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POTENTIAL ANTIMICROBIAL, ANTIOXIDANT AND WOUND HEALING PROPERTIES OF THE DIFFERENT EXTRACT OF *PEDILANTHUS TITHYMALOIDES* (L.) POIT. LEAVES

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ABSTRACT

The study aimed to identify the phytoconstituents and to establish the antimicrobial, anti-oxidant and wound healing activity of methanolic and chloroform extracts of leaves of *Pedilanthus tithymaloides* (L.) Poit. The dried and size reduced leaves were subjected to sequential extraction using different solvents in order of their increasing polarity in soxhlet extractor, each of the extracts were then collected and were dried in reduced pressure using a rotary vacuum evaporator. The dried extracts were then subjected to preliminary chemical and pharmacological studies. Each type of components present in the extracts was identified by different chromatographic techniques using specific reagent. The results support some of the folklore claims about usefulness of this plant in the treatment of human ailments, such as in inflammation, in the treatment of wound. From the observation chloroform extract was found to have greater antimicrobial, anti-oxidant and wound healing activity than methanolic extract in terms of percentage wound contraction and period epithelialization in excision model. The results indicate that the different extracts of leaves of *Pedilanthus tithymaloides* (L.) Poit. has significant wound healing activity.

Key words: *Pedilanthus tithymaloides*, Phytosterols, Flavonoids, Wound healing activity.

INTRODUCTION

Nature has been a source of medicinal treatments for thousands of years and plant-based systems continue to play an essential role in the primary health care of 80% of the world's underdeveloped and developing countries (Kalpana K *et al*., 2011)**.** There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine.

The *Pedilanthus tithymaloides* (L.) Poit. belonging to the family Euphorbiaceae is commonly known as "Rangchita" and Plants of Hawaii in Bengali and English language respectively. The plant is a low tropical succulent shrub with milky juice, stems green, widespread, ranging from southern Florida and Mexico to northern South America, the Caribbean to India. It is reported wide of range of healing properties, namely emetic, anti-inflammatory, antibiotic, antiseptic, antihemorrhagic, antiviral, antitumoral, and abortive (Caceres A *et al*., 1995; Renne EP, 1996).

A wound which is disrupted state of tissue caused by physical, chemical, microbial or immunological insult ultimately heals either by regeneration or fibroplasias (Padma M Parekh *et al*., 2009). Wound infections are most common in developing countries because of poor hygienic conditions. *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeroginosa*, *Streptococcus pneumoniae*,

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Klebsiella pneumoniae are some important organisms causing wound infection (Mertz P *et al*., 1993). A wide range of antibiotics is being used at present for treating wound infections, but they are now proved to have adverse effects in the human body, also these pathogens developed resistance to the antibiotics targeted against them. In view of this, so much recent attention has been paid to extracts of biologically active compounds isolated from plant species used in herbal medicine (Essawi T *et al*., 2000).

Wound healing is a complex multifactorial process that results in the contraction and closure of the wound and restoration of a functional barrier (Chattopadhyay D *et al*., 2002). Repair of injured tissues occurs as a sequence of events, which includes inflammation, proliferation and migration of different cell types (Sidhu GS *et al*., 1999). It is consented that reactive oxygen species (ROS) are deleterious to wound healing process due to the harmful effects on cells and tissues. Topical applications of compounds with anti-oxidant properties in patients have shown to improve significantly wound healing and protect tissues from oxidative damage (Thiem B *et al*., 2003).

The present study deals with evaluation of wound healing activity and antimicrobial potential of *Pedilanthus tithymaloides (L.) Poit.* leaves against microorganisms. The antioxidant activity was also studied to understand the mechanism behind the wound healing activity.

MATERIALS AND METHODS

Plant Material

The leaves of the plant were collected from Jalpaiguri, West Bengal, India during September-October. The herbarium of the plant was authenticated by Botanical Survey of India, Howrah, India (Voucher Specimen No CNH/-1-1(56) /2006/ Tech-11/ 1450). As a part of the pharmacognostic study of ash analysis and extractive value determination was performed (Kokate CK, 2000).

Extraction Procedure

The dried leaves of *Pedilanthus tithymaloides* were successively extracted with Petroleum Ether, Chloroform and methanol by continuous hot extraction process using Soxhlet apparatus. The solvent was completely removed under reduced pressure from the different extracts such as Petroleum Ether extract (PEPT), Chloroform extract (CEPT) and methanol extract (MEPT) and stored in vacuum desiccators.

Preliminary Phytochemical screening

The crude chloroform extract and methanolic extract were subjected to preliminary phytochemical screening using specific reagents; the extracts were also subjected to TLC using different solvent systems and specific derivatizing reagents were used to confirm the identity of the phytoconstituents (Harborne JB, 2007).

In Vitro Antioxidant Activity

The antioxidant activity of the leaf extract of plant *Pedilanthus tithymaloides* (Euphorbiaceae) was determined by the assay of reducing power as reported by Naznin Ara and Hasan Nur (2009), 1 ml of plant extract solution (different concentration) was mixed with 2.5 ml phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide [$K_3Fe(CN_6)$] (10g/l), then mixture was incubated at 50°C for 20 minutes. Then, 2.5 ml of trichloroacetic acid (100g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (1g/l) and absorbance measured at 700nm in a laboratory colorimeter. Ascorbic acid was used as standard and phosphate buffer used as blank solution. The absorbance of the final reaction mixture of two parallel experiments was expressed as mean ± standard deviation. Increased absorbance of the reaction mixture indicates stronger reducing power (Naznin Ara *et al*., 2009).

Antimicrobial study

The antimicrobial study was conducted by cup-plate agar diffusion method (Aida P *et al*., 2001; Perez C *et al*., 1990). The different antibacterial stains used in the study were *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacilus subtilis*. Culture media was prepared and after sterilization, was taken in sterilized Petri dishes and the microorganisms were grown by pouring microbial suspension on the solidified media in Petri dishes and incubating them at 30^0C temperature for 24 hrs. After growth of the microorganism, pours were made using borer and different concentration of the extracts were placed in each hole, 10 ppm standard ciprofloxacin solution was used as standard. The Petri dishes were further incubated at 300C for a period of 24 hrs and the diameter of the zone of inhibition was measured. The results were then tabulated. The diameter of zone of inhibition for each concentration was measured thrice against each microbial strain and the result shows the average diameter of zone of inhibition.

Wound Healing Activity

Experimental animals

Male albino Wister rats weighing 140 – 170 g were procured from North Bengal Medical College, Siliguri, West Bengal, India, were placed in wire netted cages in a controlled room temperature 22 ± 1^oC, relative humidity 60 - 70% and with 12 h light and dark cycle. The animals were maintained with pellet diet and water *ad libitum*. The animals were deprived of food for 24 h before experimentation but allowed free access to tap water throughout. Diet pellet was obtained from Hindustan Lever Ltd. All studies were carried out using six rats in each group. A clearance from the institutional animal ethical committee has been obtained for the study.

Experimental methods

For determination of the wound healing activity excision wound model was used (Singh SDJ *et al*., 2005). The animals were divided into six groups (N = 6); group I served as control and received only the ointment base (Paraffin ointment base), the animals of group II were treated with standard Povidone Iodine ointment, the animals of group III received 1%w/w chloroform extract ointment, the animal of group IV received 2%w/w chloroform extract ointment, the animal of group V received 1%w/w methanolic extract ointment and the animal of group VI received 2%w/w methanolic extract ointment. All the animals were treated with twice a day topical application of the ointments.

For development of the wounds, the animals were anesthetized using stabilized diethyl ether and under mild anesthesia the skin of the impressed area were excised to full thickness (about 2 mm) to obtain a wound area of about 500 mm^2 (**Figure 1**). The application of the ointments started from the day after the operation and was continued until the full epithelialization. The areas of the wounds were measured periodically to obtain the percent wound closure. The total time required for the complete epithelialization was also measured (Morton JJP *et al*., 1972). The wound contraction was calculated by percentage of wound contraction= [(Initial wound size - specific day wound size) / Initial wound size] × 100 (Srivastava P *et al*., 2008).

Statistical analysis

The results of these experiments were expressed as mean ± SEM of six animals in each group. The data were statistically evaluated by one-way ANOVA followed by Tukey's pair-wise comparison test. The values of $P<0.01$ were considered as statistically significant.

RESULTS

Phytochemical screening

The preliminary qualitative phytochemical screening of *Pedilanthus tithymaloides* leaves showed the presences of various phytochemical which can be attributed to have antioxidant, antimicrobial and wound healing properties, are represented in Table 1. The results revealed the presence of phytosterols, terpenoids, in Chloroform extract, whereas alkaloids, amino acids, carbohydrates, flavonoids and tannins, in Methanol extract.

***In vitro* antioxidant activity**

Absorbance of chloroform and methanolic extract of *Pedilanthus tithymaloides* leaves and ascorbic acid against concentration has been presented in Table 2. Reducing power of both the extracts of leaves has been shown in Figure 2. The results revealed that the chloroform extract of the leaves possess higher anti-oxidant property than the standard Ascorbic acid and methanolic extract.

Antimicrobial activity

Wound healing may be hampered by the microbial activity that is present on the wounds. The organism Pseudomonas aeroginosa grows as an endosymbiont on the Paederus fuscipes which produces the acid pederin causing the blister dermatitis on the skin. So the antimicrobial activity was tested against five organisms and the results of the antimicrobial activity by the cup-plate agar diffusion method of the plant extracts were presented in Table 3. Both the chloroform and methanolic extract showed remarkable inhibitory effect against five microorganisms, however, chloroform extract exerted greater inhibition towards microorganisms than that of methanolic extract.

Amongst the test organisms used, *Candida albicans* was found to be most sensitive to chloroform extract at all three concentration, followed by *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus and Bacilus subtilis*. For methanolic extract, *Pseudomonas aeruginosa* showed least susceptibility, whereas *Candida albicans* showed higher sensitivity followed by *Staphylococcus aureus*, *Bacilus subtilis* and *Escherichia coli*.

Wound healing activity

After checking for anti-oxidant and antimicrobial activity of the extracts, the wound healing activity of the extracts from the *Pedilanthus tithymaloides* leaves has been observed. Topical application of both the chloroform and methanolic extract ointments at 1% and 2% concentration in excision wound model significantly increased ($p<0.001$) wound contraction rate and shortened the epithelialization time compared with control animals (Table 4). The epithelialisation of the wound was observed on different days for different extracts of leaves, control and standard which are shown in Figure 3 that indicates 2%w/w chloroform extract significantly lowered the time of epithelialization. Figure 4 indicates the % wound healing of chloroform extract, methanol extracts, standard and control at an interval of four days, where chloroform extract showed a very good wound healing activity as compared to that of standard and methanolic extract. However, methanolic extract too has shown significant wound healing activity.

Table 1. Preliminary chemical investigation of these extracts

Phytoconstituents	Chloroform extract	Methanol extract
Fixed oil and Fats	-	-
Phytosterols	+	-
Terpenoids	+	-
Alkaloids	-	+
Amino acids	-	+
Carbohydrates	-	+
Flavonoids	-	+
Tannins	-	+
Saponins	-	-

+: present; - : Absent;

Table 2. Antioxidant Activity of Chloroform and Methanolic Extract of *Pedilanthus tithymaloides* (Euphorbiaceae)

Extract/ Standard	Concentration (ppm)	Absorbance
Chloroform extract	100	0.324
	200	0.426
	400	0.652
Methanol extract	100	0.264
	200	0.342
	400	0.522
Ascorbic acid	100	0.293
	200	0.386
	400	0.596

Table 3. Antimicrobial Activity of Chloroform (CEPT) and Methanolic (MEPT) Extract of *Pedilanthus tithymaloides* (Euphorbiaceae)

Microorganism	Diameter of Zone of Inhibition (mm)						
	10 mg/ml		20 mg/ml		50 mg/ml		Standard
	MEPT	*CEPT*	*MEPT*	*CEPT*	*MEPT*	*CEPT*	
Escherichia coli	06	12	08	18	12	26	32
Pseudomonas aeruginosa	06	10	06	17	10	22	28
Staphylococcus aureus	08	08	10	12	14	16	28
Bacilus subtilis	06	07	09	10	12	15	30
Candida albicans	08	14	15	19	27	30	34

Table 4. Wound Healing Activity of Chloroform and Methanolic Extract of *Pedilanthus tithymaloides* (Euphorbiaceae)

Treatment	Closure of Wound Area (%)				Epithelialization In Days
	Day 4	*Day 8*	*Day 12*	*Day 16*	
Control group	12.10±0.28	28.36±0.11	58.16±0.09	81.50±0.18	26
Std drug treated	15.63±0.09	40.04±0.04	74.75±0.16	98.93±0.15	19
1%w/w chloroform extract	18.15±0.15	38.82±0.07	73.68±0.12	99.16±0.13	18
2%w/w chloroform extract	20.20±0.25	45.07±0.06	99.02±0.13	99.99±0.01	17
1%w/w methanolic extract	12.65±0.16	29.91±0.14	62.61±0.22	91.06±0.23*	24
2%w/w methanolic extract	14.22±0.11	39.22±0.09	72.99±0.08	91.88±0.20*	22

Values are expressed as mean ± SEM (N=6). * $P < 0.001$

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Fig 1. Photographic representation of wound contraction on different post-excision days (1–16 days) Group I—Paraffin ointment base only, Group II— Standard Povidone Iodine ointment, Group III —1%w/w chloroform extract ointment, Group IV—2%w/w chloroform extract ointment, Group V—1%w/w methanolic extract ointment, Group VI—2%w/w methanolic extract ointment.

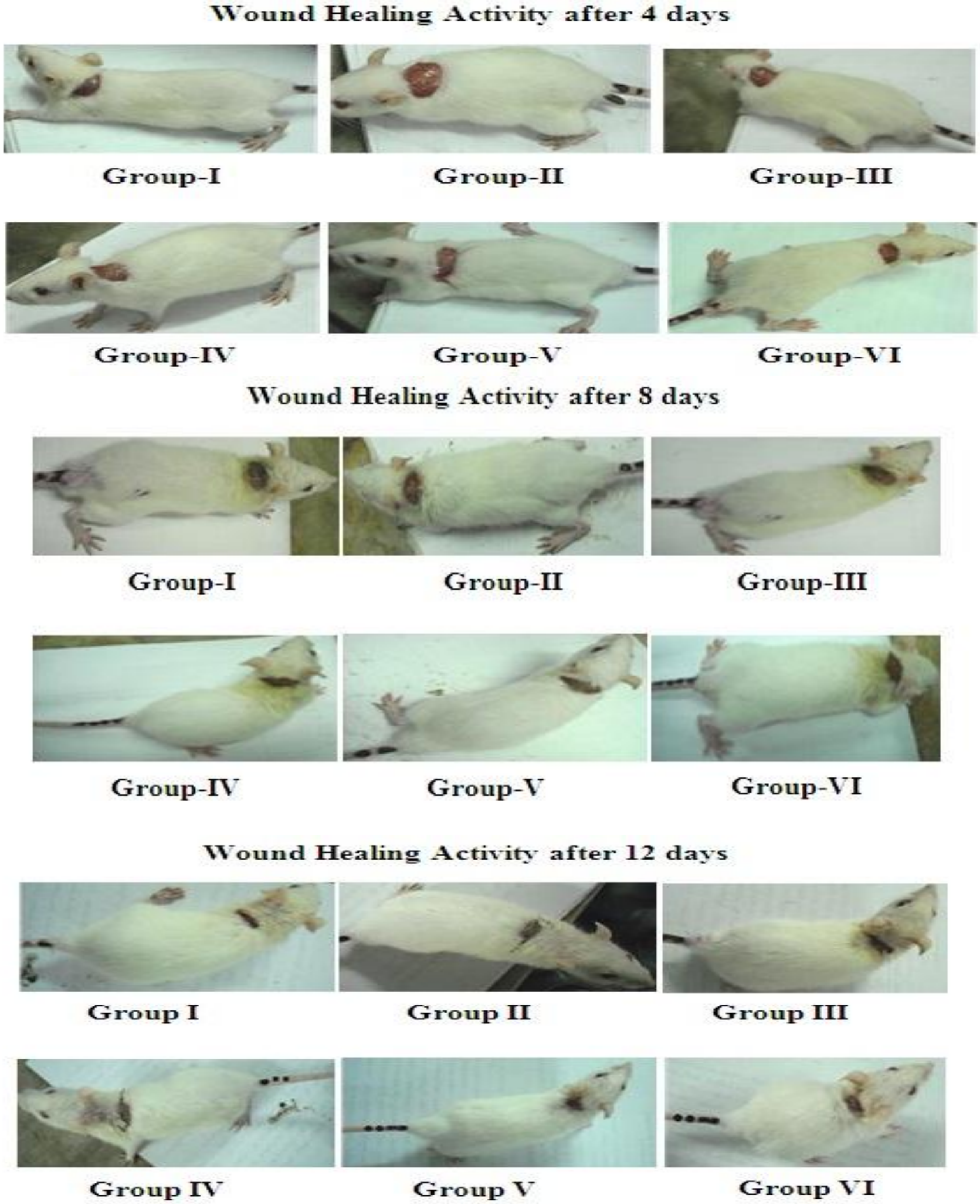


Fig 2. Reducing power of chloroform and methanolic extract of *Pedilanthus tithymaloides* leaves as compared to Ascorbic acid.

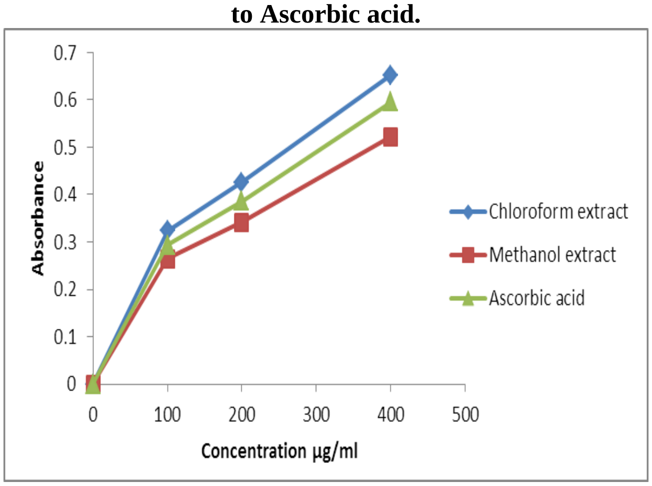


Figure 3. Epithelialization of the wound area in rats. Values are mean ± SD of six determinations.

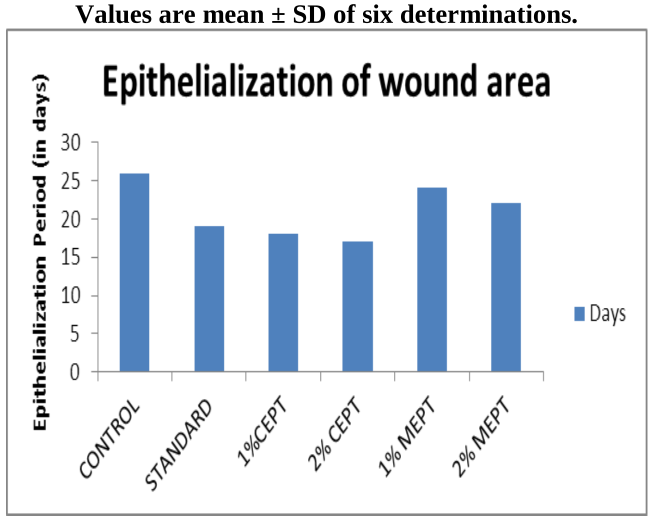
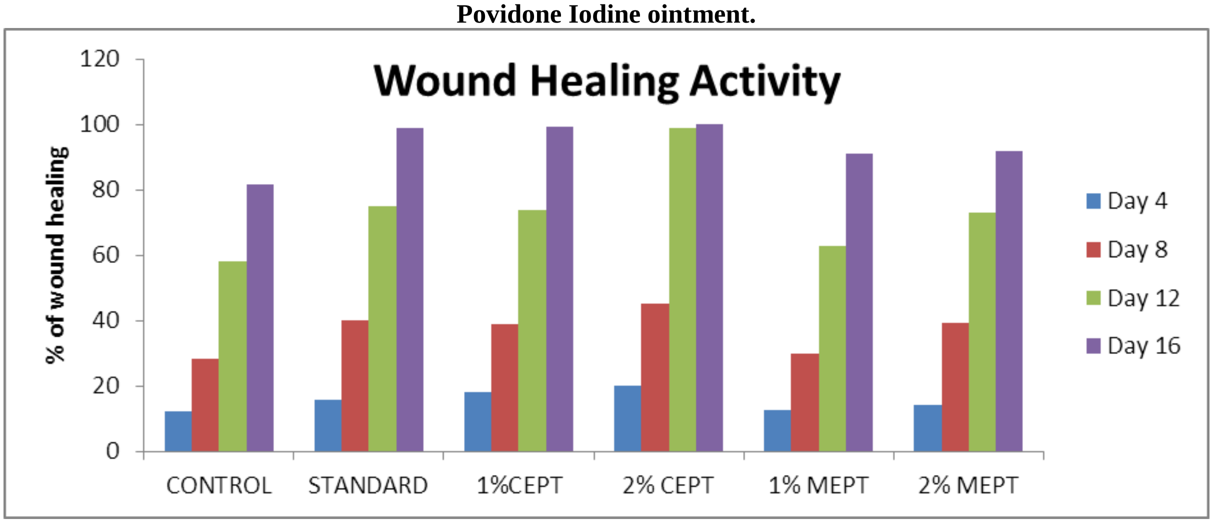


Fig 4. Wound healing activity of CEPT and MEPT at two different concentrations with respect to the standard Povidone Iodine ointment.



DISCUSSION AND CONCLUSION

Many traditional plants remedies are known in folk medicine and used for treatment and some have been validated by scientific studies to actually exert biological action against wound healing or its complications (Atangwho IJ, 2009). This study therefore provided bases to the folkloric use of different plants as a remedy for skin disease caused by the pathogens. It also justifies the folklore medicinal uses about the therapeutic values of these plants as curative agent and therefore, the purification and characterization of the phytochemicals that can be isolated from this plant will be useful as a chemotherapeutic agent (Reuben KD *et al*., 2009). Oxidative stress has been shown to play an important role in the development of wound healing. Indeed, increase in total antioxidant status has been shown to be important in recovery from wound. The plant exhibited potent antioxidant activity in our study. The results suggest that the antioxidant activity of this plant may contribute to its claimed wound healing property. Possibly, the constituents like triterpenoids and alkaloids may play a major role in the process of wound healing. However, triterpenoids and flavonoids are known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialisation (Dash GK *et al*., 2011; Shivananda BN *et al*., 2009).

In excision wound healing model the methanol and chloroform extract of the plant *Pedilanthus tithymaloides* showed significant increase in percentage closure by enhanced epithelialization. This enhanced epithelialization may be due to the effect of *Pedilanthus tithymaloides* extracts on enhanced collagen synthesis. Thus it supports the wound healing activity of *Pedilanthus tithymaloides*. The study reveals that methanolic and chloroform extracts treated groups possess good wound healing properties which may be attributed to the individual or combined action of phytoconstituents like, phytosterols, flavanoids, alkaloids, triterpenoids and tannins.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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