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EXTRACTION, PHYTOCHEMICAL ANALYSIS AND WOUND HEALING ACTIVITY OF EXTRACT OF PLANT *PISTACIA INTEGRRIMA*

Shubham Soni*, Dr. Satkar Prasad
RKDF School of Pharmaceutical Sciences, Bhopal (M.P.)

***Correspondence Info:**
Shubham Soni
RKDF School of Pharmaceutical Sciences, Bhopal (M.P.)
Email:
sonishubham199898@gmail.com

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ABSTRACT

According to current estimates, roughly 6 million people worldwide suffer from chronic wounds. Topical antibiotics are often administered in clinical settings in wounds, cuts, and burns for their potential use in localised cutaneous infections. However, regular and excessive use of topical antibiotics can lead to complications such as the development of resistant organisms. Plants are more effective healers since they naturally aid in the recuperation process. Thus, this study focus on checking wound healing activity of extract of plant *Pistacia integrrima.* The plant material was collected, extracted and analysed in in vitro & in vivo settings for wound healing activity. Results showed that plant contain carbohydrate, proteins, saponin, flavonoid, tannin & phenol. The total phenol & flavonoid content was found to be 0.316 & 0.244 respectively. The tensile strength in hydroalcoholic extract of *Pistacia integerrima* 200 & 400 mg/kg was found to be 1.92±0.32 Kg & 2.15±0.14 kg respectively. In the group treated with standard drug Cipladine the tensile strength was found to be 1.89±0.15 Kg. The wound contraction on 15 th day was found to be 6.3±6.45 for Standard drug Cipladine. While for HAPI (200mg/kg & 400mg/kg) was wound diameter was found to be 17±3.65 & 4.5±3.47 respectively. There was a significant increase in hydroxyproline content, which was 80.25 & 86.65 µg/gm in the HAPI 200mg/kg & 400mg/kg respectively, which was significantly higher than the disease control and standard drug treated groups, which had values 43.58 & 67.78 µg/gm respectively. In the current investigation, control and standard medication treated animals had substantially lower collagen content 315.47 & 502.32 µg/gm respectively than HAPI 200mg/kg & 400mg/kg treated groups, which had collagen concentrations of 601.74 & 635.85 µg/gm, respectively. The hexosamine level in animal tissues was determined to be 26.65 28.74 mg/gm HAPI 200mg/kg & 400mg/kg treated groups, respectively, while 9.65 21.45 mg/gm in the disease control and conventional medicine treated groups. Thus from results it is clear that *Pistacia integrrima* possess effective wound healing activity.

Keywords: Wound healing, Medicinal plants, *Pistacia integerrima,* Phytochemicals, Cipladine, hexosamine, hydroxyproline, collagen

INTRODUCTION

The skin protects the body's outside. Skin regulates temperature, repels water, synthesises a variety of beneficial substances such as vitamin D, and most significantly, acts as a protective barrier between the external environment and inside tissue. According to the medical definition, abrasion of the skin or underlying tissue caused by an accident, act of violence, or other cause. When the skin is

wounded, the wound healing process begins. Wound healing is defined as a complex process involving several substances such as soluble mediators, blood cells, extracellular matrix, and parenchymal cells. Wound healing is classified into three stages: inflammation, tissue creation, and tissue remodelling. Platelet accumulation, coagulation, and leukocyte migration are all steps of the inflammatory phase. Tissue creation occurs in stages such as re-epithelialization, angiogenesis, fibroplasia, and wound contraction. The remodelling phase may last one month, and the dermis may adapt to injury by producing collagen and matrix proteins before returning to its pre-injury phenotype (Velnar *et al*., 2009; Enoch and Leaper, 2005).

According to current estimates, roughly 6 million people worldwide suffer from chronic wounds. There are extremely few Indian studies on wound epidemiology. The wound prevalence in the population investigated was 15.03 per 1000. Chronic wound prevalence in the community was reported to be 4.5 per 1000 people, but acute wound prevalence was nearly twice at 10.5 per 1000 population. The fundamentals of topical wound therapy today include the removal of necrotic tissue, the control of bacterial loads, the management of wound exudates, the preservation of open proliferative wound edges, and the provision of a moist and protected wound surface (Gupta *et al*., 2004; James and Rosso, 2011)

In an attempt to aid wound repair, medical treatment of wounds includes the injection of medications either locally (topical) or systemically (oral or parenteral), or both. Topically applied antimicrobial dressings such as disinfectants, antiseptics, and antibiotics have a broad spectrum of non-selective antibacterial action (Krahwinkel and Boothe, 2006).

Povidone-iodine complex, chlorhexidine, alcohol, triclosan, hydrogen peroxide, boric acid, silver nitrate, silver sulfadiazine, and sodium hypochlorite are the most often used antiseptic products in clinical practise. Topical antibiotics are often administered in clinical settings in wounds, cuts, and burns for their potential use in localised cutaneous infections. However, regular and excessive use of topical antibiotics can lead to complications such as the development of resistant organisms (Bolton and Fattu, 1994).

As a result, more effective wound healing therapies are required. Many medicinal plants play an important part in the healing process of wounds. Plants are more effective healers since they naturally aid in the recuperation process. Plant-based treatment not only accelerates regeneration but also protects beauty. More than 70% of prepared wound therapy medications are plant-based, 20% are mineral-based, and the remainder are based on animal ingredients. Antiseptic coagulants and wound wash are made from plant-based ingredients. Therapeutic herbs exhibit wound healing effects through a variety of processes, including wound healing modulation, bacterial count reduction, collagen deposition refinement, increased fibrocytes and fibroblasts, and so on (Firdous and Sautya, 2018; Budovsky *et al*., 2015).

Pistacia integerrima (Anacardiace), often known as kakarsinghi, is a well-known medicinal herb. *P. integerrima* is a medium-sized deciduous tree that can grow to be 40 feet tall and is found in the eastern Himalayan range from Indus to Kumaon. P. integerrima

is used in folk medicine to cure a variety of conditions such as hepatitis, liver disease, anti-inflammatory, antidiabetic agent, blood cleanser, gastrointestinal disorders, cough expectorant, jaundice, stomach aches, fever, and diarrhoea (Bibi *et al*., 2015; Uddin *et al*., 2011). Appraising its advantages this study focus on checking wound healing activity of extract of plant *Pistacia integrrima.*

MATERIALS & METHODS

Collection of plant materials

The fruits of selected plant namely *Pistacia integerrima* were identified and collected from various areas of Bhopal on the basis of geographical availability.

Extraction of plant materials by maceration method

Powdered plant drug were weighed (34 gm) of plant drug namely fruits of *Pistacia integerrima* and packed in extraction bottle. The defatted plant drugs were subjected to extraction by hydroalcoholic solvent (ethanol: water; 70:30). The liquid extracts were collected in a tarred conical flask. The solvent removed by distillation. Last traces of solvent being removed under vacuum. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated.

In vivo wound healing activity

Animals

Total 24 Adult Wistar rats (180-200gms) were divided into four groups (control, standard, Hydroalcoholic extract of *Pistacia integerrima* (200mg/kg and 400mg/kg) with 6 animals in each group. Animals were housed under standard environmental conditions of temperature (23^0C) and 12 hours light and dark cycle. All the animals were provided with food and water ad libitum. Study protocol was approved by Institutional Animal Ethical Committee and conducted according to the guidelines of CPCSEA.

Acute dermal toxicity

Swiss albino female mice of 18-22g weight and age of 90 days were used to determine the dermal toxicity of test extracts. The toxicological study was carried out to determine the therapeutic dose of the Hydroalcoholic extract of *Pistacia integerrima* as per the OECD guidelines. Testing of the extract was done by applying the extract at two different concentrations on the shaved dorsal sides of the rats. It was observed that the dose was safe and lower dose was considered for further study (Korani *et al*., 2011).

Animal testing

For the in vivo wound experiment incision and excision wound models were used. Test extracts were prepared and diluted in double distilled water and applied at a dose of 200 mg/kg and 400mg/kg. Test extract was applied topically on the wounded site immediately after creating circular wounds by a surgical blade. The control group of animals was not treated with any drug and wounds were kept open16. Whereas the standard drug treated group of animals were applied with reference drug cipladine (10 % W/W) (Sami *et al*., 2019).

Linear incision wound model

All the animals were anaesthetized with 1:1 ketamine hydrochloride and xylazine and the back hair of the rats were shaved by using a shaving machine and impression was made on dorsal region 1cm away from vertebral column and 5cm away from ear. Linear paravertebral incision of 5cm long was made through the full thickness of the skin.

Wounds were closed with interrupted sutures, which were removed on the 10th day after wound creation. Incision wounds were treated with the extracts daily for 14 days. The Wounds in control group of animals were kept open and was allowed to heal naturally. On 14th day after formation of wound the breaking strength of the wound (in kilograms) and was measured by using Tensiometer (Suguna *et al*., 2002).

Excision wound model

The animals were anaesthetized by injecting intramuscularly ketamine hydrochloride and xylazine in 1:1 concentration. The dorsal fur of the animals was shaved with shaving machine. Impression was made on dorsal region and area of the wound to be created was marked on the back of the animals by picric acid using circular stainless stencil. Using toothed forceps and pointed scissors circular excision wound of 300 to 400 mm2 were created to full thickness along the markings. Wound areas were measured by tracing the wound on transparency sheet with permanent marker by using millimeter based graph paper on days 0, 3rd, 6th, 9th, 12th and 15th for all groups (Diwan *et al*., 1983: Gupta and Jain 2011).

Preparation of test samples for bioassay

The extracts, the reference drug and the vehicle were applied topically once a day till the 15th day. At an interval of every three days, changes in wound area were monitored and also the wound area was evaluated by using graph paper. Percentage of the reduction in wounded area was calculated from wound contraction. Histopathological examination and biochemical parameters were carried out by using tissue specimen isolated from the healed skin of each groups of rat (Akdemir *et al*., 2011).

Biochemical parameters

Circular wound area was excised and evaluated for various biochemical parameters at the end of the study. Especially Collagen content, Hydroxyproline and Hexosamine was estimated for evaluating the healing properties of Hydroalcoholic extract of *Pistacia integerrima* (Dwivedi *et al*., 2017).

Statistical analysis

Results obtained from the two wound healing models have been expressed as Mean ± SD and were compared with the corresponding control group by one way ANOVA test for assessing statistical significance (Rojas *et al*., 2002).

RESULTS AND DISCUSSION

The Preliminary qualitative phytochemical tests revealed the presence of carbohydrate, proteins, saponin, flavonoid, tannin & phenol. Because of their antioxidant and antibacterial activities, terpenoids, flavonoids and vitamin C have been shown to aid wound healing. Furthermore, triterpenoids have been shown to boost collagen content, which is one of the components that promotes wound healing.

Furthermore, wound heating action can be related to Flavonoids and Vitamin C's free radical scavenging activity. Both of these phytochemical groups have been shown to minimise lipid peroxidation not only by preventing and reducing the start of cell necrosis, but also by enhancing vascularity.

The total phenol & flavonoid content was found to be 0.316 & 0.244 respectively. In case of Incision wound model the tensile strength of wound was evaluated. The tensile strength in hydroalcoholic extract of *Pistacia integerrima* 200 & 400 mg/kg was found to

be 1.92±0.32 Kg & 2.15±0.14 kg respectively. In the group treated with standard drug Cipladine the tensile strength was found to be 1.89±0.15 Kg. For the control group, very poor tensile strength of 1.35±0.25Kg was recorded. The tensile strength of a wound is mostly determined by the increase in collagen concentration and fibre stabilisation. The crude extract may help to increase tensile strength by promoting collagen synthesis, maturation, stabilisation, and angiogenesis.

In case of excision wound model the excision wound contraction on 15th day for every group was checked. The process by which the edges of a full thickness wound heal centripetally in order to close the defect is known as wound contraction. Wound contraction reflects the rate at which the unhealed region shrinks after therapy, indicating that the wound will close faster if the drug is effective.

The wound contraction on 15 th day was found to be 6.3±6.45 for Standard drug Cipladine. While for HAPI (200mg/kg & 400mg/kg) was wound diameter was found to be 17±3.65** & 4.5±3.47 respectively.

There was a significant increase in hydroxyproline content, which was 80.25 & 86.65 µg/gm in the HAPI 200mg/kg & 400mg/kg respectively, which was significantly higher than the disease control and standard drug treated groups, which had values 43.58 & 67.78 µg/gm respectively. In general, an increase in hydroxyproline content is responsible for an increase in collagen levels.

In the current investigation, control and standard medication treated animals had substantially lower collagen content 315.47 & 502.32 µg/gm respectively than HAPI 200mg/kg & 400mg/kg treated groups, which had collagen concentrations of 601.74 & 635.85 µg/gm, respectively. The hexosamine level in animal tissues was determined to be 26.65 28.74 mg/gm HAPI 200mg/kg & 400mg/kg treated groups, respectively, while 9.65 21.45 mg/gm in the disease control and conventional medicine treated groups.

Table 1: Preliminary qualitative phytochemical tests for *Pistacia integerrima* extract

Phytoconstituents	***Pistacia integerrima* extract**
i)Primary Metabolites	
Carbohydrates	(+)
Amino acids	(-)
Proteins	(+)
Fats and oils	(-)
ii)Secondary metabolites	
Steroids	(-)
Triterpenoids	(-)
Volatile oils	(-)
Gums and mucilage	(-)

Glycosides	(-)
Saponins	(+)
Flavonoids	(+)
Tannins & Phenol	(+)
Alkaloids	(-)
HE = Hydroalcoholic extract; '+' = Present; '-' = Absent	

Table 2: Total bioactive constituents content of *Pistacia integerrima*

S. No.	Extract	Total phenol	Total Flavonoid
1	Hydroalcoholic extract	0.316	0.244

Table 3: Results of Incision wound model Tensile Strength (kg)

S. No.	Groups	Tensile Strength (kg)
1.	Control	1.35±0.25
2.	Standard Cipladine	1.89±0.15
3.	HAPI (200mg/kg)	1.92±0.32
4.	HAPI (400 mg/kg)	2.15±0.14

Table 4: Effect of Hydroalcoholic extract of *Pistacia integerrima* (HAPI) in excision wound contraction

Group	0 Day	3rd Day	6th Day	9th Day	12th Day	15th Day
Control	386.5±0.28	315.0±4.25	289.0±3.65	138.0±2.85**	60.4±3.6**5	45.5±7.9**
Standard Cipladine	405.30±0.85	236.7±3.26***	149.8±2.85*	61.8±3.45***	23.3±3.47*	6.3±6.45**
HAPI (200mg/kg)	404.8±2.85	259.7±2.15***	187.3±3.41*	75.2±3.65**	53.5±2.58**	17±3.65**
HAPI (400 mg/kg)	398±3.65	202.3±3.54***	90.3±3.26**	38.2±3.47**	18.7±3.45**	4.5±3.47**

Data: Mean± SD *** P<0.05 when compared with control group.

Table 5: Effect of Hydroalcoholic extract of *Pistacia integerrima* on biochemical parameters of wound healing

Group	Hydroxyproline (µg/gm)	Collagen (µg/gm)	Hexosamine (mg/gm)
Control	43.58	315.47	9.65
Standard	67.78	502.32	21.45
HAPI (200mg/kg)	80.25	601.74	26.65
HAPI (400 mg/kg)	86.65	635.85	28.74

Data: Mean± SD *** P<0.05 when compared with control group.

CONCLUSION

The results of this study reveal that the HAPI have the ability to improve wound healing. This observation supports its usage in traditional medicine to treat wounds. The observed efficacy could be due to the presence of various chemicals in the extracts that are known to contribute to this plant's wound healing characteristics. More fractionation and isolation experiments are therefore suggested to find active compound(s) in *Pistacia integerrima* that are responsible for wound healing activity.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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