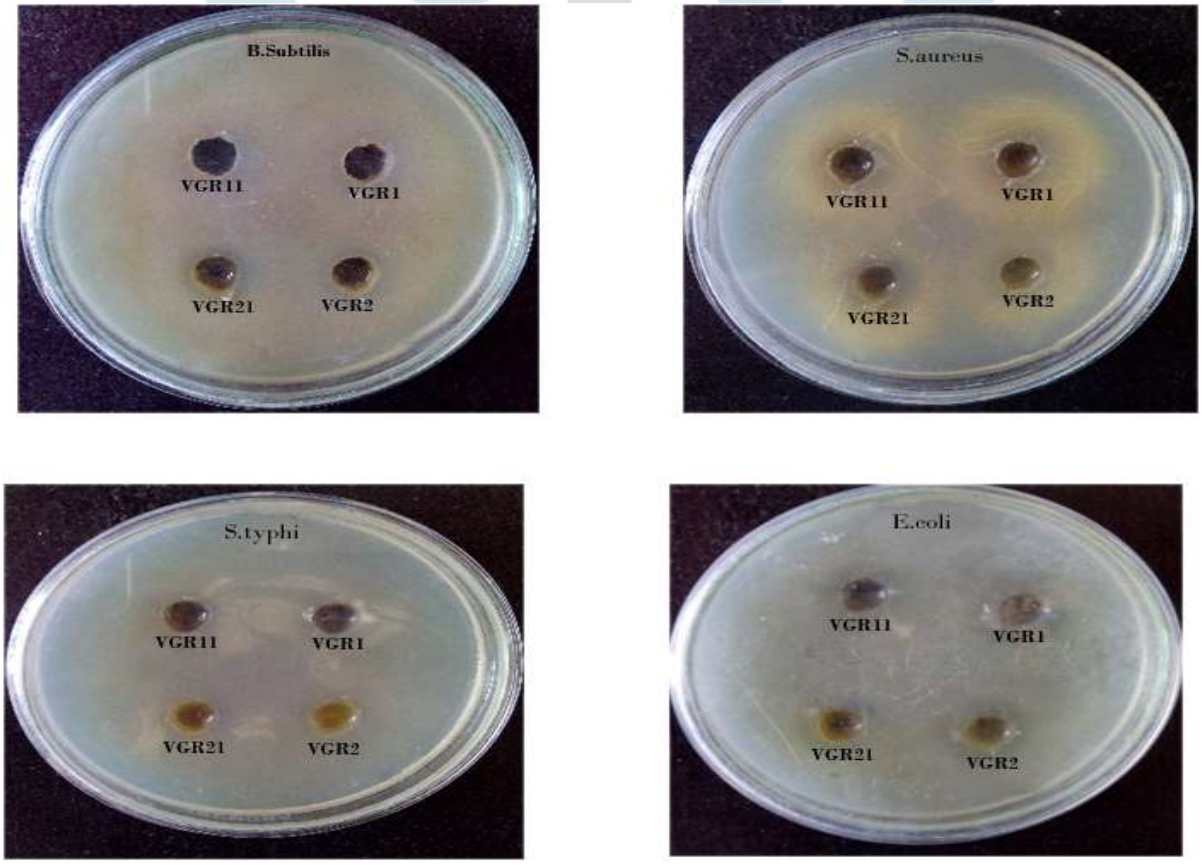

Fig no.5- Zone of inhibition of *Vigna Radiata* stem extracts at different concentrations

Table no.9-In- Vitro Antimicrobial activity of *Vigna Radiata* extracts

Sr. No.	Dose (mg/ml)	Compound	Escherichia coli	Salmonella typhi	Staphylococcus aureus	Bacillus subtilis
1.	100	VGR1	16mm	-ve	-ve	-ve
2.	100	VGR2	16mm	15mm	-ve	-ve
3.	50	VGR11	15mm	-ve	-ve	-ve
4.	50	VGR21	14mm	13mm	-ve	-ve
5.	100	DMSO	-ve	-ve	-ve	-ve
6.	100	Penicillin	11mm	24mm	36mm	30mm

Legends:- '-ve' : No antibacterial activity, Zone of inhibition: in mm

Anti- fungal activity:

Fig no.6- Effect of reference Standard (Griseofulvin) **Effect of DMSO (Negative control)**

Fig no.7- Effect of extracts at different concentration

Table no.10-Antifungal activity

Sr. No.	Dose (mg)	Compound	Aspergillus niger	Penicillium chrysogynum	Fusarium moneliforme	Aspargillus Flyus
1.	100	VGR1	RG	RG	RG	+ve
2.	100	VGR2	RG	RG	RG	RG
3.	50	VGR11	RG	RG	RG	+ve
4.	50	VGR21	RG	RG	RG	RG
5.	100	DMSO	+ve	+ve	+ve	+ve
6.	100	Griseofulvin	-ve	-ve	-ve	-ve

Legends -

+ ve - Growth (Antifungal Activity absent)

- ve - No Growth (More than 90% reduction in growth Antifungal activity present)

RG - Reduced growth (More than 50 % and less than 90 % reduction in growth)

3.*In- Vivo* Wound healing activity:

Fig no.8-Day to day progress of wound healing effect:

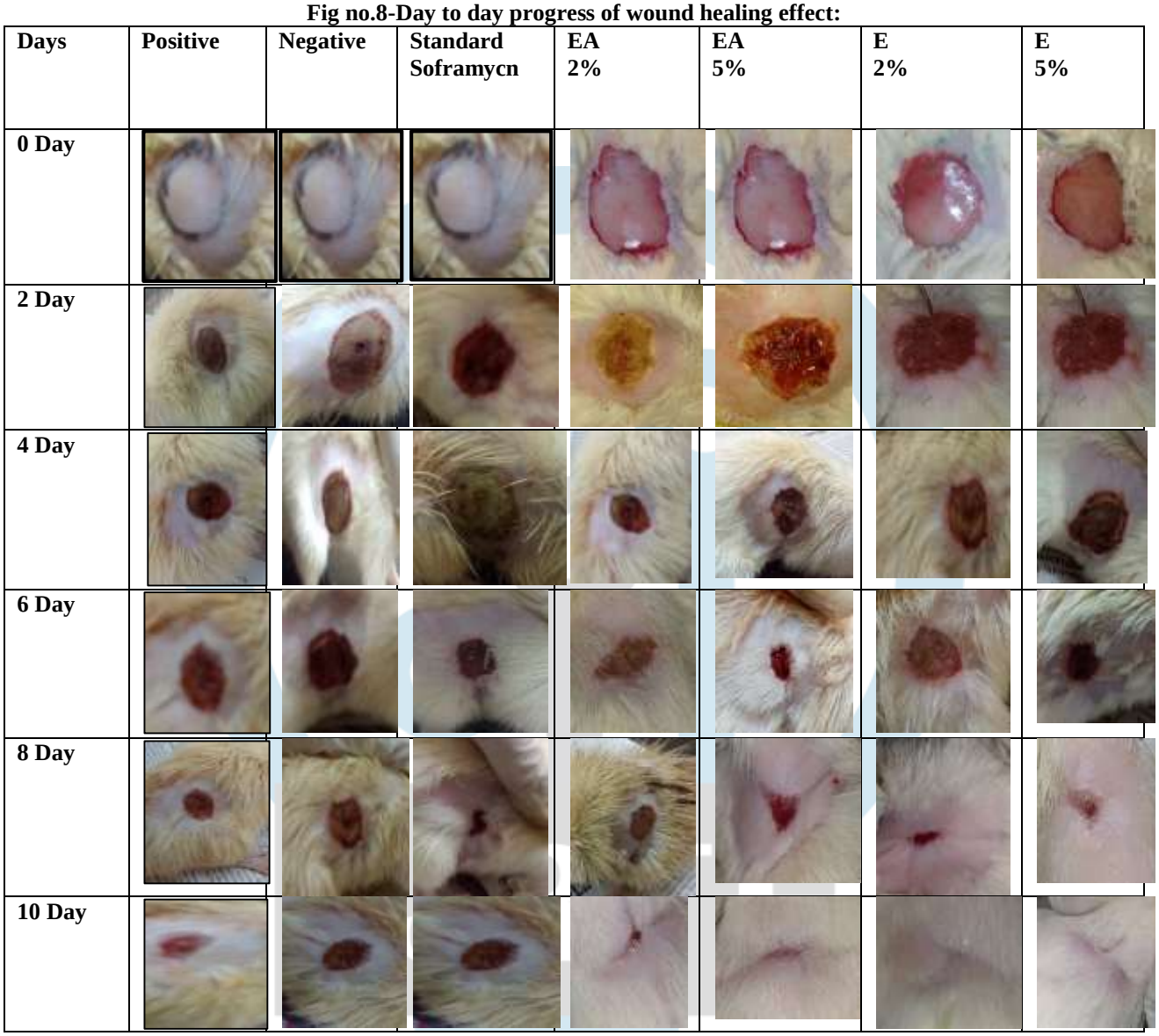

Table no.11- Evaluation of wound healing activity of *Vigna Radiata stem Bark*

7. Discussion

No methodical reports on wound healing activity of *Vigna Radiata* stem bark are available.Preliminary phytochemical evaluation of both two extracts was carried out for the determination of presence of phytoconstituents. It reveals that all two extracts (i.e. ethyl acetate and ethanol) contain carbohydrates, steroids, tannins, flavonoids. The total Phenolic and Flavonoid content were also determined. It was found that the ethanol extract has more phenols and flavonoid as compared with the aqueous extract.The *in vitro* Antibacterial property of *Vigna Radiata* stem bark was carried out by using agar cup and plate method. In this method increase the zone of inhibition was calculated and compared with standard (*Penicillin*) by using this method the ethanolic extract showed highest zone of inhibition at 100mg/ml than the aqueous extract. Antifungal property of *Vigna Radiata* stem bark was carried out by using poison plate method. In this method reducing growth of fungi (moderate antifungal activity) and no growth of fungi of test sample was calculated and compared with standard i.e (*Griseofulvin*). Ethanolic extract showed the reduced growth (more than 50% and less than 90% reduction in growth) at 100 mg/ml.The acute oral toxicity study was also determined according to OECD guidelines. After administration of 2000mg/kg of dose animals does not showing any adverse reaction.*In-vivo* wound healing activity of Aqueous and ethanol extract of *Vigna Radiata* was evaluated by using the excision wound model. VRE 2% and VRE 5% showed highly significant decrease wound area when compared with positive control group and significant difference when compared with standard but more effect than standard.

	Post wounding days					
rats	**0 day**	**2nd day**	**4th day**	**6th day**	**8th day**	**10th day**
Positive control	2±0.0	1.82±0.01	1.7±0.02	1.5±0.02	1.21±0.00	1±0.01
Negative control	2±0.0	1.9±0.01	1.8±0.01	1.5±0.01	1.15±0.00	1±0.00
standard	2±0.0	1.7±0.03#	1.3±0.04*	1±0.04**	0.68±0.00**	0.06±0.02**
VGR-EA 2%	2±0.00	1.60±0.07*≠	1.5±0.02#,#	1.2±0.02**,#	0.84±.10*≠	0.1±0.06**≠
VGR-EA 5%	2±0.00	1.56±0.08*≠	1.1±0.10**,#,▫	1±0.04**,#,▫	0.71±0.07**≠	0.04±0.02**≠
VGR-ME 2%	2±0.00	1.40±0.08*Δ	1.3±0.03*,#	1.2±0.03**,*	0.52±0.10**Δ	0.04±0.02**≠
VGR-ME 5%	2±0.00	1.14±0.10*Δ	1.2±0.09**,#,▫	1.1±0.02**,#	0.40±0.04**Δ	0.02±0.01**Δ

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