Research Article

Assessment of wound healing potential of *Momordica charantia*, *Pongamia glabra* and their combination with *Piper nigrum* on anemic albino rats using excision wound model

Rashmi Shukla, Varsha Kashaw*

SVN Institute of Pharmaceutical Sciences, Swami Vivekanand University, Sagar, Madhya Pradesh, India

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Abstract

Objective: The objective of present work was to assess the wound healing potential of *Momordica charantia, Pongamia glabra* and their combination with *piper nigrum* using as ointment formulation on anemic albino rats using excision wound model. These all have exhibit antidiabetic, anti-inflammatory, anti-microbial, antileukemic, antimycobacterial, antioxidant, antitumor, antiulcer, antiviral, anticancer, antimalarial, cytotoxic, antiprotozoal and wound healing properties. **Materials and Methods:** Solvent extraction method has been utilized for the separation of the constituents of interest. Herbal ointment formulation has been prepared by mixing the extract of *Momordica charantia, Pongamia glabra* and their combination with *piper nigrum*. Excision wound model on anemic rats has been used for the assessment of wound healing potential. **Results and Conclusion:** The studies on excision wound healing model reveals that all twelve groups showed decreased wound area on the time and there was no mortality observed in the course of study. **Discussion:** These studies have indicated that ointment formulation of *Momordica charantia, Pongamia glabra* and their combination with *Piper nigrum* has been utilized for wound healing and it is safer for topical application. No toxicity and mortality has been observed during the experimental tenure.

Keywords: Wound healing, *Momordica charantia, Pongamia glabra, Piper nigrum,* excision wound

Introduction

Wound infection is one of the most common diseases in developing countries because of poor hygienic conditions (Kumar et al., 2006). Wounds are the physical injuries that result in an opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin.Wound healing process holds several steps which involve coagulation, inflammation, formation of granulation tissue, matrix formation, remodeling of connective tissue, collagenization and aquisation of wound strength (Enoch et al., 2005; Guo et al., 2010).

Momordica charantia belong to *Cucurbitaceae* family (Jayasooriya et al., 2000). Fruit having ovoid, ellipsoid, or spindle shaped, usually ridged or warty, dehiscent irregularly as a 3 valve fleshy capsule or indehiscent (Figure 1: Fruit, leaves and seeds of *M. Charantia*). The fruit has a distinct warty looking exterior and an oblong shape. It is hollow in cross section, with a relatively thin layer of flesh surrounding a central seed cavity filled with large flat seeds and pith. *M. Charantia* consist of the following chemical constituents those are Alkaloids, charantin, diosgenin, elaeostearic acids, erythrodiol, galacturonic acids, gentisic acid, gypsogenin, hydroxytryptamines, lanosterol, lauricacid, linoleic acid, linolenic acid, momorcharasides, momorcharins, momordenol, multiflorenol, myristic acid, nerolidol, oleanolic acid, oleic acid and oxalic acid. *Momordica charantia* is used as anthelmentic, antibacterial, antibiotic, anti-diabetic, anti-inflammatory, anti-microbial, anti-mycobacterial, antioxidant, antitumor, wound healing, antiulcer, antiviral, hypoglycemic and immune-stimulant (Toeh et al., 2009; Patel et al., 2010; Satish et al., 2010; Chia et al., 2011; Leelaprakash et al., 2011; Rajaram et al., 2012; Rawat et al., 2012).

Pongamia glabra (Figure 2) belong to *Fabaceae*family (Singh et al., 1996; Sharma et al., 2004). Fruit contains furano-flavonoids, pongapinnol A–D, and a new coumestan, pongacoumestan. Oil constituentshave consists of furan flavones, karanjin, pongapin, kanjone and a

Address for Corresponding Author:
Dr. (Mrs.) Varsha Kashaw
Professor
SVN Institute of Pharmaceutical Sciences,
Swami Vivekananda University, Sagar (M.P.) India
E-mail: varshakashaw@gmail.com

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diketonepongamol. It have been also contains alkaloids, tannins, flavonoids, steroids, glycosides & carbohydrates. *P. glabra* has been reported to contain a large number of furanoflavonoids e.g. karanjin, pongapin, kanjone, pongamol, and pongaglabrone, along with a number of simple flavonoids and lipid like arachidonic acid (Bandivdekar et al., 2002). It used as bacteriocidal activity against V. cholerae and E. coli, as well an anti-inflammatory, anti-nociceptive (reduction in sensitivity to painful stimuli) and antipyretic (reduction in fever) properties (Chopade et al., 2008; Herror et al., 2012; Krishnan et al., 2013).

Figure 1. Fruit, leaves and seeds of *M. charantia*

Figure 2. Leaves, flowers, seeds and pods of *Pongamia glabra*

Piper nigrum (Figure 3) belonging to family *Piperaceae* (Sunila et al., 2004). The fruits are small globose drupes (a fleshy fruit containing a seed with a hard, stony covering), 3 to 4 mm (less than 0.25 in) in diameter, that ripen to red.The fruit was known as a peppercorn when dried (Sudjarwo et al., 2005). Pepper plants grow easily in the shade and require little maintenance until harvest, so they are frequently cultivated for supplemental income on even small farms.

Figure 3. Unripe and ripe seeds of *Piper nigrum*

Antioxidant active chemicals isolated from black pepper includes camphene, carvacrol, eugenol, gamma terpinene, lauric-acid, linalyl-acetate, methyl-eugenol, myrcene myristic-acid, myristicin, palmitic-acid, piperine, terpinen-4-ol and ubiquinone were the major chemical compounds responsible for the aroma, pungency and medicinal property of the black pepper. Black pepper is used to improve digestion, stimulate appetite, and treat gastrointestinal problems, including diarrhea, dyspepsia and flatulence. It is also used to treat colds, coughs and sore throats (Karsha et al., 2009; Shaik et al., 2013). The object of the present paper is to determine the wound healing potential of *Momordica charantia, Pongamia glabra and piper nigrum*. The excision wound healing model has been utilized for the assessment of the wound healing potential.

Materials and methods

Plant material collection and authentication

The fruits of *Momordica charantia* were collected at in the month of July, 2016 from local field areas of Bhopal, Madhya Pradesh. Leaves of Pongamia glabra collected from Garden and Fruits of Piper nigrum from local market. The specimens were submitted and identified as fruits of *Momordica charantia* (MC) family of *Cucurbitaceae*, leaves of *Pongamia glabra* (PG) family of *Fabaceae*, fruits of *Piper nigrum* (PN) family of Pipereacea and authenticated by Dr. Zia ul Hassan, Department of Botany, Saifia Science College, Bhopal.

Successive solvent extraction

Generally, three methods are employed in the extraction of plant materials as (1) Maceration (2) Percolation (3) Soxhlet extraction. Maceration and percolation may be employed in extraction of thermo labile constituents. Soxhlet extraction is rapid, continuous, an easy and may be employed in extraction of sparingly soluble constituents due to repeated extraction, which cannot be done by either maceration or percolation methods. This advantages offered by soxhlet extraction promote to selected for present study. Successive Soxhlet extraction by using solvents, selected on the basis of polarity after defatting of the crude drug by petroleum ether. Solvents used were ethyl acetate, ethanol and water.

Soxhlet extraction

The crude drugs were dried in shade. Then moderately coarse powder of the drugs e.g. *Momordica charantia* (MC), *Pongammia glabra* (PG) and *Piper nigrum* (PN) were subjected to successive soxhlet extraction with different solvents in increasing order of polarity from non-polar to polar. Soxhlet extraction has carried as follows, by taking 80 gm of dried coarsely powdered drug was packed in soxhlet apparatus and defatted with 1000 ml of petroleum ether (40-60˚C) till complete defatted. Complete defatting ensured by placing a drop by thimble on the filter paper which did not exhibited any oily spot.The defatted material was removed from the soxhlet apparatus and air dried to

remove the last traces of petroleum ether. The defatted material was subjected to extraction by ethyl acetate and then with ethanol as solvent by soxhlet apparatus and finally with water by maceration process. The completion of extract was confirmed by evaporating a few drops of the extract on the watch glass and ensuring that no residue remained after evaporating the solvent.

The marc was air dried before extracted with the next solvent. Dried marc was macerated with water for 24 h. The extracts were evaporated under reduced pressure at low temperature (30˚C) to dryness to yield different extracts, stored in an airtight container in refrigerator for further experimental studies. They were weighed to a constant weight and percentage w/w basis was calculated.

Herbal ointment formulation and evaluation

Herbal preparations were formulated by combination of three plant extract of *Momordica charantia, Pongamia glabra* and their combination with *piper nigrum*. An ointment with water soluble base was of first choice due to their ease of preparation and eases of cleaning after application. The alcoholic and aqueous extracts were selected for formulation as they have the higher content of flavanoids and phenolic compounds.

Preparation of Simple Ointment (B.P.)

The simple ointment base was prepared by mixing the wool fat, hard paraffin, cetostearyl alcohol and white softparaffin with gentle heating with stirring. The obtained ointment base is then cooled and stored. Quantity take of the ingredients was shown in table 1. Two formulations were prepared by Fusion method e.g. one containing all three extracts of above mentioned plants parts in equal ratios of alcoholic and aqueousextracts i.e. 3.33% w/w of each extract for the preparation of 10 % w/w ointment in ointment base (Treated as herbal-I or F-1) and the other one containing all three extracts of above mentioned plants parts in equal ratios of alcoholic and aqueous extracts i.e. containing 5.0% w/w of each extract, equal to total 15% w/w in ointment base (Treated as herbal-II or F2). Two ointment formulations have been prepared and quantity specified in table 2. The prepared formulations were then evaluated by various parameters e.g. consistency, stability etc.

Table1. Ingredient for ointment formulation

Ingredients	Quantity (g)	Percentage
Wool Fat	1	5
Cetostearyl alcohol	1	5
Hard Paraffin	1	5
White soft paraffin	17	85
Total	20	100

Table 2. Formulation of ointment

Content	Quantity (%)	
	F1	F2
MCAQ (1:1)	3.33	5.0
PGAQ (1:1)	3.33	5.0
PNAQ (1:1)	3.33	5.0
Ointment base	Q.S.	Q.S.

Where, MCAQ- Alcoholic and Aqueous extracts of *Momordica charantia* in 1:1 quantity; PCAQ- Alcoholic and aqueous extracts of *Pongamiaglabra* in 1:1 quantity; PNAQ- Alcoholic and aqueous extracts of *Piper nigrum* in 1:1 quantity.

Pharmacological activity

Anemic wound healing activity

Excision wound model using anemic albino rats was selected for assessing the wound healing activity. This model was employed to study the rate of wound contraction, time required for full epithelization and tensile strength. These parameters were selected and albino rats were selected by easy availability and simplicity in handling them.

Selection and procurement of animals

Institutional Animal Ethics Committee (IAEC), Registration number CPCSEA/1413/ PO/ES) has been permitted for animal studies. Albino rats were procured and rats of either sex weighing 150-200 gm were selected and maintained at 24-28˚C, housed individually with free access to food and water. They were fed with standard diet and kept in well-ventilated animal house with alternate dark-light cycle of 12 hrs throughout the studies.

Oral toxicity studies

Albino rats of weight 150-200gm were selected for acute oral toxicity study and it was conducted according to the "Organization for Environmental Control Development" guidelines (OECD: Guidelines 420; Fixed Dose Method) for oral administration of extracts. Eighteen hour overnight fasted animals were subjected to oral administration of extracts at a dose of 2000 mg/kg body weight. All the animals were kept under observation for first 3h for any changes or toxic effects like neurological, gross behavioral and lethality. The animals were observed and confirmed the absence of any toxic effects, hence two dose of combination 10% and 15% ointment were prepared with simple ointment base for topical application and 10mg/ml oral dose taken for study.

Sample preparation

Sample solutions each of 10 mg/ml of extracts was prepared separately and mixed so as each combination will contain 20 % w/v aqueous extract of *Piper nigrum* - PNAQ.

Excision wound model

For the excision wound studies, seventy two albino rats were taken, divided in two main groups: non-anemic group and anemic group. The anemic group was further divided into eleven groups of six each (Khan et al., 2013).

To perform the experiment, the rats were divided into Eleven groups (n=6).

Group I: Control group which received simple vehicle (ointment base) [Control]

Group II: Standard group received Povidone iodine ointment [Standard]

Group III: Test group received MCAQ extract orally [OAQMC]

Group IV: Test group received MCAQ and PNAQ extract orally [OAQ (MC+PN)]

Group V: Test group received PGAQ extract orally [OAQPG]

Group VI: Test group received PGAQ and PNAQ extract orally [OAQ (PG+PN)]

Group VII: Test group received MCAQ, PGAQ and PNAQ orally [OA (MC+PG+PN)]

Group VIII: Test group received MCAQ, PGAQ and PNAQ formulation F1 (10%) topically [TF1]

Group IX: Test group received MCAQ, PGAQ and PNAQ formulation F2 (15%) topically [TF2]

Group X: Test group received MCAQ, PGAQ and PNAQ orally and formulation F1 (10%) topically [O3TF1]

Group XI: Test group received MCAQ, PGAQ and PNAQ orally and formulation F2 (15%) topically [O3TF2]

Group XII: Anemic Group received no treatment [GXII]

Where, AQ stands for alcoholic and aqueous extract of drugs in 1:1 amount; O stands for ointment base.

Induction of anemic conditions with diabetes

Anemic conditions with diabetes were induced by intravenous injection of freshly prepared Streptozotocin (Sigma, USA) (50 mg/kg, dissolved in 0.9% normal saline) via the tail vein following overnight fasting. The control group was injected with 0.9% normal saline (Toeh et al., 2009). Fasting blood sugar levels were determined at day 3 after streptozotocin induction using a glucometer (Accu-Check Advantage, Roche, Germany). The rats with fasting blood sugarsmore than 8mmol/L were included in the anemic group. The fasting blood sugar levels were monitored every third day to ascertain the diabetic status (Khan et al., 2013). For anemia the blood samples were collected before and after the induction of anemia by retro orbital sinus with glass capillary tube and used for the estimation of hematological parameters.

Creation of wound

Rats depilated by removing hairs at the dorsal thoracic regionbefore wounding. Rats were locally anaesthetized by xylocaine Jelly (Lignocaine hydrochloride gel I.P., 2%w/v) prior to excisioncircular wound of about 2.5 cm diameter was made ondepilated dorsal thoracic region of rats under asepticconditions and were observed throughout the study. Theareas of the wounds were measured (in sq. mm) immediately by placing a transparent polythene graphpaper over the wound and then tracing the area of thewound on it (Approx. area 300 sq mm). This was takenas the initial wound area reading. Thereas of the wounds were measured, immediately by placing a transparent polythene graphpaper over the wound and then tracing the area of thewound on it (Approx. area 300 sq mm). This was takenas the initial wound area reading (Nayak et al., 2007).

Treatment of wounds

All the samples were applied once daily for 10 days, starting from the day of wounding. The wound sizes of all wounds were measured on days 0, 1, 5, 10 days and the average readings were recorded as wound area. The rate of wound closure (percentage wound closure) was then determined at days 1, 5 and 10. The wound area of each animal was measured at intervals of 24-48 h using tracing paper method. The percentage of wound contractionwas calculated from the days of measurements of wound area.The wound contraction was calculated as percentage reduction in wound area with respect to initial wound area while the epithelization time was noted as the number of days after wounding required for scar to fall off leaving no raw wound behind.

Wound contraction measurement

The wound contraction was calculated as percentage reduction in wound area with respect to initial wound area while the epithelization time was noted as the number of days after wounding required for scar to fall off leaving no raw wound behind (Mehanni et al., 2003). Wound contraction (WC) was calculated as a percentage change in the initial wound size i.e. epithelization period was monitored by noting the number of days required for scar to fall away, leaving no raw wound behind.

$$\text{Percentage Wound Closure} = \frac{(\text{Initial wound size} - \text{Wound size at specific day})}{\text{Initial Wound size}} \times 100$$

Statistical analysis

The statistical analysis of experimental data was done with one-way analysis of variance, and SPSS software (version 11.5). $P< 0.001$ was considered as highly significant.

Results and discussion

Plant material collection and authentication

The collected and authenticated *Momordica charantia* fruits, *Pongamia glabra* leaves and *Piper nigrum* fruits were studied and confirmed based on the organoleptic properties. The crude drugs were dried and powdered in shade to stop the enzymatic metabolic process within them and to prevent them from degradation of metabolic products. The specimens were submitted and identified as fruits of *Momordica charantia* family of Cucurbitaceae, leaves of *Pongamia glabra* family of Fabaceae and fruits of *Piper nigrum* family of Pipereacea.

Extraction of plant material

The extraction was done by successive solvent extraction, to increase the extraction, to achieve separation of compounds in different extracts and decrease the time taken by extraction process the flask and soxhlet apparatus was covered by cotton to increase the insulation. The drying of extract containing solvent (Petroleum ether, ethyl acetate, ethanol and water) was done by vacuum distillation process. Percent yield of all extract was depicted in table 3.

Wound healing activity on anemic rats

Increased fasting blood sugar levels were observed on day 3 after streptozotocin administration. The fasting blood sugars remained high throughout the study period, with a mean value of 14.77 ± 0.08mmol/L compared to the normal fasting blood sugar level, which was 6.94± 0.52mmol/L before streptozotocin administration. The fasting blood sugar (FBS) levels and were estimated on initial and final day of experimental protocol to confirm anemic state. A little change in FBS was observed. Increased fasting blood sugar levels were observed on day 3 after streptozotocin administration. The fasting blood sugars remained high throughout the study period, with a mean value of 14.77 ± 0.08mmol/L compared to the normal fasting blood sugar level, which was 6.94± 0.52mmol/L before streptozotocin administration. The fasting blood sugar levels of different groups are shown in table 4 and figure 4.

There is a decrease in RBC count and Haemoglobin level after the administration of streptozotocin in negative control group GXII. And these levels are increased with the administration of test extracts and formulations of various selected drugs and combinations, and the P values are also significant. Anemic rats showed decrease in RBC count whereas the RBC count of the control rats remained the same. Administration of various extracts and combination of drugs significantly ($P<0.001$) increase the RBC count and brought back RBC count towards normal. Hematological parameters after treatment with extracts and formulations were tabulated in table 5.

Anemic rats showed decrease in Hb level whereas the Hb level of the control rats remained the same shown in Figure 5. Administration of various extracts significantly ($P<0.001$)

Table 3. Percentage yield of different extracts

Parts	Solvents	Extract color	Yield (in gm)	% Yield w/w
FMC (fruits of *Momordica charantia*)	PFMC	Yellowish green	5.54	6.93
	EFMC	Brown	4.72	5.9
	AFMC	Dark Brown	16.05	20.06
	QFMC	Greenish brown	13.08	16.35
LPG (leaves of *Pongamia glabra*)	PLPG	Greenish brown	1.624	2.03
	ELPG	Brown	2.94	3.675
	ALPG	Dark Brown	6.264	7.83
	QLPG	Brownish Black	5.024	6.28
FPN (fruits of *Piper nigrum*)	PFPN	Yellowish green	2.304	2.88
	EFPN	Brown	1.664	2.08
	AFPN	Dark Brown	10.158	12.698
	QFPN	Greenish brown	8.904	11.13

Where, PFMC- Petroleum ether Extract of *Momordica charantia* fruits, EFMC-Ethyl acetate extract of *Momordica charantia* fruits, AFMC- Ethanolic extract of *Momordica charantia* fruits, QFMC- Aqueous extract of *Momordica charantia* fruits, PLPG- Petroleum ether extract of *Pongamia glabra*leaves, ELPG-Ethyl acetate extract of *Pongamia glabra* leaves, ALPG- Ethanolic extract of *Pongamia glabra* leaves, QLPG- Aqueous extract of *Pongamia glabra* leaves, PFPN-Petroleum ether extract of *Piper nigrum* fruits, EFPN-Ethyl acetate extract of *Piper nigrum* fruits, AFPN- Ethanolic extract of *Piper nigrum* fruits, QFPN-Aqueous extract of *Piper nigrum* fruits.

Table 4. Fasting blood sugar level of experimental groups

Groups	Before STZ induction mmol/L	At day 3 after STZ Induction	At day 10 of the Treatment
Control	5.78±0.31	6.32±0.22	5.77±0.29
GXII	5.55±0.21	18.05±2.13	21.04±2.88
Standard	6.15±0.31	21.54±2.42	14.53±2.25
OAQMC	7.94±3.12	15.21±0.89	15.84±3.14
OAQ(MC+PN)	7.58±2.46	16.53±2.45	15.08±3.44
OAQPG	8.05±2.11	15.71±3.49	16.04±4.25
OAQ(PG+PN)	7.76±1.24	15.98±1.88	15.46±0.21
OAQ(MC+PG+PN)	7.39±3.50	15.44±2.46	14.77±1.72
TF1	7.08±1.25	14.96±2.58	14.15±2.63
TF2	6.93±1.11	14.45±3.47	13.84±0.25
O3TF1	6.78±3.45	14.2±3.29	13.57±1.85
O3TF2	6.39±0.28	13.41±2.74	12.79±2.14

Where, AQ stands for alcoholic and aqueous extract in 1:1 amount,**Group I:** Control group which received simple vehicle (Ointment base), **Group II:** Standard group received Povidone iodine ointment, **Group III:** Test group received MC extract orally **[AQMC]**, **Group IV:** Test group received MC and PN extract orally **[OAQ(MC+PN)]**, **Group V:** Test group received PG extract orally **[OAQPG]**, **Group VI:** Test group received PG and PN extract orally **[OAQ(PG+PN)]**, **Group VII:** Test group received MC, PG and PN orally **[OAQ(MC+PG+PN]**, **Group VIII:** Test group received MC, PG and PN extract formulation F1 (10%) topically **[TF1]**, **Group IX:** Test group received MC, PG and PN extract formulation F2 (15%) topically **[TF2]**, **Group X:** Test group received MC, PG and PN orally and formulation F1 (10%) topically **[O3TF1],Group XI:** Test group received MC, PG and PN orally and formulation F2 (15%) topically **[O3TF2]**, **Group XII:** Anemic DiabeticGroup received no treatment **[GXII]**

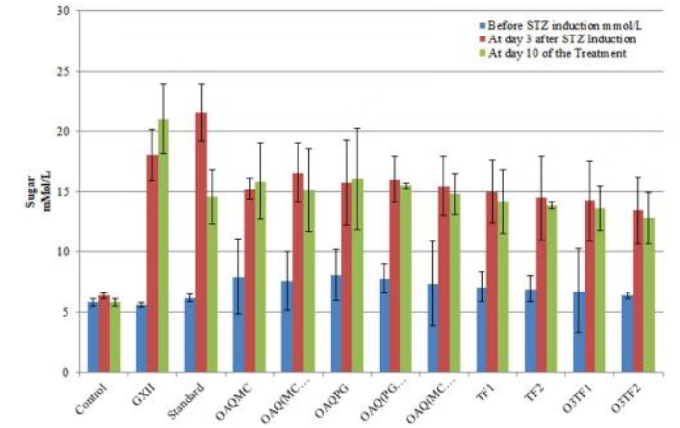

Figure 4. Fasting blood sugar level of experimental groups

increase the Hb level and brought back Hb level towards normal. Streptozotocin is able to cause anemia in animals due to its oxidative stress. Appearance of Heinz bodies in red blood cell morphology proves the presence of anemia and it maybe a type of hemolytic anemia.

Histology of excision biopsy at day 10 demonstrated healed skin structures of skin wounds with normal epithelization, the restoration of adnexa and fibrosis within the dermis in all of the formulations and standard treated groups. The control group lagged behind the treated groups with respect to the formation of the amount of ground substance in the granulation tissue, as observed in tissue sections (Figure 6.1 to 6.6).

Table 5. Hematological parameters after treatment with extracts and formulations

Groups	RBC Count (X10^12/L)	Haemoglobin (g/Dl)
Control	8.76±0.65	15.82±0.59
GXII	6.18±0.47*	9.82±0.49*
Standard	8.13±0.53**	14.84±0.68**
OAQMC	6.21±0.42	13.2±0.56*
OAQ(MC+PN)	6.23±0.37*	13.26±0.74*
OAQPG	6.19±0.55*	13.17±0.80*
OAQ(PG+PN)	6.35±0.61*	13.43±0.89*
OAQ(MC+PG+PN)	6.77±0.70**	13.62±0.70**
TF1	6.84±0.51**	13.78±0.83**
TF2	6.98±0.64**	13.82±0.99**
O3TF1	7.88±0.80**	14.58±0.42**
O3TF2	8.04±0.23**	14.72±0.19**

Values are expressed as mean ± SD (n=6).,* P<0.001 compared with control group, **P<0.001 compared with negative control group

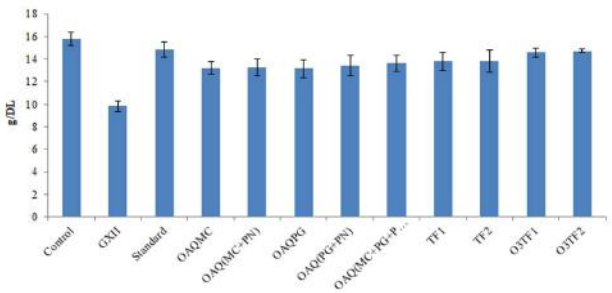

Figure 5. Haemoglobin (g/dl) Level after Treatment with various samples

Table 6. Excision wound model on anemic rats

Groups (n)	0th day	1st day	5h day	10th day	Epitheliza-tion period (Days)
Control	191.43±1.21	190.12±0.82 (0.68%)	139.45±0.78 (27.15%)	59.74±0.50 (68.79%)	18.23±0.19
Standard	191.76±1.20	191.54±0.56 (0.11%)	167.38±0.58 (12.71%)	46.87±0.69* (75.56%)	15.51±0.21*
OAQMC	190.8±1.14	189.22±1.17 (0.83%)	170.58±1.21 (10.60%)	50.55±1.67 (73.51%)	17.54±1.67
OAQ(MC+PN)	192.25±1.69	191.03±2.14 (0.63%)	169.44±1.57 (11.86%)	48.58±0.92* (74.737%)	15.98±0.98*
OAQPG	189.79±1.60	188.24±1.49 (0.82%)	171.88±1.59 (9.44%)	52.49±1.41 (72.34%)	17.76±2.03
OAQ(PG+PN)	190.48±1.77	189.56±1.74 (0.48%)	169.86±1.93 (10.83%)	48.57±1.49 (74.50%)	16.27±1.85
OAQ(MC+PG+PN)	188.56±2.00	187.44±2.03 (0.59%)	168.77±2.07 (10.50%)	46.92±2.53* (74.77%)	15.88±2.27*
TF1	191.52±2.03	190.88±2.48 (0.33%)	160.79±1.91 (16.05%)	38.65±1.26* (79.82%)	14.98±1.49*
TF2	190.74±1.88	189.11±1.77 (0.85%)	162.46±187 (14.83%)	38.89±1.69* (79.61%)	14.75±2.13*
O3TF1	193.51±2.14	192.06±2.56 (0.75%)	164.89±2.30 (14.79%)	38.48±1.86* (80.1%)	14.06±2.57*
O3TF2	189.82±2.53	188.93±2.94 (0.47%)	165.28±2.60 (12.93%)	37.24±3.06* (80.38%)	13.81±2.76*
GXII	187.88±2.49	186.79±2.57 (0.58%)	167.41±2.37 (10.90%)	59.73 ±1.72 (68.21%)	18.87±2.55

Note: n =6 animals in each group, values are expressed as Mean ±SEM, If *=p<0.05, **=p<0.01, ***=p<0.001 when compare to control.

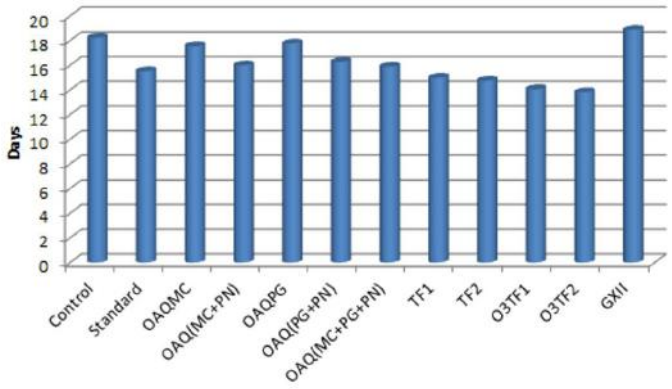

Figure 6.1 Epithelization period (days) of different groups

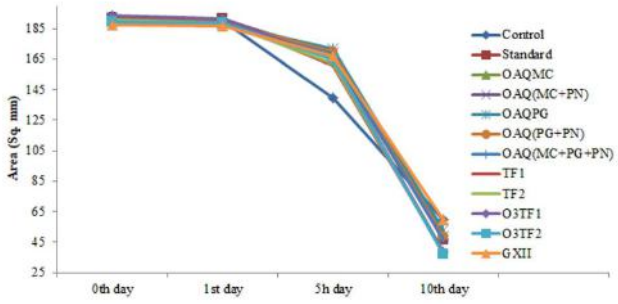

Figure 6.3 Effect of extracts and formulations on excision wound model

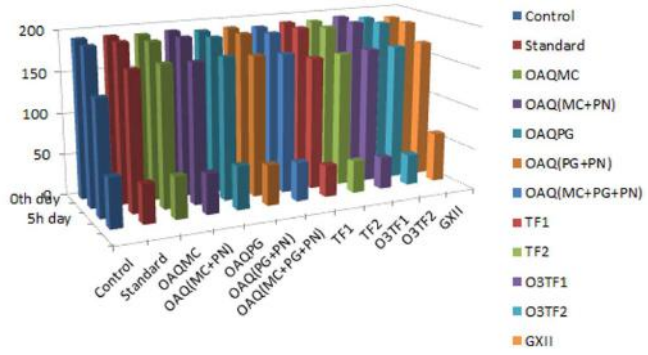

Figure 6.2 Area of wound closure in different groups on excision wound model

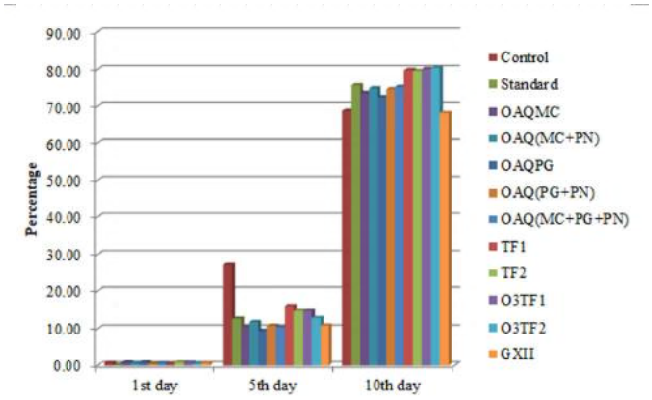

Figure 6.4 Percentage of wound closure in animal groups at different days

Discussion

Wound contraction can be defined as the centripetal movement of the edges of a full-thickness wound in order to facilitate closure. The data obtained on wound size reduction revealed that the percentage of wound contraction ranged from 17.44% to 33.56% in the period from 1 to 5 days and from 0.11% to 27.15% in the period from 05 to 10 days in the control (ointment base) group of rats, whereas whole epithelisation and healing were shown. The shedding of Eschar lasted for an average of 18.23 days without leaving any residual raw

Table 7. Percentage of wound closure in animal groups at different days

Groups (n)	0^{th} day	1^{st} day	5^{th} day	10^{th} day
Control	0.00	0.68	27.15	68.79
Standard	0.00	0.11	12.71	75.56*
OAQMC	0.00	0.83	10.60	73.51
OAQ(MC+PN)	0.00	0.63	11.86	74.73
OAQPG	0.00	0.82	9.44	72.34
OAQ(PG+PN)	0.00	0.48	10.83	74.50
OAQ(MC+PG+PN)	0.00	0.59	10.50	75.12*
TF1	0.00	0.33	16.05	79.82*
TF2	0.00	0.85	14.83	79.61*
O3TF1	0.00	0.75	14.79	80.11*
O3TF2	0.00	0.47	12.93	80.38*
GXII	0.00	0.58	10.90	68.21

Note: n =6 animals in each group, values are expressed as Mean ±SEM, If *=p<0.05, **=p<0.01, ***=p<0.001 when compare to control.

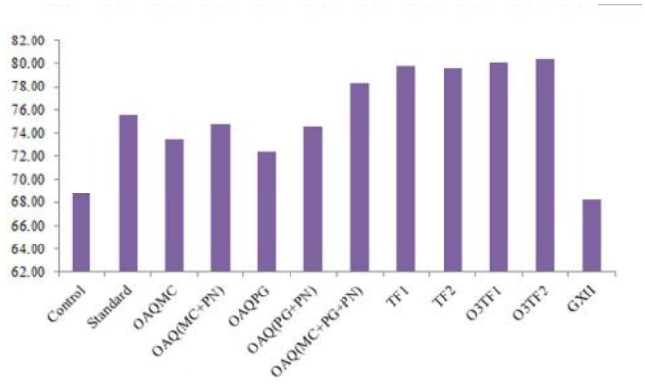

Figure 6.5 Comparison between different groups on percentage of wound closure on 10^{th} day

wound in the control rats.

The % of wound contraction in rats treated externally with TF1 ranged from 0.33% to 16.05% in the period from 0 to 5 days and from 16.05% to 79.82% in the period from 05 to 10 days, respectively. The % rate of wound contraction in rats treated externally with F-2 ranged from 0.85% to 14.83% in the period from 01 to 05 days and from 14.83% to 79.61% in the period from 05 to 10 days, respectively (Figure 6.5)**.** The povidone iodine treated rats showed increased wound contraction from 0.68% to 27.15% in the period from 01 to 05 days and from 27.15% to 68.79% in the period from 05 to 10 days, respectively.The % rate of wound contraction in rats treated externally with OAQMC ranged from 0.83% to 10.60% in the period from 01 to 05 days and from 10.60% to 73.51% in the period from 05 to 10 days, respectively.

The % rate of wound contraction in rats treated externally with GXII ranged from 0.58% to 10.90% in the period from 01 to 05 days and from 10.90% to 68.21% in the period from 05 to 10 days, respectively. The overall epithelialisation time can be presented as: GXII > Control > OAQPG > OAQMC >OAQ (PG+PN)> OAQ(MC+PN)> OAQ(MC+PG+PN) > Standard > TF1 > TF2 > O3TF1 > O3TF2. The studies on excision wound healing model revealsthat all the twelve groups showed decreased wound areafrom day to day (Figure 6.1 to 6.6). There was no mortality observed in the course of study. However, on 10^{th} post wounding day,Group-I animals showed 68.79% of healing (which may be due to self-immunity of the animals) where as Povidone iodine treated animals showed 75.56% healing.On the other hand, the herbal formulation treated group test-1 (TF-1) showed79.82% of wound healing. Also, hebral treated group test-2 (TF-2) showed 79.61% healing (Table 8 and Figure 6.4).

The wound healing contraction and epithelialisation were faster in O3TF2 followed by O3TF1 when compared to control. In the drug treated rats the wounds were completely healed in less than 15 days whereas in the control animals it took more than 15 days. Even on the 10th day the wound contraction was 80.38% and 80.12% in the treated rats O3TF2 and O3TF1 respectively whereas it was only 68.79% in the control. Statistical analysis showed very encouraging results. When compared with control and drugs alone (OAQMC and OAQPG), they showed significant activity (P<0.01). A highly significant activity of O3TF2 and O3TF1 was observed, when compared to control (P<0.001). It was also observed that when the formulations were given through oral and topical route the wound healing was highly significant when compared to control (P<0.001).

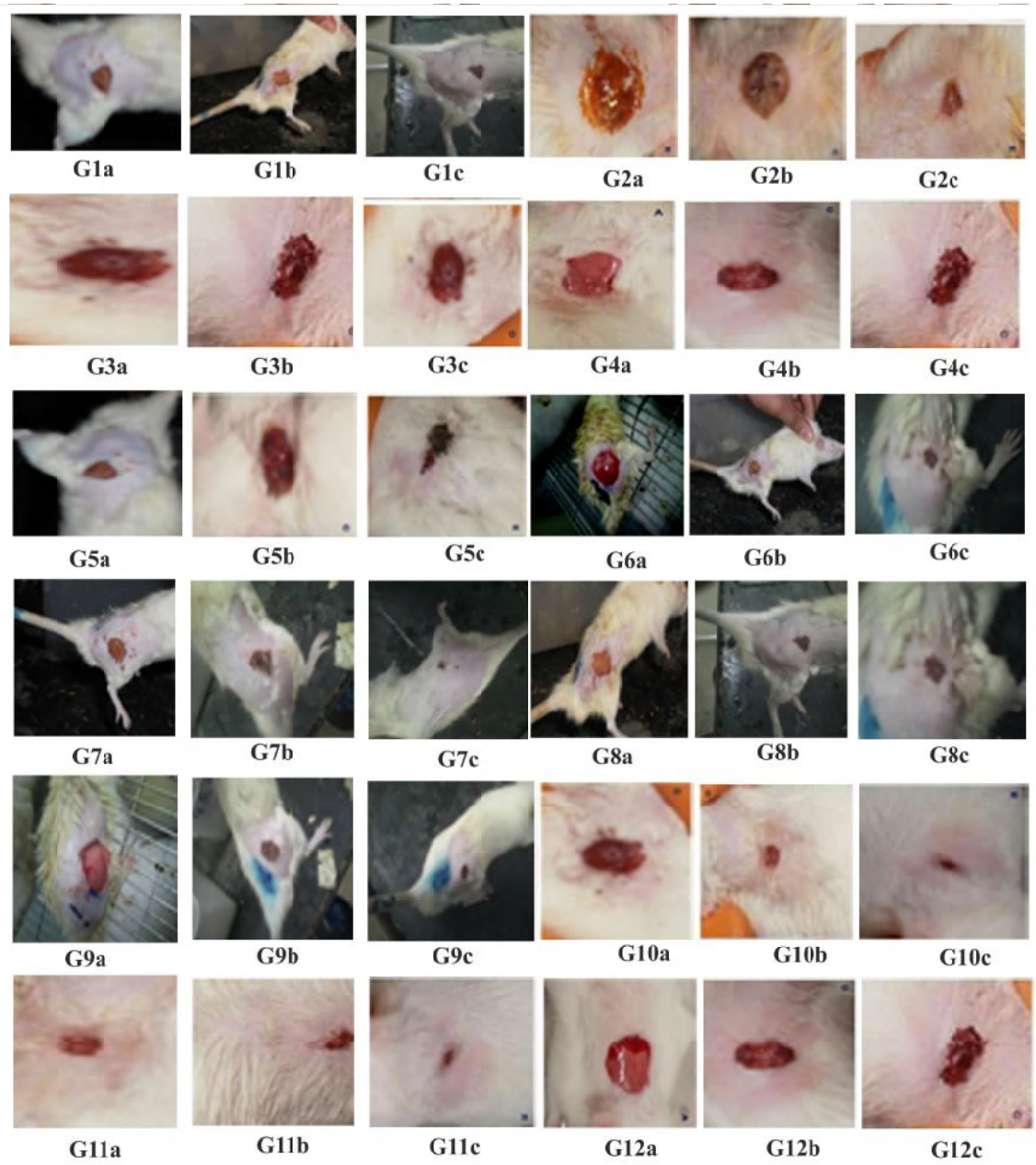

Figure 6.6. Excision wound healing activity: Excision wounds on 0, 5 and 10th day a-c for G1 Control group, G2 = Standard group, G3 = OAQMC group, G4 = OAQ(MC+PN) group, G5 = OAQPG, G5 = OAQPG, G6 = OAQ(PG+PN), G7 = OAQ(MC+PG+PN), G8-=TF1, G9- TF2, G10=O3TF1, G11=O3TF2, G12=XII respectively

Conclusion

Wound healing in diabetes is a complicated and delayed process in which wound healing follows granulation, collagenation and scar formation. Hyperglycemia suppress cell proliferation collagen production.In the present research work ointment formulations with extract of three herbal drugs was prepared and evaluated for the aneamic and wound healing activity by excision wound model in animals. The three individual drug extracts e.g. *Momordica charantia* fruits, *Pongamia glabra* leaves and *Piper nigrum fruits* has shown wound healing properties but the formulation F2 has shown significant results in wound healing. The extracts prepared by using soxhletion method were incorporated in the ointment base for formulation. After completion of formulation they were evaluated for its physicochemical parameters like colour, odour, pH, spreadability, extrudability, consistency, diffusion study, solubility, washability. Also the formulations were evaluated for its stability at various temperature conditions which shows no change in the irritancy, spreadability and diffusion study. Thus it could become a medium to use the medicinal properties of extracts effectively and easily as a simple dosage form.

All the results indicated the effectiveness of herbal ointment F2 in enhancing wound healing activities. The drug

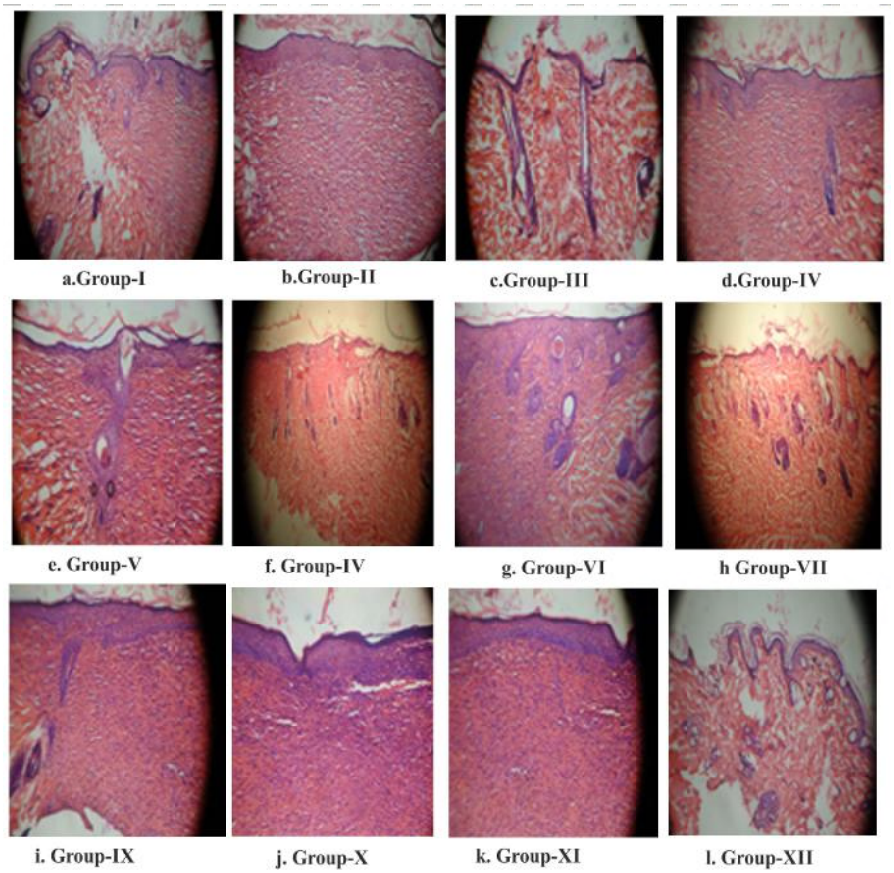

Figure 7. Histopatholgical observation of wound tissues: Where, AQ stands for alcoholic and aqueous extract in 1:1 amount, **Group I:** Control group which received simple vehicle (Ointment base), **Group II:** Standard group received Povidone iodine ointment, **Group III:** Test group received MC extract orally **[AQMC]**, **Group IV:** Test group received MC and PN extract orally **[OAQ(MC+PN)]**, **Group V:** Test group received PG extract orally **[OAQPG]**, **Group VI:** Test group received PG and PN extract orally **[OAQ(PG+PN)]**, **Group VII:** Test group received MC, PG and PN orally **[OAQ(MC+PG+PN]**, **Group VIII:** Test group received MC, PG and PN extract formulation F1 (10%) topically **[TF1]**, **Group IX:** Test group received MC, PG and PN extract formulation F2 (15%) topically **[TF2]**, **Group X:** Test group received MC, PG and PN orally and formulation F1 (10%) topically **[O3TF1], Group XI:** Test group received MC, PG and PN orally and formulation F2 (15%) topically **[O3TF2]**, **Group XII:** Anemic Diabetic Group received no treatment **[GXII].**

Momordica and Pongamia when administered orally and topically with piper they had shown significant synergistic activity, The herbal ointment prepared from extract of *Momordica charantia* fruits, *Pongamia glabra* leaves and *Piper nigrum* fruits showed marked reduction in wound area in comparison to control group when examined for wound healing activity by topical application in albino rats. The formulation will be helpful in anemic and wound healing with no side effects and will be beneficial for society and industry with standardization approaches. The present studies concluded through the excision model that the wound healing property of individual drug extracts *Momordica charantia* and *Pongamia glabra* can be enhanced by the concomitant use of *Piper nigrum* within the oral and topical formulations. The topical formulation containing 15% of extracts of *Momordica charantia, Pongamia glabra* and *Piper nigrum* in ointment formulation can be used as marketed formulations and its effect can be enhanced with intake of its oral doses besides the topical application.

Conflicts of interest

The author declares no conflicts of interest.

References

Bandivdekar AH, Moodbidri SB. 2002. Spermicidal activity of seed oil of Pongamia glabra. Archieve of Andrololgy, 48(1):9-13.

Chia YY, Yap WS. 2011. In vitro Antimicrobial activity of hexane: petroleum ether extracts from fruits of *Momordica charantia* L. International Journal of Pharmaceutical and Biological Archives, 2(3):868-873.

Chopade VV, Tankar AN. 2008. Pongamia Pinnata: Phytochemical constituents, traditional, and pharmacological properties: A review. International Journal of Green Pharmacy, 2:72-75.

Enoch S, John LD. 2005. Basic Science of wound healing. Ethnopharmacology, 23:37-42.

Guo S, Dipietro LA. 2010. Factors affecting wound healing. Journal of Dental Research, 89(3):219-229.

Herror S, Beknal S, Mahurker N. 2012. Immunomodulatory activity of methanolic extracts of Pongamia glabra Vent seeds and bark in cyclophosphamide induced mice. RGUHS Journal of Pharmaceutical Science, 2(1):74-77.

Jayasooriva AP, Sakono P. 2000. Effects of Momordica charantia powder on serum glucose levels and various lipid parameters in rats fed with cholesterol-free and cholesterol-enriched diets. Journal of Ethanopharmacology, 72(1-2):331-336.

Karsha P, Bhagyalaxmi O. 2009. Antibacterial activity of Piper Nigrum Linn with special reference to its mode of action on bacteria, Indian Journal of Natural Products and Resources, 1:213-215.

Khan AW, Kotta S, Ansari SH, Sharma RK, Kumar A, Ali J. 2013. Formulation development, optimization and evaluation of aloe vera gel for wound healing. Pharmacognosy Magzine, 1:S6-S10.

Krishnan SK, Pavan KG. 2013. Evaluation of antimicrobial activity of roots of Pongamia glabra vent. International Journal of Pharmaceutical and Chemical Sciences, 2(1):303-304.

Kumar S, Sripriya R, Raghavan VH. 2006. Wound healing potential of Cassia fistula on infected albino rat model. Journal of Surgical Research, 23:283-289.

Leelaprakash G, Caroline Rose, Gautham BM, Jivaji PK, Shivram P. 2011. In-vitro, antimicrobial and antioxidant activity of *Momordica charantia* leaves. Pharmacophore, 2(4):244-252.

Mehanni SS, Ibrahim NF, Hassan AR, Rashed LA. 2013. New approach of bone marrow-derived mesenchymal stem cells and human amniotic epithelial cells applications in accelerating wound healing of irradiated albino rats. International Journal of Stem Cell, 6(1):45-54.

Nabi SA, Kasetti RB, Sirasanagandla S, Tilak K,Venkateshwarulu JK, Appa RC. 2013. Anti diabetic and antihyperlipidemic activity of Piper longum root aqueous extract in STZ induced diabetic rats. BMC Complementary and Alternative Medicine, 2:13-37.

Nayak BS, Sandiford S, Maxwell A. 2007. Evaluation of the wound-healing activity of ethanolic extract of *Morinda citrifolia* L. leaf. Evidence Based Complement Alternative Medicine, 6(3):351-356.

Patel R, Mahobia N, Patel Z. 2010. Analgesic and antipyretic activities of *Momordica charantia* Fruits. Journal of Advance Pharma Technology Research, 1(4):415-418.

Rajaram P, Mohan N, Vijayan G. 2012. Wound healing property of aqueous extract of seed and outer layer of *Momordica charantia* L. on albino rats. Indian Journal of Pharmaceutical Science and Technology, 5(1):1936-1940.

Rawat P, Gill NS, Arora R. 2012. Study of *Momordica charantia* seeds for their antioxidant Potential. International Journal of Natural Product Science, 1:223-227.

Sathish KD, Vamshi SK, Yogeswaran P, Sudhakar K, Sudha P. 2010. A Medicinal property of *Momordica charantia*. International Journal of Pharmaceutical Sciences Review and Research, 1(2): 95.

Sawant SE, Tajane MD. 2016. Formulation and evaluation of herbal ointment containing Neem and Turmeric extract. Journal of Scientific and Innovative Research, 5(4):149-151.

Sharma MC, Joshi C. 2004. Plants used in skin diseases of animal. Natural product radiance, 3(4):293-299.

Singh RK, Pandey BL. 1996. Anti-inflammatory activity of seed extracts of Pongamiapinnata in rat. Indian Journal of Physiology and Pharmacology, 40(4):355-358.

Sudjarwo SA. 2005. The potency of piperine as anti-inflammatory and analgesic in rats and Mice. Folia MedicaIndonesiana, 194.

Sunila ES, Kuttan G. 2004. Immunomodulatory and antitumor activity of *Piper longum* Linn. and piperine. Journal of Ethnopharmacology, 90(2-3):339-346.

Toeh SL, Latiff AA, Das S. 2009. The effect of topical extract of *Momordica charantia* (bitter gourd) on wound healing in nondiabetic rats and in rats with diabetes induced by streptozotocin. Clinical Experimental Dermatology, 34(7):815-822.