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Wound healing property of *Flacourtia jangomas* plant on incision and dead space wound model

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Abstract
The present study was undertaken to evaluate the wound healing property of medicinal plant *Flacourtia jangomas* on incision and dead space wound model. A total of 96 numbers of Wistar albino rats weighing 120-150 gm were divided into eight groups containing six animals per group for each incision and dead space wound model. Hydro-ethanolic (H/E) and chloroform (C/H) extracts were prepared from the bark of the plant *Flacourtia jangomas*. Plant extracts were administered topically at 1%, 2.5% and 5%. Observations were compared with the control group and povidone iodine (5%) ointment treated group. Results of the present study revealed that wet weight, dry weight, hydroxyprolein and protein content of granulation tissue significantly increases with the above-mentioned plant extract treatment in a dose-dependent manner.

Keywords: *Flacourtia jangomas*, Hydro-ethanolic (H/E), Chloroform (C/H), Hydroxyprolein, povidone iodine (5%)

Introduction
Skin, performs various functions like environment sensing, maintaining homeostasis, being a reservoir of essential nutrients, providing defense against foreign body or pathogens, and responding to trauma and injury (Xu *et al*., 2015) [1]. Maintenance of these critical functions calls for robust and effective mechanisms not only to protect it from trauma but to repair and replace it when damaged or lost.
Wound is defined as a break in the continuity of the tissue brought about by several factors like chemical, physical, thermal, microbial, or immunological injury to the tissue. Wound healing is the natural process by which body regenerates the lost dermal and epidermal tissue. Repair of an injured tissue can be attributed to a sequence of events *viz*. inflammation, proliferation, and infiltration of numerous inflammatory cells (Singh *et al*., 2006) [2].
Antibiotics used in wound infections produce many untoward effects. Scientists have thus started looking for alternatives like biologically active compounds from plant species which are endowed with wound healing properties (Essawi *et al*., 200) [3].
The process of using traditional medicinal remedies and plant based products while treating wounds and burns has not only provided beneficial health effects but has helped reduce the financial burden substantially. Various plants have shown amazing results against several skin disorders.
Flacourtia jangomas, has been used as a traditional medicine for treating various skin ailments, diarrhoea, toothache, jaundice, diabetics, asthma and tumours (Shirona *et al*., 2014) [4]. *F. jangomas* is pharmacologically regarded as diaphoretic, acrid, astringent, analgesic, stomachic, anti-inflammatory, and antimicrobial. Fruits have got antidiabetic property (Jeyachandran and Mahesh, 2007) [5]. *Flacourtia jangomas* contains different phytochemicals which include tannins, carbohydrate, fats minerals, ascorbic acids, tartaric acids, proteins, amino acids and phenolic compounds (Ghani, 2003) [6].
So we have taken up this study with an objective to evaluate wound healing property of *Flacourtia jangomas* plant in rats.

Materials and Methods
Experimental animals
The study was performed by following the guideline of IAEC (Approval No.

770/GO/Re/S/03/CPCSEA/FVSc/AAU/IAEC/18-19/694). A total of 384 numbers of Wistar albino rats weighing 120-150 g were procured from Chakraborty Enterprise, Kolkata. All the animals were kept in polypropylene cages in a small group of 6 rats per cage. Animals had free access to standard balanced ration and clean drinking water *ad libitum* and were maintained in a standard laboratory conditions (12:12 hour light/ dark cycle at ambient temperature ranging between (22-25 ^{0}C).

Preparation of plant extracts

For preparation of hydro-ethanolic extract, about 250 grams of bark was soaked in a 1000 ml mixture of Ethanol and water (7:3 v/v) in a beaker. For chloroform extract, about 250 grams of powdered plant material was soaked in a 1000 ml of solvent chloroform. The mixture was intermittently stirred and kept for 3 days. On 3rd day, the content was filtered with Whatman filter paper No- 1. The process was repeated for three times using fresh solvent. All the three filtrates were collected and concentrated by Rotary Vacuum evaporator and dried on an evaporative dish at a temperature of 50-60 ^{0}C. Semi solid plant extract so obtained was kept in the refrigerator at 4 ^{o}C.

Qualitative analysis of phytochemicals

Preliminary Phytochemical screening was carried out using procedures described by Sofowara (1993), and Harborne (1998) [7, 8].

Determination of *in vitro* antioxidant properties

The plant extracts were assessed *in vitro* for the presence of following antioxidant properties:

1. DPPH (2, 2 diphenyl 2 picryl hydrazyl hydrates) Radical Scavanging Acivity (Cotelle *et al.*, 1996) [9].
2. Superoxide radical scavenging activity (Robak and Gryglewki, 1998) [10].

Preparation of ointments for topical administration

1% (w/w), 2.5% (w/w) and 5% (w/w) concentrations of both extracts were taken to prepare ointments (Mahmood *et al*., 2005) [11] and stored at 4 °C.

Acute dermal toxicity

No visible sign of skin irritation, inflammation and swelling were seen. Therefore, topical application of the plant extracts was found to be safe for the present study.

Incision wound model

Animals were divided into eight groups each containing six animals. Group I was treated with vehicle control. Group II was treated with povidone Iodine (5%) ointment as standard. Group III, IV and V were treated with 1%, 2.5% and 5% w/w H/E ointments prepared from *Flacourtia jangomas* respectively. Group VI, VII and VIII animals were treated with 1%, 2.5% and 5% w/w C/E ointments respectively. Animals were anaesthetized using a combination of xylazine and ketamine i/p before creating wounds. A longitudinal paravertebral incision of three centimetres was made through the skin and muscle of the back (Ehrlich and Hun, 1968) [12]. Experiment was performed in absence of asepsis and antimicrobials (Saha *et al*., 1997) [13]. Wounds were closed using nylon threads (No. 000) and left undressed. The sutures were removed on 10th day and the treatment was carried on till 12th day. On 12th day, breaking strength was measured by following technique (Lee, 1968) [14]

Measurement of healing

The breaking strength is the force required to open a healing wound. How much the healed tissue resists to breaking under tension indicates the quality of healing tissue. Tensiometer was the instrument used to study this parameter (Evans, 1980) [15]. The animals were anaesthetized prior to testing. Sutures were removed. The animal was placed on a stack of adjustable paper towels. The clamps of tensiometer were then clamped on the skin of opposite sides of the wound at a distance of 0.5 cm away from the wound. The longer piece of fishing line was placed on pulley and position of the board was so adjusted that the polyethylene bottle could freely hang in the air. Water was added to the polyethylene bottle till the wound starts opening. The water in the polyethylene bottle was measured and considered as the breaking /tensile strength of the wound. The result was recorded in grams of weight added and the values were expressed as Mean ± SEM.

Dead space wound model:

Animals were divided into a total of eight groups containing 6 in each group. Group I served as control. Group II was treated with Himalaya Purim® tablets @100 mg/kg body weight. Group III, IV and V were administered H/E of 100, 300 and 900 mg/kg body weight P.O. Group VI, VII and VIII were administered C/E of 100, 300, and 900 mg/kg body weight p.o. Animals were anaesthetized prior to creating wounds.

All animals were inflicted by implanting two sterilized cotton pellets (50 mg), one on either side of pectoral region of each rat and kept for 12 days. On the 12th day, the granulation tissue so formed was removed and wet weight was noted. These granulation tissues were dried at 60 °C for 12 hours and the dry weight was recorded. 5 ml 6 N HCl were added to dried tissue and kept at 110 °C for 24 hours. The neutralized acid hydrolysate of the dry tissue was used for hydroxyproline determination.

Estimation of hydroxyproline

10mg dried tissue was placed in an ampoule. 2 ml of 6 N HCl was added and incubated at 110 ^{o}C for 18 hours. The ampoule was broken and a pinch of activated charcoal was added. After 30 minutes, it was filtered and neutralized with Na2CO3 solution (pH 6.5 – 7.0). 1ml of neutralized solution was taken in a test tube along with blank and 2 ml isopropyl alcohol was added to all the test tubes and mixed well. 1ml of Chloramin T (7%) and 2ml of Ehrlich's reagent was added to all the test tubes. The sample was then incubated at 60 ^{o}C for 25 minutes and allowed to cool. OD was measured at 560 nm and the amount of hydroxyproline was determined and expressed in mg/g of dry tissue.

Estimation of protein content in granulation tissue:

Protein was estimated in granulation tissue homogenate with the help of commercially available kit by the Biuret method (Gornall *et al.*, 1949 and Doumas *et al.*, 1975) [16].

Collection of blood and skin tissue

Blood was collected on 12th day by puncturing retro-orbital plexus for dead space wound model and used for estimating antioxidant biomarkers *viz*. superoxide dismutase, reduced glutathione, catalase and malondialdehyde. For this, skin tissue was collected for incision wound model on 12th day.

Results and Discussion

Phytochemical study of the plant extracts

H/E and C/E of *F. jangomas* were found positive for the presence of flavonoids, terpenoids, saponin and steroids. Glycosides were positive only for H/E while alkaloid and tannin was positive only for C/E. Results were presented in Table 1.

DPPH free radical scavenging activity (% inhibition) of hydro-ethanolic (H/E) and chloroform (C/H) extracts of *Flacourtia jangomas*

The DPPH is a stable free radical with maximum absorbance at 517 nm. It can be scavenged easily by any antioxidant and is used for testing the compound's scavenging and antioxidant activity (Lu and Yeap, 2001) [17]

Antioxidant activity of the plant was estimated against the percent inhibition of DPPH at the concentration of 20-100 µg/ml, considering Vit C as standard reference. The maximum activity was reported by the chloroform extract of *Flacourtia jangomas* at the concentration of 100 µg/ml. Hydro-ethanolic extract of the plant manifested significant % inhibition (p<0.05) of 29.67±3.18 and significant % inhibition of (p<0.01) 57.15±3.49 at 20 µg/m and 80 µg/ml respectively, while Vit C activity at 20 µg/m was 42.51±3.21 and 70.91±3.21at 80 µg/ml. At the concentration of 40, 60 and 100 µg/ml, significant (p<0.001) % inhibition of DPPH free radical were 33.11±2.29, 45.86±2.42 and 65.36±5.67% compared to the activity of Vit C which were reported as 53.27±2.34, 66.35±1.96 and 82.43±2.74% respectively. At the concentration of 40 µg/ml, chloroform extract of the plant, manifested significant (p<0.01) % inhibition of 41.22±3.01. At the concentration of 20, 60, 80 and 100 µg/ml chloroform extract didn't show any significant difference with Vit C activity. Maximum activity was recorded at 100 µg/ml which was 73.12±3.98% compared to 82.43±2.74% activity of Vit C.

The antioxidant activity in terms of DPPH radical scavenging activity of H/E and C/E of *Flacourtia jangomas* were presented in Table 2 and Fig. 1.

George *et al.,* 2017, found that at the concentration of 100 µg/ml methanolic extract of *Flacourtia jangomas* flower had maximum scavenging activity of (89.34%) when compared with the standard Quercetin (93.45%) [18] Kumar *et al.,* 2018 found that DPPH IC_{50} value of ethanol and aqueous extract of leaves of *Flacourtia jangomas* were 34.32±0.81 and 37.42±0.37 µg/ml respectively in comparison with Vit C (11.42±0.08 µg/ml). The IC_{50} value of the chloroform, methanol and pet ether extracts of fruit of *Flacourtia jangomas* were recorded as 523.15 µg/ml, 1623.87 µg/ml and 5811.35 µg/ml respectively whereas, the IC_{50} value of antioxidant Vit C was 13.37 µg/ml. Scavenging of DPPH radical was directly proportional with the concentration of the extracts. [19]

Superoxide radical scavenging activity (% inhibition) of hydro-ethanolic (H/E) and chloroform (C/H) extracts of *Flacourtia jangomas* plant

Superoxide radical induces tissue destruction. It generates ROS like hydroxyl radical, hydrogen peroxide, singlet oxygen in living systems. Superoxide anion were produced in a non-enzymatic PMS-NADH system by the reaction of PMS, NADH, and oxygen that can be analysed by the decline of nitro-blue-tetrazolium (NBT).

Antioxidant activity of *Flacourtia jangomas* was evaluated against the % inhibition of superoxide free radical at a concentration of 20-100 µg/ml, considering Vit C as standard reference. Current experiment reveals that, peak activity was seen in the chloroform-extract of the plants at maximum concentration of 100µg/ml. At the concentration of 20-100 µg/ml, superoxide free radical scavenging activity of hydro-ethanolic extract of *Flacourtia jangomas* plant manifested a significant (p<0.001) rise in % inhibition. The activities were recorded as 12.03±2.28, 23.57±3.18, 46.02±2.30, 50.12±1.92 and 57.55±1.8 in comparison with Vit C activity 29.37±1.61, 52.18±1.79, 61.06±2.22, 69.70±2.32 and 73.67±1.88 respectively. At 20, 40, 60, 80 and 100 µg/ml concentration, inflated % inhibition of superoxide free radical for chloroform-extract of *Flacourtia jangomas* plant were recorded to be 18.20±1.72 (p<0.01), 35.13±1.73 (p<0.001), 48.10±4.04 (p<0.001), 58.52±1.61 (p<0.01) and 63.15±2.87 (p<0.01) respectively when compared to Vit C activity.

The antioxidant activity in terms of superoxide radical scavenging activity of hydro-ethanolic and chloroform-extract of *Flacourtia jangomas* plant are presented in Table 3 and Fig. 2.

Kumar *et al.,* 2018 recorded that IC_{50} value of superoxide scavenging activity of ethanol and aqueous extract of leaves of *Flacourtia jangomas* were 148.42±5.83 and 176.82±6.71 µg/ml respectively when compared to Vit C (214.31±5.68 µg/ml) [19].

Effect of hydro-ethanolic (H/E) and chloroform (C/H) extract of *Flacourtia jangomas* plant on breaking strength (gm) for incision wound model

Both hydro-ethanolic and chloroform-extract of *F. jangomas* bark revealed escalating breaking strength in incision wound model. At 1% ointment, hydro-ethanolic extract of *F. jangomas*, exhibited a breaking strength of 246.80±1.78 (P<0.01) gm. Breaking strength was significantly (p<0.001) raised to 248.80±1.45 and 250.00±1.32 gm at 2.5% and 5%, in comparison to the normal control group 234.80±1.70 gm. At 1%, 2.5% and 5%, Chloroform extracts ointment significantly (p<0.001) magnified the breaking strength and they were recorded as 323.70±1.84, 418.30±2.17 and 482.70±1.89 gm respectively when compared with the normal control group 234.80±1.70 gm. Significant (p<0.001) breaking strength was found (518.20±2.70 gm) in povidone iodine (5%) treated group. Results were presented in Table 4 and Fig. 3.

Effect of hydro-ethanolic (H/E) and chloroform (C/H) extract of *Flacourtia jangomas* plant on oxidative biomarkers in tissues for incision wound model

1. Superoxide dismutase (SOD) activity in tissues for incision wound model

In a dose dependent manner, hydro-ethanolic extract ointment of *Flacourtia jangomas* plant increased the activity of superoxide dismutase (SOD) enzyme. In tissues at 1%, 2.5% and 5%, it were recorded as 1.21±0.07, 1.51±0.10 and 1.53±0.17 U/mg protein respectively when compared to the control group 1.17±0.11 U/mg protein. At 1%, 2.5% and 5%, superoxide dismutase activity of chloroform extract treated group were recorded as 2.13±0.14 (p<0.01), 2.54±0.23 (p<0.001) and 2.60±0.22 (p<0.001) U/mg protein respectively, when compared to the control group 1.17±0.11 U/mg protein. SOD activity was recorded to be 2.71±0.08 (p<0.001) U/mg protein in povidone iodine (5%) treated group. Results were presented in Table 5 and Fig. 4.

2. Reduced glutathione (GSH) activity in tissues for incision wound model

In a dose dependent manner reduced glutathione activity was found to be increased after treatment with hydro-ethanolic and chloroform extract-ointments of *Flacourtia jangomas* plant. The activities were recorded as 1.20±0.04 and 2.01±0.08 (p<0.001), 1.81±0.11 (p<0.001), 2.22±0.13 (p<0.001), 2.19±0.09 (p<0.001) and 2.71±0.07 (p<0.001) µgm/mg protein at 1%, 2.5%, 5% respectively, when compared with the control group 1.20±0.09 µgm/mg protein. Activity of GSH was recorded as 2.90±0.11 (p<0.001) µgm/mg protein in povidone iodine (5%) treated group. Results were presented in Table 6 and Fig. 5.

3. Catalase activity in tissues for incision wound model

Treatment with hydro-ethanolic and chloroform-extract ointment of *Flacourtia jangomas* plant, increased the activity of catalase enzyme in tissues in a dose dependent manner. Elevated enzyme activity were recorded as 4.50±0.18 and 5.12±0.17 (p<0.05), 4.93±0.11 and 5.62±0.27 (p<0.001), 5.03±0.14 (p<0.05) and 6.02±0.12 (p<0.001) U/mg protein at 1%, 2.5%, 5% H/E and C/H extracts respectively, when compared to the control group 4.20±0.18 Activity of the catalase enzyme activity was recorded as 6.33±0.16 (p<0.001) U/mg protein in povidone iodine (5%) treated group. Results were presented in Table 7 and Fig. 6.

4. Maondialdehyde (MDA) concentration in tissue lipid peroxidation for incision wound model

MDA concentration in tissue of control group was recorded to be 6.99±0.16 µmol/mg protein, which was reduced by the topical application of H/E and C/H extracts ointment of *Flacourtia jangomas* plant. At 1%, 2.5%, 5% H/E and C/H extract, MDA concentration was found to be 5.97±0.17 and 5.09±0.32 (p<0.05) µmol/mg protein, 5.32±0.35 and 4.04±0.45 (p<0.001) µmol/mg protein, 4.50±0.34 (p<0.001) and 3.40±0.39 (p<0.001) µmol/mg protein respectively in comparison to control group 6.99±0.16 µmol/mg protein. MDA concentration was found to be 3.34±0.07, p<0.001 µmol/mg protein in povidone iodine (5%) treated group. Results were presented in Table 8 and Fig. 7.

Effect of hydro-ethanolic (H/E) and chloroform (C/H) extract of *Flacourtia jangomas* plant on oxidative stress biomarkers in blood for dead space wound model

1. **Superoxide dismutase (SOD) activity in blood hemolysate for dead space wound model**

Hydro-ethanolic extract of *Flacourtia jangomas* plant increased the activity of superoxide dismutase (SOD) enzyme in blood in a dose dependent manner. Activities were recorded as 1.62±0.03, 2.38±0.14 (p<0.01) and 2.43±0.03 (P<0.01) U/mg protein at 100, 300 and 900 mg/kg body weight respectively, in comparison to the control group 1.65±0.03 U/mg protein. For 100, 300 and 900 mg/kg body weight activity of Superoxide dismutase enzyme for chloroform-extract treated group was recorded to be 1.94±0.04, 2.44±0.06 (p<0.01) and 2.59±0.17 (p<0.001) U/mg protein respectively, when compared to the control group 1.65±0.03 U/mg protein. Activity of SOD was recorded as 2.65±0.29, p<0.001 U/mg protein in Himalaya Purim (100 mg/kg) treated group. Results were presented in Table 9 and Fig. 8.

2. **Reduced glutathione (GSH) activity in blood hemolysate for dead space wound model**

Reduced glutathione activity was found to be increased in blood hemolysate after treatment with H/E and C/E of *Flacourtia jangomas* plant in a dose dependent manner. The activities were recorded as 2.90±0.12 and 2.92±0.25 µgm/mg protein. Significant (p<0.001) elevation in the activity of GSH were noted as 3.50±0.03 and 3.40±0.04 µgm/mg protein, 3.29±0.02 and 3.87±0.110 µgm/mg protein at 300 mg/kg body weight and 900 mg/kg body weight respectively when compared to the control group 2.29 ±0.02 µgm/mg protein. Activity of GSH was recorded as 4.38±0.07 (p<0.001) µgm/mg protein in Himalaya Purim (100 mg/kg) treated group. Results were presented in Table 10 and Fig. 9.

3. **Catalase activity in blood hemolysate for dead space wound model**

In a dose dependent manner hydro-ethanolic and chloroform-extract of *Flacourtia jangomas* plant, increased the activity of catalase in blood. The activities were recorded as 1.90±0.0.03 (p<0.01) and 2.39±0.03 U/mg protein (p<0.01), 2.26±0.02 and 2.79±0.05 (p<0.001) U/mg protein, 2.51±0.01 (p<0.001) and 3.21±0.06 (p<0.001) U/mg protein at 100 mg/kg, 300 mg/kg, 900 mg/kg body weight respectively. Activity of catalase enzyme was recorded as 3.34±0.06 (p<0.001) U/mg protein in Himalaya Purim (100 mg/kg) treated group. Results were presented in Table 11 and Fig. 10.

4. **Malondialdehyde (MDA) concentration in blood lipid peroxidation for dead space wound model**

MDA concentration in tissues of control group was found to be lowered after treatment with H/E and C/H extract of *Flacourtia jangomas* plant. At 100 mg/kg body weight of H/E and C/H extract, level of MDA was recorded as 7.60±0.25 and 6.83±0.03 (p<0.001) µmol/mg protein. MDA level was significantly (p<0.001) decreased to 6.64±0.03 and 5.30±0.19 µmol/mg protein, 5.50±0.02 and 3.89±0.21 µmol/mg protein at 300 mg/kg, 900 mg/kg body weight respectively. MDA concentration was recorded as 3.35±0.04, p<0.001 µmol/mg protein in Himalaya Purim (100 mg/kg) treated group. Results were presented in Table 12 and Fig. 11.

ROS comprises both oxygen-derived radical and non-radical oxidants, which are termed as "oxidants or free radicals". They act as cellular messengers and drives biological pathways for healing. Various experiment exhibit relations between oxidative stress and diabetes mellitus (Bartosikova *et al*., 2003) [20] The oxidative stress is elevated in diabetes because hyperglycemia increases the formation of free radicals and hinders wound healing (Colman *et al*., 1989) [21] Hence, I have decided to estimate the antioxidant enzymes for evaluating the healing potency of *F. jangomas* plant. The results revealed that H/E and C/E of *F. jangomas* treatment had remarkably elevated the level of antioxidant enzymes in the healing process. The elevated activity of superoxide dismutase (SOD) hinders formation of free radicals. A rise in intracellular antioxidant GSH detoxifies the deleterious radicals. A rise in catalase enzyme, catalyses the hydrogen peroxide decomposition and guard the cell from oxidative damage. The antioxidant activity is due to the scavenging activity of phenolics present in the extracts of both the plant. Flavonoids regulate the activities of several enzyme systems (Devipriya *et al*., 1999) [22] Tannins possess antioxidant, wound healing, and antimicrobial properties (Kipngeno *et al*., 2014) [23] Triterpenoids possess astringent, antimicrobial and immunomodulatory property (Bodenstein *et al*., 2012) [24]

Polyphenols, phenolic compounds, favonoids possess both antimicrobial and· anti-infammatory properties. The synergistic effect of both antimicrobial and antioxidant properties foster the wound healing process (Akkol *et al*., 2009) [25]

The wound healing effect of H/E and C/E of *Flacourtia jangomas* could be because of the presence of bioactive compounds like carbohydrates, protein, lipid, minerals, ascorbic acid, amino acids, phenolics, alkaloids, glycosides, tannins, terpenoids, flavonoids like quercetin, luteolin and rutin, saponin, sterois and terpenoids. Findings of Murthy *et al.*, 2013; Bandarupalli *et al.*, 2014; Dwivedi *et al.*, 2016; supported the results of the present study (Murthy *et al*., 2013; Bandarupalli *et al*., 2014; Dwivedi *et al*., 2009) [26, 27, 28].

Effect of hydro-ethanolic (H/E) and chloroform (C/H) extract of *Flacourtia jangomas* plant on wet and dry weight of granulation tissue: In dead space wound model, H/E and C/E of *F. jangomas* plant showed dose dependent result. At 100 mg/kg body weight, hydro-ethanolic extract increased the wet and dry weight of the granulation tissue to 367.20±2.65 (p<0.01) and 134.20±1.89 mg when compared to the normal control group of 350.80 ±1.62 and 130.20±1.11 mg. Wet and dry weight of the granulation tissue was found significantly (p<0.001) increase to 383.80±3.44 and 151.00±2.84 mg for hydro-ethanolic extract (300 mg/kg body weight) when compared to the normal control group of 350.80 ±1.62 and 130.20±1.11 mg. At 900 mg/kg body weight, wet and dry weight of granulation tissue was significantly (p<0.001) increase to 394.30±2.50 and 160.50±2.50 mg, when compared to the normal control group of 350.80±1.62 and 130.20±1.11 mg. Here chloroform-extract revealed a better result than hydro-ethanolic extract. Chloroform-extract significantly (p<0.001) increased the wet weight of granulation tissue at 100, 300 and 900 mg/kg body weight and were found as 377.80±2.06, 400.50±3.66 and 421.20±2.63 mg respectively when compared to the normal control group 350.80 ±1.62 mg. On the other hand, dry weight of the granulation tissue was found 143.20±2.65 (p<0.01) mg for 100 mg/kg body weight of chloroform-extract treatment. At 300 and 900 mg/kg body weight of chloroform-extract dry weight was significantly (p<0.001) increased to 171.30±1.86 and 187.00±2.03 mg respectively when compared to the normal control group 130.20 ±1.11 mg. Significant (p<0.001) increased in wet and dry weight of granulation tissue for Himalaya Purim tablet (100 mg/kg body weight) treated group was found to be 443.30±1.40 and 211.70±2.01 mg. Findings are presented in Table 13 and Fig. 12.

Effect of hydro-ethanolic (H/E) and chloroform (C/H) extract of *Flacourtia jangomas* plant on hydroxyprolein and protein content of granulation tissue

Hydroxyprolein content was increased by H/E extract of *Flacourtia jangomas* plant at the dose rate of 100, 300 and 900 mg/kg body weight to 47.33±1.56, 69.56±0.99 (p<0.01) and 78.33±0.88 (p<0.001) mg/gm tissue when compared to the normal control group 42.00±1.46 mg/gm tissue. Hydroxyprolein content was increased significantly (p<0.001) on C/E administration than H/E and were recorded as 62.83±1.64, 77.83±1.30 and 88.50±0.72 mg/gm tissue at 100, 300 and 900 mg/kg body weight respectively compared to the normal control group 42.00±1.46 mg/gm tissue.

It was found that treatment with H/E extract of *Flacourtia jangomas* plant increased the protein content at 100, 300 and 900 mg/kg body weight and were 58.16±1.18, 66.83±1.58 (p<0.001) and 77.67±1.48 (p<0.05) mg/gm tissue, when compared to the control group 54.50±2.33 mg/gm tissue. In himalaya purim (100 mg/kg body weight) treated group protein content was found to be 92.66±1.87 (p<0.001) mg/gm tissue. Similarly protein content was significantly (p<0.001) increased with the treatment of 100, 300 and 900 mg/kg body weight of C/E of *Flacourtia jangomas* plant and were recorded as 62.83±1.45, 78.87±1.81 and 85.17±1.78 mg/gm tissue respectively when compared to the control group 54.50±2.33 mg/gm tissue. It has been observed that both the H/E and C/E treatment of *Flacourtia jangomas* plant showed more effective result at higher dose of 900 mg/kg body weight. C/E increased the protein content at greater amount than that of H/E extract treatment of *Flacourtia jangomas.* Findings are presented in Table 14 and Fig. 13.

The present study, reveal that after treatment with H/E and C/E of *Flacourtia jangomas* plant, there was significant increase in wet and dry weight of granulation tissue, which indicated high protein content(collagen). Granulation tissue is composed of edema, fibloblast, collagen, and new blood vessels. Collagen provides a structural framework for tissue to regenerate [29]. Increased proliferation of granulation tissue, higher concentration of hydroxyproline and protein content in tissue indicated faster rate of healing with *Flacourtia jangomas* plant extracts treatment. Healing potential could be due to phytoconstituents and free radical scavenging activity. The results of the present study were supported by the findings of Murthy *et al*., 2013; Sahoo *et al*., 2015 [26, 30].

Flavonoids increase collagen synthesis. Oxygen diffusion, diminishing oxygen free radical overproduction, and increased collagen synthesis account for improved healing (Inan *et al*., 2006) [31]. Collagen contributes to wound strength. Collagen helps in homeostasis and epithelialisation at the latter phase of healing (Suntar *et al*., 2010) [32]. The result showed potent wound healing capacity as evident from the wound contraction; increased tensile strength and increased hydroxyproline content in healing tissue. The results of the present study agreed with Barua *et al.*, 2013; Murthy *et al.*, 2013; Kundu *et al*., 2016; Lodhi *et al.*, 2016; Dwivedi *et al.*, 2016 [26, 33, 34, 35].

Table 1: Results of phytochemical screening of *Flacourtia jangomas* Plant

Extract	Alkaloids	Flavonoids	Terpenoids	Tannin	Saponins	Glycosides	Steroids
F. jangomas	Wagner's Test	Lead acetate Test	Salkowski Test	Ferric Chloride Test	Frothing Test	Sodium Hydroxide Test	Salkowski Test
Hydro-ethanolic	-ve	+ve	+ve	-ve	+ve	+ve	+ve
Chloroform	+ve	+ve	+ve	+ve	+ve	-ve	+ve

Table 2: DPPH free radical scavenging activity (% inhibition) of hydro-ethanolic (h/e) and chloroform (c/h) extracts of *Flacourtia jangomas* plant

Concentrations (µg/ml)	**Vit C**	*Flacourtia jangomas*	
		H/E	C/H
20	42.51±3.21	29.67±3.18*	37.11±2.70
40	53.27±2.34	33.11±2.29***	41.22±3.01*
60	66.35±1.96	45.86±2.42***	58.12±1.65
80	70.91±3.21	57.15±3.49**	67.02±3.32
100	82.43±2.74	65.36±5.65***	73.12±3.98

Values are expressed as Mean ± SEM. *p<0.05; **p<0.01; ***p<0.001 compared with Vit c group

Table 3: Superoxide radical scavenging activity (% inhibition) of hydro-ethanolic (h/e) and chloroform (c/h) extracts of *Flacourtia jangomas* plant

Concentrations (µg/ml)	**Vit C**	***Flacourtia jangomas***	
		H/E	C/H
20	29.37±1.61	12.03±2.28***	18.20±1.72**
40	52.18±1.79	23.57±3.18***	35.13±1.73***
60	61.06±2.22	46.02±2.30***	48.10±4.04***
80	69.70±2.32	50.12±1.92***	58.52±1.61**
100	73.67±1.88	57.55±1.84***	63.15±2.87**

Values are expressed as Mean ± SEM. *p<0.05; **p<0.01; ***p<0.001 compared with vit c group

Table 4: Effect of hydro-ethanolic (h/e) and chloroform (c/h) extract of *F. Jangomas* plant on breakingh strength

Groups	**Breaking strength (gm)**
I (control)	234.80±1.70
II (povidone iodine 5% Ointment)	518.20±2.70***
III (H/E 1% Ointment)	246.80±1.78**$^{\#\#\#}$
IV (H/E 2.5% Ointment)	248.80±1.45***$^{\#\#\#}$
V (H/E 5% Ointment)	250.00±1.32***$^{\#\#\#}$
VI (C/H 1% Ointment)	323.70±1.84***$^{\#\#\#}$
VII (C/H 2.5% Ointment)	418.30±2.17***$^{\#\#\#}$
VIII (C/H 5% Ointment)	482.70±1.89***$^{\#\#\#}$

Values are expressed as Mean ± SEM. *p<0.05; **p<0.01; ***p<0.001 compared with normal control group; $^{\#}p$<0.05; $^{\#\#}p$<0.01; $^{\#\#\#}p$<0.001 compared with povidone iodine treated group

Table 5: Effect of hydro-ethanolic (h/e) and chloroform (c/h) extract of *F. jangomas* plant on tissue sod (u/mg protein) activity for incision wound model on 12th day

Groups	***Flacourtia jangomas***
	SOD (U/mg protein)
	12th day
I (Control)	1.17±0.11
II (Povidone iodine 5% Ointment)	2.71±0.08***
III (H/E 1% Ointment)	1.21±0.07$^{\#\#\#}$
IV (H/E 2.5% Ointment)	1.51±0.10$^{\#\#\#}$
V (H/E 5% Ointment)	1.53±0.17$^{\#\#\#}$
VI (C/H 1% Ointment)	2.13±0.14**
VII (C/H 2.5% Ointment)	2.54±0.23***
VIII (C/H 5% Ointment)	2.60±0.22***

Values are expressed as Mean ± SEM. *p<0.05; **p<0.01; ***p<0.001 compared with normal control group; $^{\#}p$<0.05; $^{\#\#}p$<0.01; $^{\#\#\#}p$<0.001 compared with povidone iodine treated group

Table 6: Effect of hydro-ethanolic (h/e) and chloroform (c/h) extract of *F. Jangomas* plant on tissue gsh (µgm/MG PROTEIN) activity for incision wound model on 12TH Day

Groups	***Flacourtia jangomas***
	GSH ((µgm/mg protein)
	12TH DAY
I (Control)	1.20±0.09
II (Povidone iodine 5% Ointment)	2.90±0.11***
III (H/E 1% Ointment)	1.20±0.04$^{\#\#\#}$
IV (H/E 2.5% Ointment)	1.81±0.11***$^{\#\#\#}$
V (H/E 5% Ointment)	2.19±0.09***$^{\#\#\#}$
VI (C/H 1% Ointment)	2.01±0.08***$^{\#\#\#}$
VII (C/H 2.5% Ointment)	2.22±0.13***$^{\#\#\#}$
VIII (C/H 5% Ointment)	2.71±0.07***

Values are expressed as Mean ± SEM. *p<0.05; **p<0.01; ***p<0.001 compared with normal control group; $^{\#}p$<0.05; $^{\#\#}p$<0.01; $^{\#\#\#}p$<0.001 compared with povidone iodine treated group

Table 7: Effect of hydro-ethanolic (h/e) and chloroform (c/h) extract *of F. jangomas* plant on tissue catalase (u/mg protein) activity for incision wound model on 12TH Day

Groups	*Flacourtia jangomas*
	Catalase (U/mg Protein)
	12th day
I (control)	4.20±0.18
II (Povidone iodine 5% Ointment)	6.33±0.16***
III (H/E 1% Ointment)	4.50±0.18###
IV (H/E 2.5% Ointment)	4.93±0.11###
V (H/E 5% Ointment)	5.03±0.14*###
VI (C/H 1% Ointment)	5.12±0.17*###
VII (C/H 2.5% Ointment)	5.62±0.27***
VIII (C/H 5% Ointment)	6.02±0.12***

Values are expressed as Mean ± SEM. *p<0.05; **p<0.01; ***p<0.001 compared with normal control group; #p<0.05; ##p<0.01; ###p<0.001 compared with povidone iodine treated group

Table 8: Effect of hydro-ethanolic (h/e) and chloroform (c/h) extract *of F. Jangomas* plant on tissue lipid peroxidation (µmol/mg protein) for incision wound model on 12TH Day

Groups	*Flacourtia jangomas*
	MDA (µmol/mg protein)
	12TH DAY
I (Control)	6.99±0.16
II (Povidone iodine 5% Ointment)	3.34±0.07***
III (H/E 1% Ointment)	5.97±0.17###
IV (H/E 2.5% Ointment)	5.32±0.35##
V (H/E 5% Ointment)	4.50±0.34***
VI (C/H 1% Ointment)	5.09±0.32*#
VII (C/H 2.5% Ointment)	4.04±0.45***
VIII (C/H 5% Ointment)	3.40±0.39***

Values are expressed as Mean ± SEM. *p<0.05; **p<0.01; ***p<0.001 compared with normal control group; #p<0.05; ##p<0.01; ###p<0.001 compared with povidone iodine treated group

Table 9: Effect of hydro-ethanolic (h/e) and chloroform (C/H) Extract *of F. Jangomas* plant on tissue SOD (U/mg protein) activity for dead space wound model on 12TH Day

Groups	*Flacourtia jangomas*
	SOD (U/mg protein)
	12TH DAY
I (Control)	1.65±0.03
Himalaya Purim (100 mg/kg p.o.)	2.65±0.29***
III (H/E 100 mg/kg p.o.)	1.62±0.03###
IV (H/E 300 mg/kg p.o.)	2.38±0.14**
V (H/E (900 mg/kg p.o.)	2.43±0.03**
VI (C/H 100 mg/kg p.o.)	1.94±0.04#
VII (C/H 300 mg/kg p.o.)	2.44±0.06**
VIII (C/H 900 mg/kg p.o.)	2.59±0.17***

Values are expressed as Mean ± SEM. *p<0.05; **p<0.01; ***p<0.001 compared with normal control group; #p<0.05; ##p<0.01; ###p<0.001 compared with Himalaya purim treated group

Table 10: Effect of hydro-ethanolic (H/E) and chloroform (C/H) extract of *F. Jangomas* plant on GSH (µgm/mg protein) activity in blood hemolysate for dead space wound model on 12TH Day

Groups	*Flacourtia jangomas*
	GSH (µgm/mg protein)
	12th day
I (Control)	2.29 ±0.02
Himalaya Purim (100 mg/kg p.o.)	4.38±0.07***
III (H/E 100 mg/kg p.o.)	2.90±0.12**###
IV (H/E 300 mg/kg p.o.)	3.50±0.03***###
V (H/E 900 mg/kg p.o.)	3.29±0.02***###
VI (C/H 100 mg/kg p.o.)	2.92±0.25**###
VII (C/H 300 mg/kg p.o.)	3.40±0.04***###
VIII (C/H 900 mg/kg p.o.)	3.87±0.11***

Values are expressed as Mean ± SEM. *p<0.05; **p<0.01; ***p<0.001 compared with normal control group; #p<0.05; ##p<0.01; ###p<0.001 compared with Himalaya purim treated group.

Table 11: Effect of hydro-ethanolic (h/e) and chloroform (C/H) extract *of F. Jangomas* plant on catalase (U/mg protein) activity in blood hemolysate for dead space wound model on 12TH Day

Groups	*Flacourtia jangomas*
	Catalase (U/mg protein)
	12th day
I (Control)	2.14±0.03
Himalaya Purim (100 mg/kg p.o.)	3.34±0.06***
III (H/E 100 mg/kg p.o.)	1.90±0.0.03**###
IV (H/E 300 mg/kg p.o.)	2.26±0.02###
V (H/E 900 mg/kg p.o.)	2.51±0.01***###
VI (C/H 100 mg/kg p.o.)	2.39±0.03**###
VII (C/H 300 mg/kg p.o.)	2.79±0.05***###
VIII (C/H 900 mg/kg p.o.)	3.21±0.06***

Values are expressed as mean ± sem. *p<0.05; **p<0.01; ***p<0.001 compared with normal control group; #p<0.05; ##p<0.01; ###p<0.001 compared with himalaya purim treated group

Table 12: Effect of hydro-ethanolic (H/E) and chloroform (C/H) extract of *F. Jangomas* plant on lipid peroxidation (µmol/mg protein) in blood hemolysate for dead space wound model on 12TH day

Groups	*Flacourtia jangomas*
	MDA(µmol/mg protein)
	12TH Day
I (Control)	6.36±0.062
Himalaya Purim (100 mg/kg p.o.)	3.35±0.04***
III (H/E 100 mg/kg p.o.)	7.60±0.25###
IV (H/E 300 mg/kg p.o.)	6.64±0.03***###
V (H/E 900 mg/kg p.o.)	5.50±0.02***###
VI (C/H 100 mg/kg p.o.)	6.83±0.03***###
VII (C/H 300 mg/kg p.o.)	5.30±0.19***###
VIII (C/H 900 mg/kg p.o.)	3.89±0.21***

Values are expressed as Mean ± SEM. *p<0.05; **p<0.01; ***p<0.001 compared with normal control group; #p<0.05; ##p<0.01; ###p<0.001 compared with himalaya purim treated group

Table 13: Effects of hydro-ethanolic (H/E) and chloroform (C/H) extract of *F. Jangomas* plant on wet weight and dry weight of granulation tissue

Groups	Granulation tissue	
	Wet weight (mg)	Dry weight (mg)
I (Control)	350.80 ±1.62	130.20±1.11
II Himalaya Purim (100 mg/kg p.o.)	443.30±1.40***	211.70±2.01***
III (H/E 100 mg/kg p.o.)	367.20±2.65**###	134.20±1.89###
IV (H/E 300 mg/kg p.o.)	383.80±3.44***###	151.00±2.84***###
V (H/E 900 mg/kg p.o.)	394.30±2.50***###	160.50±2.50***###
VI (C/H 100 mg/kg p.o.)	377.80±2.06***###	143.20±2.65**###
VII (C/H 300 mg/kg p.o.)	400.50±3.66***###	171.30±1.86***###
VIII (C/H 900 mg/kg p.o.)	421.20±2.63***###	187.00±2.03***###

Values are expressed as Mean ± SEM. *p<0.05; **p<0.01; ***p<0.001 compared with normal control group; #p<0.05; ##p<0.01; ###p<0.001 compared with himalaya purim treated group

Table 14: Effect of hydro-ethanolic (H/E) and chloroform (C/H) extract of *F. Jangomas* plant on hydroxyprolein (mg/g tissue) and protein content

Groups	*Flacourtia Jangomas*	
	Hydroxyprolein	Protein
I (Control)	42.00±1.46	54.50±2.33
II Himalaya Purim (100 mg/kg p.o.)	94.78±1.89***	92.66±1.87***
III (H/E 100 mg/kg p.o.)	47.33±1.56###	58.16±1.18###
IV (H/E 300 mg/kg p.o.)	69.56±0.99**###	66.83±1.58***###
V (H/E 900 mg/kg p.o.)	78.33±0.88***###	77.67±1.48*###
VI (C/H 100 mg/kg p.o.)	62.83±1.64***###	62.83±1.45***###
VII (C/H 300 mg/kg p.o.)	77.83±1.30***###	78.87±1.81***###
VIII (C/H 900 mg/kg p.o.)	88.50±0.72***#	85.17±1.78***

Values are expressed as Mean ± SEM. *p<0.05; **p<0.01; ***p<0.001 compared with normal control group; #p<0.05; ##p<0.01; ###p<0.001 compared with himalaya purim treated group

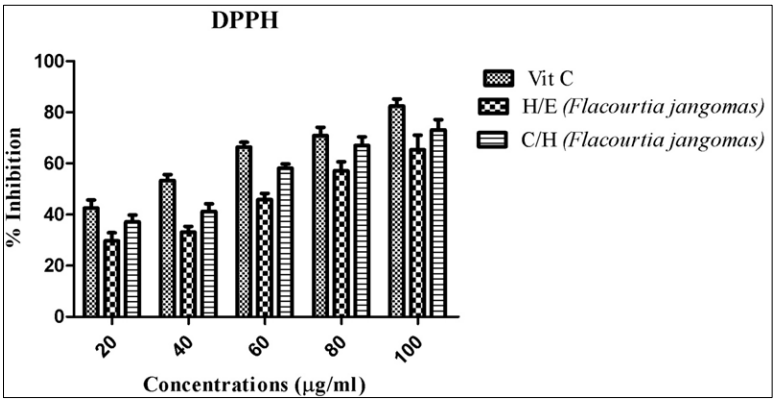

Fig 1: Graphical representation of DPPH free radical scavenging activity of hydro-ethanolic and chloroform extracts of F. *jangomas* plant

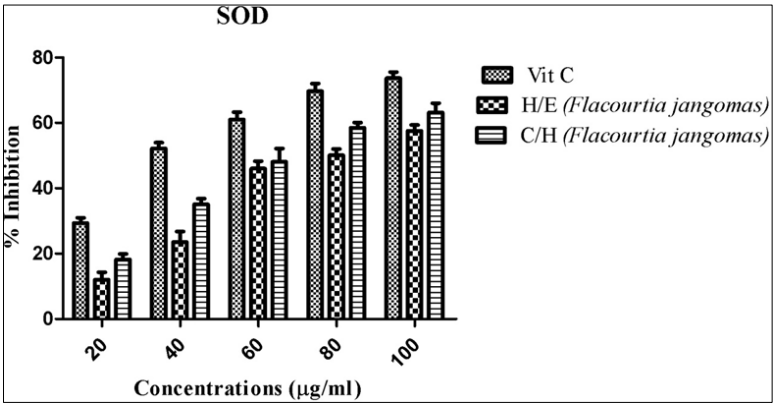

Fig 2: Graphical representation of superoxide radical scavenging activity of hydro-ethanolic and chloroform extracts of *F. Jangomas*

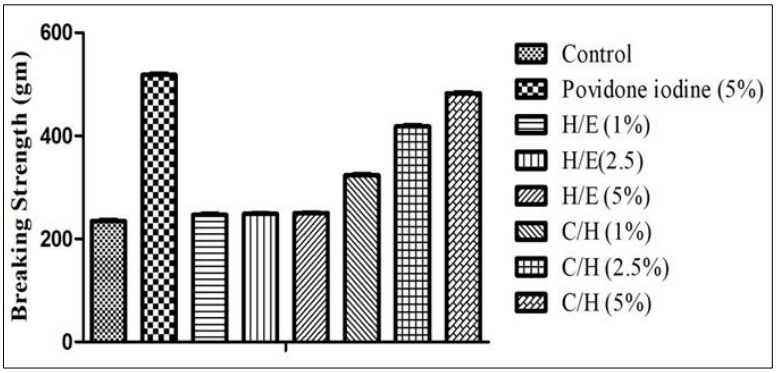

Fig 3: Graphical representation of effect of (H/E) and (C/H) extract of *F. jangomas* plant on breaking strength for incision wound model

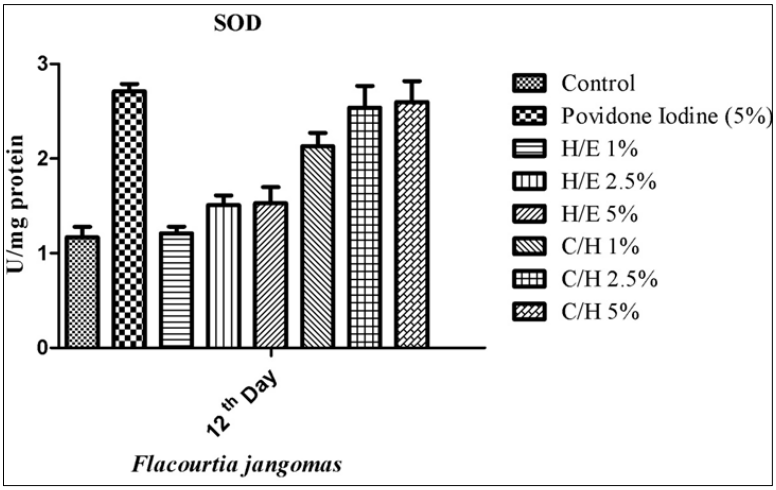

Fig 4: Graphical representation of effect of H/E and C/H extract of *F. jangomas* plant on tissue sod (u/mg protein) activity for incision wound model

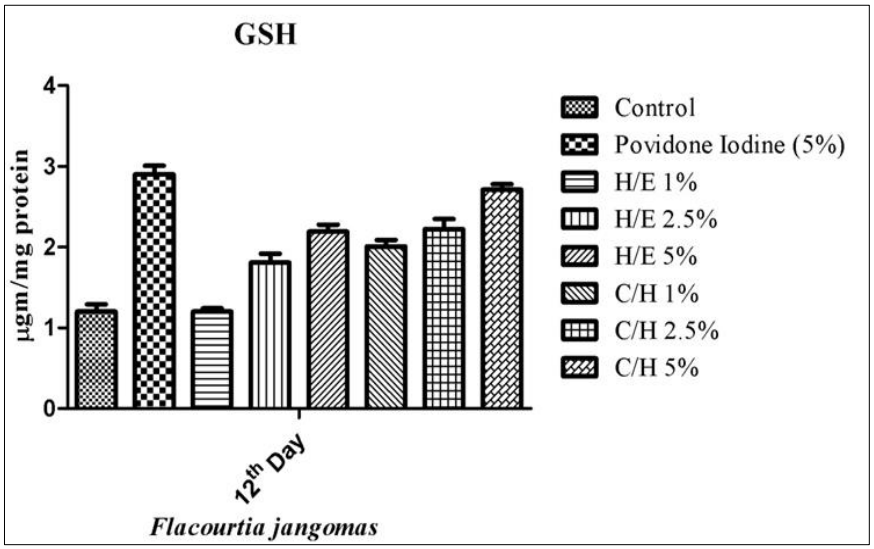

Fig 5: Graphical representation of effect of H/E and C/H extract of *F. Jangomas* plant on tissue gsh (µgm/mg protein) activity for incision wound model

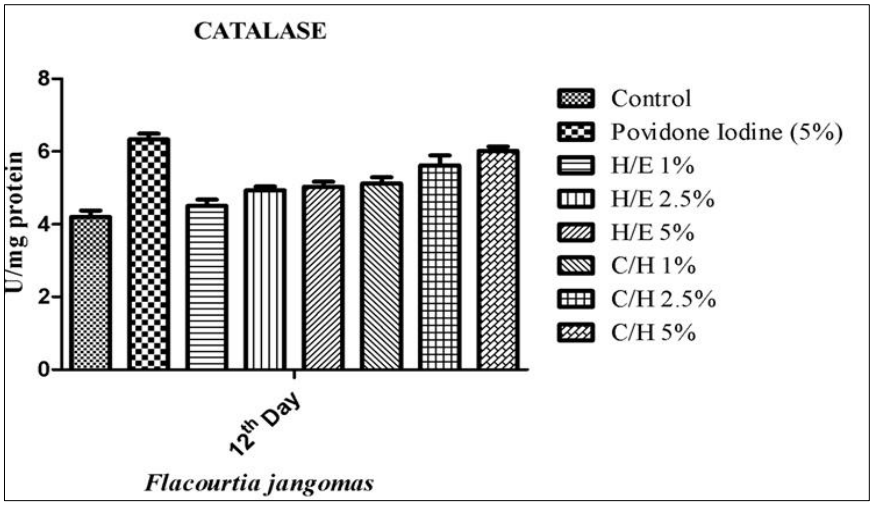

Fig 6: Graphical representation of effect of H/E and C/H extract of *f. Jangomas* plant on tissue catalase (u/mg protein) activity for incision wound model

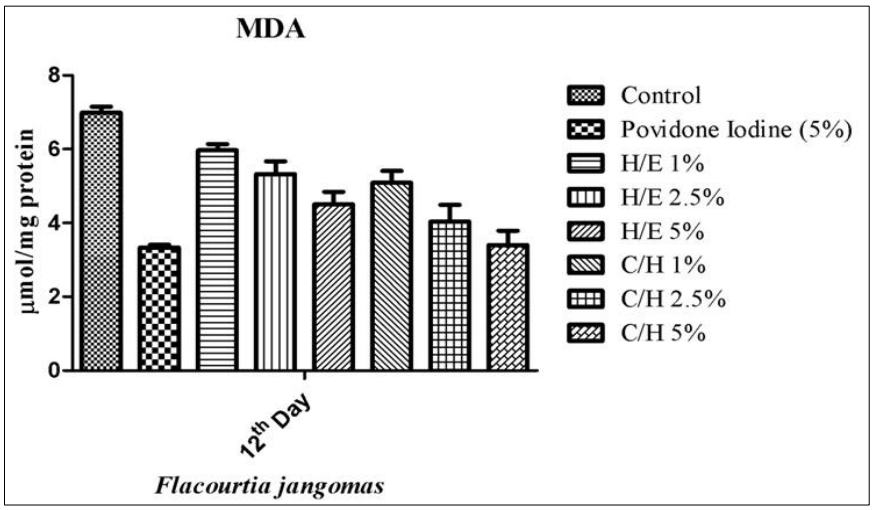

Fig 7: Graphical representation of effect of H/E and C/H extract of *F. jangomas* plant on tissue lipid peroxidation (µmol/mg protein) for incision wound model

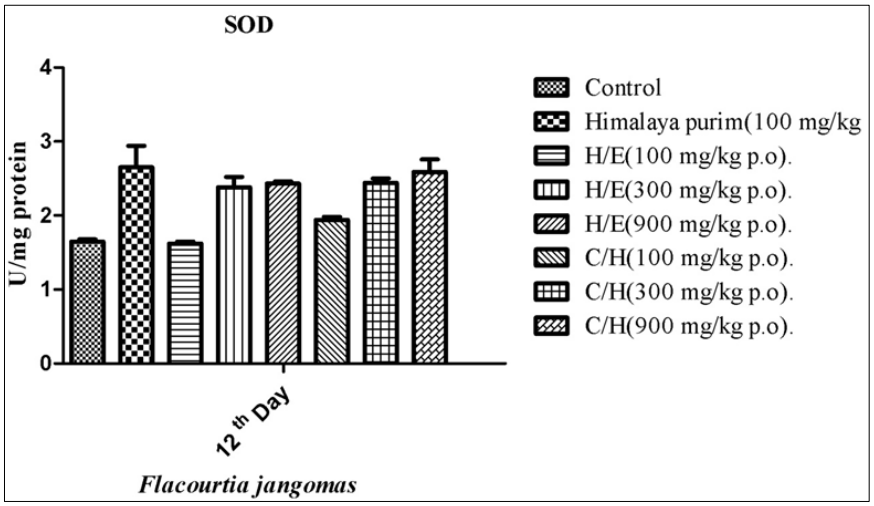

Fig 8: Graphical representation of effect of H/E and C/H extract of *F. jangomas* plant sod (u/mg protein) activity in blood hemolysate for dead space wound model

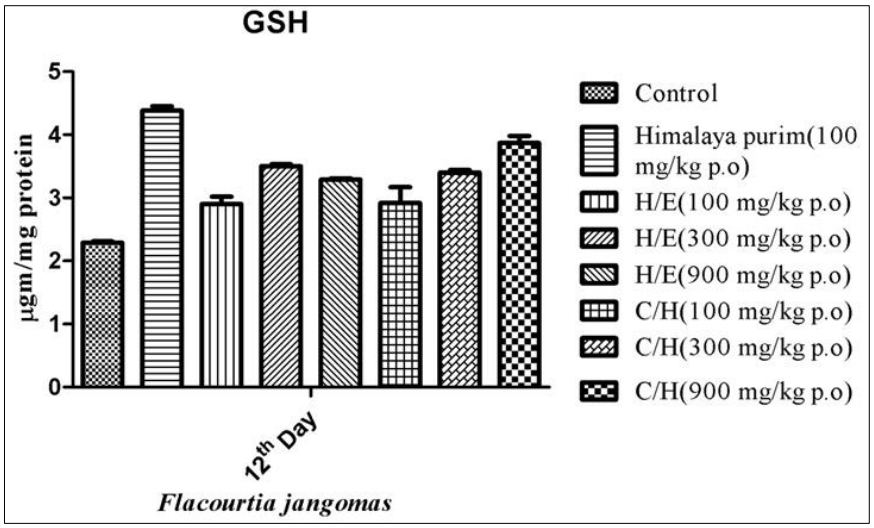

Fig 9: Graphical representation of effect of H/E and C/H extract of *F. jangomas* plant on gsh (µgm/mg protein) activity in blood hemolysate for dead space wound model

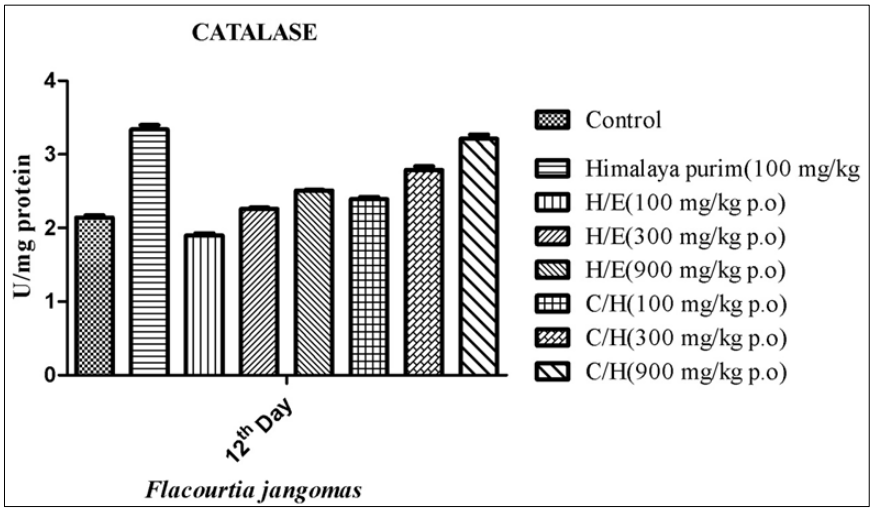

Fig 10: Graphical representation of effect of H/E and C/H extract of *F. jangomas* plant on catalase (u/mg protein) activity in blood hemolysate for dead space wound model

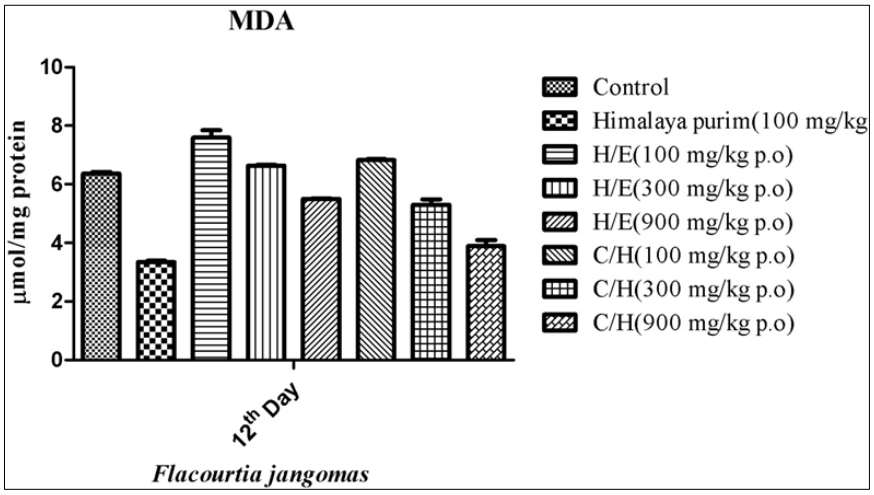

Fig 11: Graphical representation of effect of H/E and C/H extract of *F. jangomas* plant on lipid peroxidation (µmol/mg protein) in blood hemolysate for dead space wound model

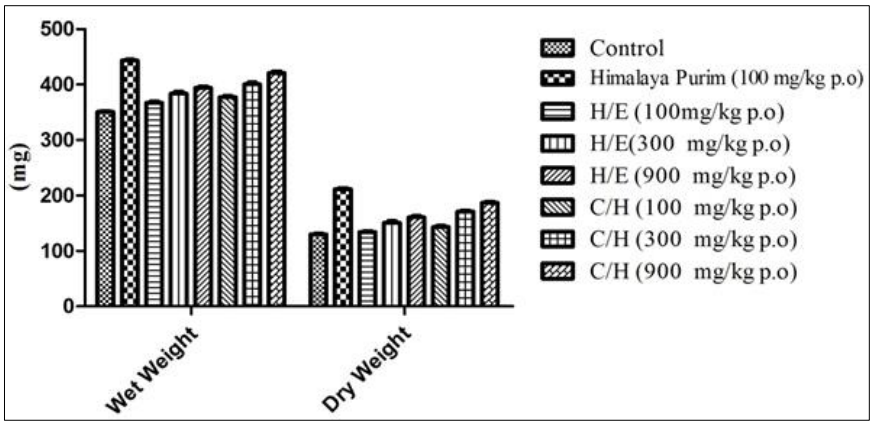

Fig 12: Graphical representation of effect of (H/E) and (C/H) extract of *F. Jangomas* plant on wet and dry weight of granulation tissue for dead space wound model

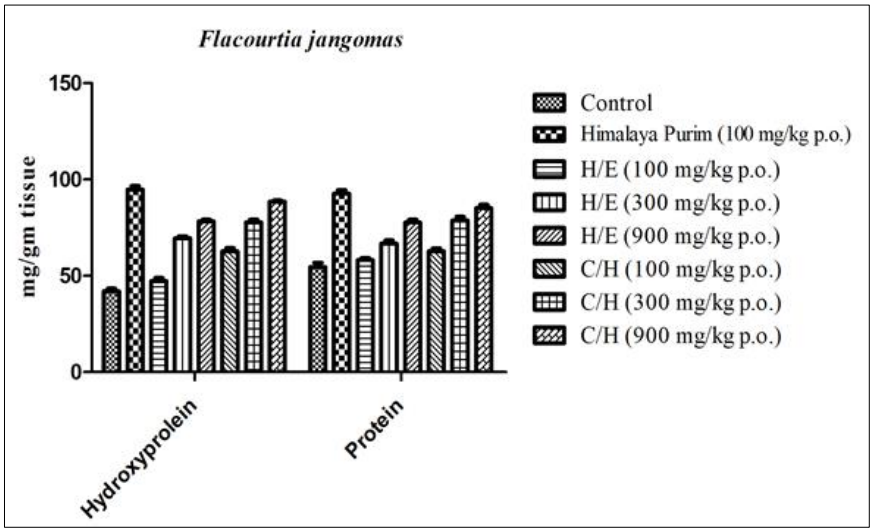

Fig 13: Graphical representation of effect of (H/E) and (C/H) extract of *F. Jangomas* plant on Hydroxyprolein and protein content of granulation tissue for dead space wound model

Conclusion

The results of the present study demonstrated that the plant possesses various phytochemical constituents that may contribute to the wound healing activity. Both the H/E and C/E of *Flacourtia jangomas* plant established its wound healing property in regards to increased wet and dry weight, Hydroxyprolein and protein content of granulation tissue in a dose dependent manner when compared with control group. It was observed that C/E of the plant showed better wound healing efficacy than H/E extract.

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