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RESEARCH ARTICLE

Identification of Quercetin, Kaempferol and Luteolin from Methanolic Extract of *Corchorus depressus* and its Pre-Clinical Wound Healing

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Abstract

Corchorus depressus Linn. (Tiliaceae) has been extensively utilized in Odisha, India, and is considered sacred and religious in addition to being used in Pakistan, Baluchistan, and Sind for ethnopharmacological purposes. Ethno-pharmacological claims and lack of sufficient scientific data with respect to the wound-healing activity of the plant encouraged us to the preparation of herbal formulation and its evaluation against wound-healing models with the identification of the phytoconstituents present in the plant extract. The physiochemical properties of the plant like ash value, the preliminary phytochemical screening, the HPLC, HPTLC, FTIR analysis, and the dermal toxicity of the methanolic extract were carried out using standard methods. A stability study was conducted on the prepared ointment formulations. With the help of *in vivo* excision, incision, dead-space wound, mice-burn model, and along with *in vitro* stimulation of Interleukin-10 models, the wound healing potential was evaluated. The granulation tissue was collected for nitric oxide estimation and histopathological analysis. The tensile strength of the granuloma tissue and hydroxyproline content were estimated using standard methods. The methanolic extract ointment resulted in a steady contraction of wounds with time and caused significant inhibition in the level of nitric oxide in the scar tissue and aggregation of macrophages in histological examination. The incision model showed a significant (381.33±4.58, $p<0.05$) increase in the tensile strength of granuloma tissue when compared to the control (156.5±5.75). The dead space wound model demonstrated that the plant extract significantly increased (75.43±2.97, $p<0.001$) hydroxyproline content against the control. Methanolic extract ointment caused significant wound healing and reduced epithelialization time in the mice burn model from the 12^{th} day till the 24^{th} day as compared to the negative control. Observations of hematological and serum biochemical changes in the dermal toxicity study suggested that the herbal extracts are safe for use topically. In the accelerated stability study, there were no marks of physical instability in the prepared ointment. IR spectral analysis was successfully used to detect flavanones like Luteolin and flavonol such as Quercetin and Kaempferol in methanolic extract. The present findings provide scientific evidence that the plant *Corchorus depressus* Linn. (Tiliaceae) displayed wound healing activity, corroborating its traditional use by the Tribals of Odisha and the Indian system of medicine.

Keywords: *Corchorus depressus*, HPLC, Hydroxyproline, Nitric-oxide, Quercetin

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1. Introduction

Wound healing disorders are a severe clinical concern that is anticipated to worsen as more diseases such as hypertension, diabetes, and obesity are linked to them. Current growth factors and cytokine-based therapy methods have a number of disadvantages, including a lengthy purification and expression procedure, high cost, burst release at the wound site, and harmful side effects. As a result, developing possible therapeutic medications from natural materials to speed wound healing will be beneficial for treating wounds because natural resources provide cost-effective, accessible, and dependable medical compounds[1].

The selected plant, *Corchorus depressus* Linn. (Tiliaceae) is treated as religious and worshipped by the married women of Odisha, India, in the rituals called "Jama Jutia (Jama Dutia)". This is the traditional ancient festival of worshipping the Lord "Yamaraj" (The Lord of Death). The women of Odisha make a daylong fasting, prepare different sweets, and cakes and worship the plant in the evening (Figure 1).

An old man of the society will sit at a distance and act as "Yamaraj". The women sweep the road with the plants up to the old man and offer the cakes and sweets and it is believed that by doing so the family members will be free from attack of any disease and have a long life. The worshipped plants were taken up by the women and softly swept over the body of their family members.

In the Indigenous system of medicine, the above plant is used as a medicine for fever, as a tonic; mucilage of the plant is utilized in gonorrhea. On stone, the roots are rubbed and spread on the forehead for getting relief from migraine; to cure leucorrhoea powdered dried fruits are taken orally with milk. To treat body aches, uterine protrusion, and urinary irritation and to prevent abortion, the plant is mashed with tender twigs of *Prosopis cineraria,* mixed with whey and sugar, and consumed as a drink.

Figure 1. The photographs of the plant, worshipped by the people of Odisha in the festival 'JAMA JUTIA'.

The paste of the leaves is mixed with curd and given orally to cure diarrhea in children[2]. For increasing the viscosity of the seminal fluid and to regularize the menstrual disorder *C. depressus* is also utilized[3]. The plant's extract is used for its anti-diabetic activity and as a paste on wound surfaces in healing (The wealth of India)[4]. Ikram, *et al.,* studied the hexane and chloroform soluble whole plant extract of *C. depressus* which showed an antipyretic effect in rabbits undergoing subcutaneous yeast injections and it exhibited no harmful or deleterious effects up to oral dose 1.6g/kg[5].

Vohora, *et al* found that at 100 mg/kg, Cordepressic acid has antipyretic activity on yeast-induced pyrexia in albino rats when administered intraperitoneally[6].

In $HepG_2$ cell line Pareek, *et al* studied the invitro hepatoprotective activity against CCl_4 induced toxicity. In a concentration-dependent manner, the ethanolic extract alleviated the changes induced by CCl_4 that may be due to a decrease in reactive oxygen species level and oxidative stress thereon[7].

Kataria, *et al* through their research demonstrated the *in vitro* and *in vivo* aphrodisiac activity of *C. depressus* Linn. on rabbit smooth muscle relaxation and normal male rats' sexual behavior[8].

From a survey of different literatures, we found that the wound healing property of *C. depressus* has not been evaluated. The aim of present research is to identify the phytochemicals present in the methanolic extract of *Corchorus depressus* and scientifically investigate its wound healing activity by different methods so as to authenticate its folkloric particulars.

2. Materials and Methods

2.1 Plant Material

The authentication of *Corchorus depressus* Linn. (Tiliaceae) was certified by BSI (Botanical Survey of India), Howrah (Authentification No – CNH/I-I/28/2009/Tech.II/93) a specimen (SJCPS-T) was kept in the herbarium, SJCPS, Naharkanta, Bhubaneswar, Odisha, INDIA after its collection from local area of Salipur, present in state Odisha, India in the month of August 2009.

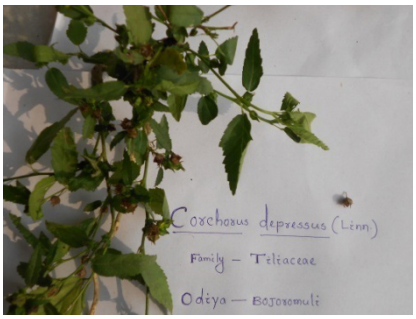


Figure 1a. Photograph of the whole Plant *Corchorus depressus* Linn. (Tiliaceae).

2.2 Chemicals and Cell Line

Cellline of Murine macrophage, penicillin, dexamethasone, Indomethacin, streptomycin, l-glutamine, fetal bovine serum, lipopolysaccharide, were procured from sigma chemical and hexane, methanol, ethyl acetate was purchased from Merck, Standard Luteolin, Quercetin and Kaempferol obtained from Chromadex, Bangalore, India and all reagents and chemicals utilized in the study belongs to analytical grade.

2.3 Experimental Animals

In the experiment, healthy Wistar rats, about (150-200 g) and albino mice of both sex (20-25 g), were procured from the Animal House, SJCPS, Naharkanta, Bhubaneswar, Odisha. The animals were kept in cages made up of polypropylene and with a stainless-steel lid. They were maintained in standard conditions (12hr light/12hr dark cycle; temperature 25±3 ºC; 30-70 % humidity). Animals were acclimatized 4 days prior to the experiment. The research was conducted according to the Committee for the purpose of control and supervision of Experiments on animals (CPCSEA) Govt. of India. Initial approval was taken from (IAEC) for conducting the animal experimental studies (678/02/A/CPCSEA).

3.1 Preparation of Plant Material

Under running water, the plants were washed thoroughly, chopped, and dried in air for a week at 35 to 40 ºC. The leaves, stem, and root was separated to prepare the powders separately. The respective plant materials were pulverized in an electric grinder separately to moderately fine powders (355/180) [Through 355 No. sieve, all particles, and through a No. 180 sieve, not more than 40% should pass][9]. The respective powders were immediately vaccum packed and stored at 20ºC until extraction and utilized for powder characteristic study.

3.2 Physiochemical Properties of *C. depressus*

3.2.1 Ash Values

The crude drugs produce inorganic ash after incineration, which varies considerably depending on their quality and purity.

3.2.2 Determination of Total Ash

In silica crucible, the powdered crude drugs of three grams were accurately weighed. The powdered drug was heated with a gradual increase in temperature until it is free from carbon. It was kept in desiccators after cooling. The percentage (%) 'ash value' was calculated with reference to air dried sample. The procedure as described by (Evans, 2009; WHO, 1998) for the study of acid-insoluble, sulphated and water-soluble ash was carried out and the results were tabulated[9,10].

3.2.3 Acid Insoluble Ash

The acid-insoluble ash was weighed and calculated in relation to the air-dried drug (Evans, 2009; WHO, 1998)[9,10].

3.2.4 Water Soluble Ash

With 25 ml of water, the ash was boiled for a duration of 5 minutes after that the insoluble debris was filtered, and collected and the filter papers were cleaned with hot water before being torched for 15 minutes at a temperature of not more than 450ºC. The difference between the weight of the insoluble matter from the pre-weighed ash was represented as water-soluble ash.

3.2.5 Sulphated Ash

The sulphated ash was calculated and recorded as per standard methods (Evans, 2009; WHO, 1998)[9,10].

3.2.6 Moisture Content

To determine the actual moisture content of those powdered drug materials, about 2g of powdered drug was weighed properly. These drug powders were kept separately in flat porcelain dishes, then dried in an oven at 100ºC. The process of burning of powder proceeded slowly by taking special care not allowing those materials to go up in flames during the entire process[9,10]. After

drying, these drug powders were re-weighed and the loss of weight was recorded as the previous content of moisture in the material and the results were tabulated[10].

3.2.7 Swelling Index

Under defined conditions, the volume (ml) taken up by swelling of 1g of plant material is known as the swelling index. Many medicinal plant materials, particularly gums and those having a significant quantity of mucilage, pectin, or hemicelluloses, have specialized therapeutic or pharmacological utility due to their swelling qualities.

Accurately weighed 200 mg of powdered plant material was introduced into a 25ml-glass-stoppered measuring cylinder (16mm internal diameter, length of the graduated portion is 125mm, marked in 0.2ml divisions from 0 to 25 ml in an upwards direction). 25ml of water added and shaken thoroughly every 10 minutes for 1 hr and then at room temperature, allowed to stand for 3 hours. The volume(ml) was measured, which was occupied by the plant material. The volume of the individual determinations was calculated, related to 1g of plant material.

3.2.8 Foaming Index

When an aqueous decoction is shaken, saponins in many therapeutic plant materials can create persistent foam. The Foaming index is a measurement of the ability of an aqueous decoction of plant materials and extracts to foam.

Plant material 1g was converted to a coarse powder (1250 sieve number), accurately weighed, and transferred to a 500ml conical flask that contains 100ml boiling water. For 30 minutes, medium boiling was maintained then cooled and filtered into a 100ml volumetric flask. Adequate water was added to dilute the volume through the filter. The decoction was poured into test tubes (10 stoppered tubes, height 16 cm, 16 mm diameter) in successive portions of 1ml, 2ml, 3ml, etc up to 10ml. With water, the volume of the liquid was adjusted up to 10 ml in each tube. For 15 seconds, tubes were shaken in a lengthwise motion and the foam height was measured.

According to WHO, the foaming index is considered as (<100), when the foam height in every tube is less than 1 cm[9].

3.2.9 Analysis of Fluorescence

The fluorescence analysis was carried out with different chemical reagents by standard methods as described by Kataria, *et al.*, and Usha, *et al.*, were carried out[11,12].

3.3 Extraction Process

The moderately fine powder of the plant material (1kg) was initially defatted with petroleum ether. The defatting process is concerned mainly with the elimination of less polar constituents such as chlorophyll (coloring matter), fixed oils, and proteins, which may interfere with the isolation of the other constituents in the extract. By employing solvents of different polarity in ascending order, the defatted material was subjected to successive extraction. The powder and the solvent mixture were macerated by stirring at each 4hrs interval for 48hrs at room temperature (25 ± 5 ºC) and then filtered with what man filter paper of 2 to 3 µm pore size[13]. The extraction approach chosen (maceration under agitation) allowed for a faster extraction process, reducing the amount of time the plant sample was in contact with the solvent and conserving the bioactivity of the isolated molecules. Furthermore, extraction at room temperature constituted a compromise between[14]. The flowchart of the entire extraction process is described as follows (Figure 1b).

3.3.1 Extractive Values of all the Extracts

Estimation of the extractive value of crude drugs is beneficial for a preliminary assessment. It provides information about the chemical constituents found in a crude extract. It can also be used to estimate the amount of certain elements that are soluble in the extraction solvent.

Estimation of these values tells us about the justification for whether carrying out research work further will be beneficial to the community or not. As a part of the preliminary study, the individual yield of each of these plant parts was observed and recorded.

3.3.2 The Consistency and Colour of the Extracts

The consistency and colour of these crude extracts were properly checked in daylight by organoleptic evaluation and recorded.

3.4 Qualitative Phytochemical Analysis

The Qualitative Phytochemical analysis was evaluated in the different extracts obtained from the selected plant. Different literatures were followed (Wager, 1984; Odebiyi OO, *et al.*, 1978; Trease and Evans, 1987) for preliminary identification of various phytoconstitutents[15-17].

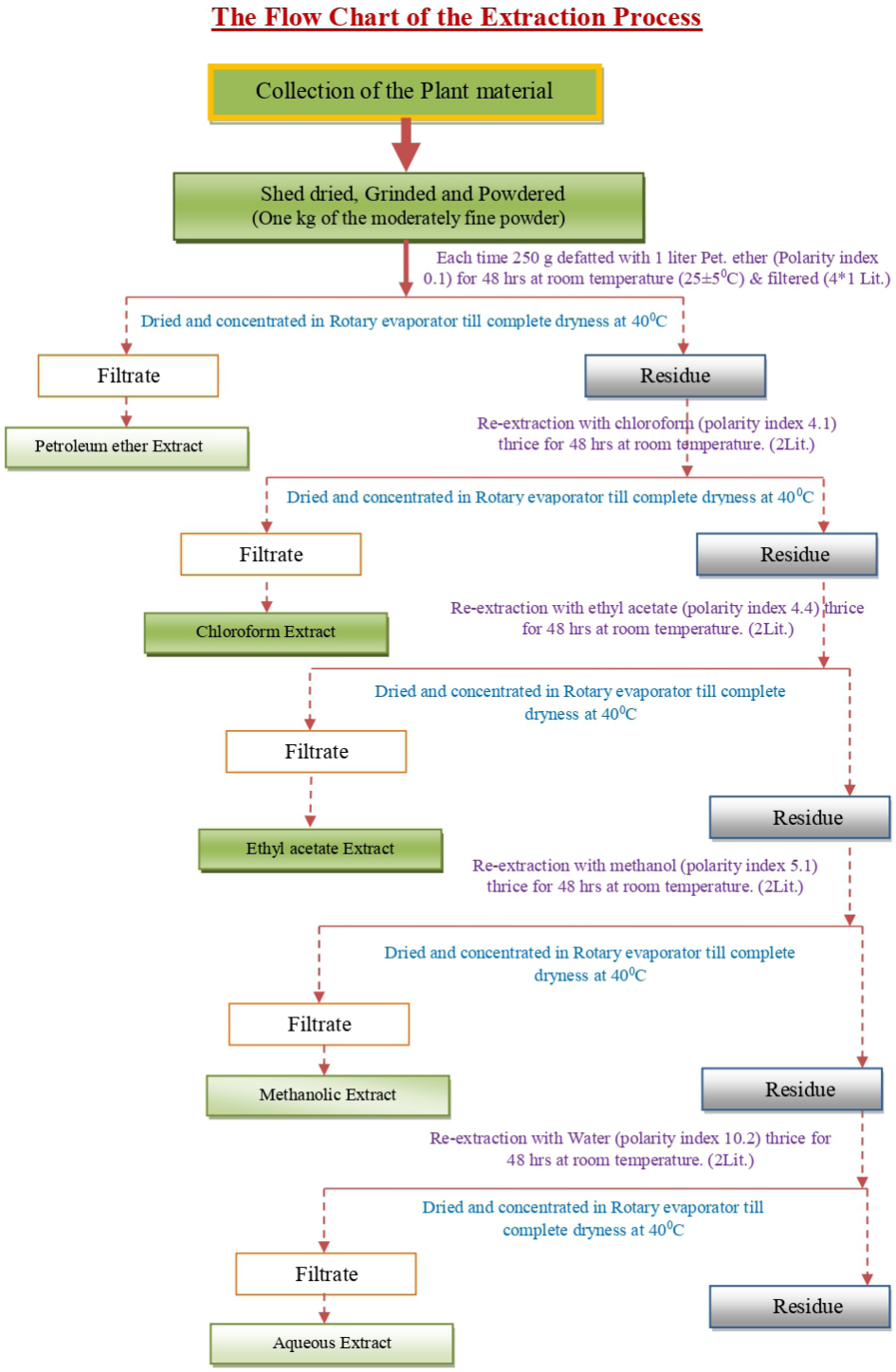


Figure 1b. The flow chart of the extraction process.

3.5 Chromatographic and Spectroscopic Study

3.5.1 HPLC

Waters 515 equipped with Empower 2 software consisting of a solvent delivery pump (quaternary), and a 2998 Photo-Diode Array (PDA) detector, were utilized for chromatographic analysis. On the Waters Spherisorb C_{18} column (4.6mm x 250mm, 5µm), all the separations were carried out. Acetonitrile: water (60:40, v/v) was used as mobile phase and ultrasonically degassed before use. The temperature of the column was 25°C, 0.5 ml/min was the flow rate and 10 µl was the volume of injection. The retention time, and the PDA spectrum provided in the chromatogram were used to identify the peaks.

3.5.2 HPTLC method

The HPTLC glass plates of size (20 cm x 10 cm) with silica gel [60 F_{254} (E-Merck)] coated, were used for HPTLC analysis. Samples were applied as 20 mm apart to the plates, 8 mm bands by means of a sample applicator ((Linomat V, fitted with 100 µl syringe). An application rate of 0.2 μls^{-1} was applied. Up to a distance of 9 cm the plates were developed in a (20 cm x 10 cm) twin through glass chamber. It had been pre-saturated with mobile phase vapors (Toluene-ethylacetae-formic acid 5:3.5:0.1, v/v/v). Twenty minutes was the optimized chamber saturation time at room temperature.

3.5.3 FTIR Fingerprint Analysis

FTIR fingerprint analysis was performed by FTIR spectroscopy (Spectrum Two), (PerkinElmer, Massachusetts, United States), taking preheated Potassium Bromide and test sample in agate motor pestle and grinding to make fine and homogenous powder. A hydraulic press of 7 tons pressure was applied for about 20 seconds to make a transparent pellet. The pressure was released and the pellet was removed from the die by manual press and was carefully placed in the pellet holder. It was scanned in a range of 450-4000 cm^{-1}. The raw data of FTIR spectrum was processed with Spectrum Quant software along with baseline correction and interactive baseline correction. The respective desired peaks were labeled. The same method was applied for standard quercetin, Kaempferol, Luteolin, and the test methanolic extract of the selected plant.

3.6 *In-vitro* Stimulation of Interleukin-10 (IL-10)

The protocol was similar to Nualkaew, *et al*., 2009[18]. The murine macrophage cell line was cultured in Dulbecco's modified Eagles' medium (DMEM) (2mM L-glutamine, 100 unit/ml penicillin, and streptomycin) and supplemented with 10% FBS (fetal bovine serum). 10mg/ml of each extract of *C. depressus* was mixed to the culture medium followed by 100 ng/ml of lipopolysaccharide, for stimulation of cytokine production. After the 24 hour incubation period, the supernatants were collected and kept at -20°C until cytokine analysis using ELISA method according to ELISA kit's standard protocol. The percentage of cytokine stimulation was calculated using dexamethasone as a positive control.

3.7 Wound – Healing Activity Study

3.7.1 Acute Dermal Toxicity

To make sure the safety of the methanolic and aqueous extract before it could be safely used for pre-clinical animal models for wound healing activity; their dermal toxicity study (acute) was investigated on Sprague-Dawley rats.

3.7.1.1 Experimental Design

A total number of 18 female rats were used. They were divided into 3 groups having 6 animals each and named as Methanolic extract treatment group, aqueous extract treatment group, and negative control (applied with 20% white soft paraffin) group. 3g both extracts were applied to the respective group on the first day of the experiment. The standard (OECD) guidelines no 402 were utilized for the experiment[19].

3.7.1.2 Skin Preparation for Dermal Toxicity Study

Under general anesthesia with i.m. injection of a mixture of Ketamine (50mg/kg) and Xylazine (5mg/kg), the skin was prepared for the experiment. On the dorsal thoracic region, the furs were shaved using a razor blade.

3.7.1.3 Euthansia and Collection of Blood Sample

At the end of the 14 day experimental period, all rats were euthanized using the carbon dioxide inhalation method. The blood samples were collected from the posterior vena cava and preserved in EDTA (ethylene diamine tetracetic acid) blood tubes for hematology analysis and again samples were preserved in simple blood tubes without

anticoagulant, for biochemical analysis. Samples were clotted for 30 minutes at normal room temperature and the sera were separated by centrifugation (5000 rpm for 5 minutes). All samples were kept at -20°C in the freezer. Hematology analysis was conducted by auto haematolyser whereas the serum biochemical analysis was carried out manually using standard methods[20].

3.7.2 Preparation of Ointment for the Test Sample (MeoH fraction of C. depressus)

The extracts were formulated into ointments as per British Pharmacopoeia (1980)[21]. The ointment base was prepared by taking soft white paraffin 85g mixed with 5g each of wool fat, hard paraffin, and cetostearyl alcohol by gentle heating not exceeding 60°C. The soft gel material was cooled to normal temperature and homogenized at 1600 rpm for 20 minutes, to form a uniform ointment base.

The ointment base was used to prepare two types of test topical formulations and one placebo control (containing only ointment base). The two test ointments contain an ointment base (100gm) with methanolic and aqueous extracts of 5.0gm each to obtain 5%w/w ointments. These formulations [Methanolic extract Ointment of *C. depressus* (MOCD)] and [Aqueous extract Ointment of *C. depressus* (AOCD)] were compared with providone–iodine ointment 5%w/w USP for evaluation.

3.7.3 Stability Studies of Ointment Formulation

By testing phase separation, color, odour, and consistency, the physical stability of the ointment was evaluated. The temperature stability study was performed at accelerated conditions (40±2 °C and 75%±5% RH) for 45 days. The samples were observed periodically for any physical changes like the development of objectionable colour, odour or phase separation according to ICH guidelines[22].

The spreadability of the ointment was determined by the method described by Prasad and Dorle. Briefly, a wooden block device had a wooden block with a fixed glass slide and a pulley on one end, and a pan coupled to a movable glass slide with a string on the other end.

The ointments were sandwiched between the two slides for 5 minutes after a measured amount of ointment was poured on the fixed glass slides and the movable slide with the pan was placed over the fixed slide. On top of two plates, 50g weight was placed and the top plate's time to transverse a distance of 10 cm was measured.

$$\text{Spreadability (S)} = M/T$$

Where; S is spreadability in g/s, M is mass (grams) and T is time (seconds)

Better spreadability is represented by a shorter interval[23].

3.7.4 Excision Wound Model

3.7.4.1 Experimental Protocol

The Wistar rats were kept in the experimental room for 4 days for acclimatization. The rats both male and female, were divided into four major groups. Initially, the fur of dorsum of was removed with electric hair clipper, and 2hrs after; the rats were anesthetized by injecting Ketamine (0.5ml/kg b.wt. i.p.). 30 mins after Ketamine administration, the back skin surface was marked with a sterile circular stainless steel stencil. In an aseptic setting, a full-thickness wound (600mm^2) was made by skin excision including panniculus carnosus with sterile scissors and forceps[24].

3.7.4.2 Study Design

After the creation of wounds to the entire animals, the two test groups receive MOCD and AOCD ointments, Positive control group animals receive Betadine whereas the control group receives ointment base only. The mode of application of substance is topical and single application per day.

3.7.4.3 Animal Distribution for Biochemical Analysis

Animals were sacrificed at different time intervals and granulation tissue was collected for biochemical analysis and for histopathological investigation, a sample of tissue was isolated from each group of animal's healed skin.

3.7.4.4 Wound Area Measurement

On the 3rd, 7th, 9th, and 14th day, animals were anesthetized and wound measurement was taken by tracing the wound using transparent paper and permanent marker.

3.7.4.5 Histopathology

The tissue samples were embedded in paraffin after being fixed in 10% formalin. Serial sections of paraffin-embedded tissues (5µm thickness) were cut. The ulceration, epithelialization, and necrosis were evaluated in the skin tissues. The macrophage aggregations were also qualitatively evaluated.

3.7.4.6 Granulation Tissue Collection for Biochemical Studies

From each group, the granulation tissue was collected in vials on the 3rd, 7th, 9th, and 14th day from three sacrificed animals and stored at – 20ºC immediately for biochemical studies with proper labels.

3.7.5 Nitric oxide Estimation

3.7.5.1 Reagents Preparation

Part-A: Sodium nitroprusside (100 mM) Stock solution.

Method of Preparation: 0.298g of Sodium nitroprusside was weighed and then dissolved in water of 10 ml.

Part-B: Sodium nitroprusside (10 mM) was made with a part-A stock solution.

3.7.5.1.1 Preparation of Griess Reagent

Part-1: 1g sulphanilamide dissolved in 100 ml of 2% phosphoric acid.

Part-2: 0.1 g Naphthlethylene diamine dihydrochloride NDD dissolved in 100 ml of water. Part 1 and Part 2 were mixed in equal volumes.

3.7.5.2 Test Substance Preparation

At different time intervals, scar tissue was isolated from the wounds of the animals during the experiment and stored at -20ºC. 2 g of tissue was weighed from each sample and soaked in the 6N Hcl overnight and the tissue was homogenized with the help of mortar and pestle. The same procedure was followed for the Control and treatment groups.

3.7.5.3 Protocol

The NO assay was measured with the following reaction mixture in a final volume of 2 ml, which contains Sodium nitroprusside and test compound. After incubation at 37ºC for 30 minutes, 0.5 ml of incubated sample was removed and an equal volume of Griess reagent was added. The chromophore generated had its absorbance measured at 546 nm. Control, positive control, and the test absorbance values were compared to determine NO inhibition[25].

% of inhibition Formula = Control value -Treated value ÷ Control × 100

3.7.6 Linear Incision Wound Model

Under ketamine Hcl (10%) anesthesia by using a shaving machine, the dorsal surface hairs were shaved. A sterile surgical blade is used to make 5cm long two linear paravertebral incisions through the whole thickness of skin. The incisions were at a distance of 1.5 cm from either side of the vertebral column[26].

Surgical interrupted sutures (3 nos), spaced 1 cm apart, were used to close the incisions. Throughout 9 days, the MOCD, AOCD, providone-iodine ointment, and the vehicle were applied topically to the respective groups once in a day. The negative control group did not receive any drugs. On the post-wound day (9th day), the sutures were removed and on the 10th day, the animals were sacrificed under anesthesia. The Tensiometer was used to determine the tensile strength of linear-paraventricular incised skin in N (Newton)[27,28].

$$\% \text{ Tensile strength (TS) of extract} = \frac{TS_{extract} - TS_{vehicle}}{TS_{vehicle}} \times 100$$

$$\% \text{ (TS) of reference} = \frac{TS_{reference} - TS_{vehicle}}{TS_{vehicle}} \times 100$$

$$\% \text{ (TS) of vehicle} = \frac{TS_{vehicle} - TS_{negative}}{TS_{negative}} \times 100$$

3.7.7 Dead Space Wound Model

Polypropylene tubes (2.5cm X 0.5cm) were implanted in the lumbar area (subcutaneously) on the dorsal side to generate a dead space wound[29].

All the groups received their respective drugs (i. e. MOCD, AOCD, and the standard drug) from 0 to the 9th day. The granuloma tissue formed was dissected carefully and utilized for the estimation of hydroxyproline content on the 10th day.

3.7.7.1 Estimation of Hydroxy Proline

The wound tissues were dissected out and dried to constant weight in a hot air oven at 60ºC, then hydrolyzed for 4 hours at 130ºC in 6 N Hcl in sealed glass tubes to determine the hydroxyproline content. Chloramines-T, Ehrlich reagent, and L-hydroxyproline were procured from Sigma-Aldrich. The hydroxylate was neutralized to P^H 7.0 and was subjected to chloramines-T oxidation for 20 minutes. Then 0.4 M perchloric acid was added to end the reaction and the colour was developed with the help of Ehrlich reagent at 60ºC. At 557 nm, the absorbance was measured using a spectrophotometer (Schimadzu 1700, Japan). By using dilutions of 1 mg/ml stock solution of hydroxyl proline, a standard curve was prepared with the

range of concentrations from 2 to 200 µg/ml (2, 10, 25, 50, 100, 200 µg/ml). By using a standard curve, results were determined and denoted as µg of hydroxyproline per mg of skin[30,31].

3.7.8 Invivo Mice-burn Model

The Swiss albino mice (48 nos) weighing about 25-30 g. were given diethyl ether anesthesia and had their dorsal surfaces shaved using a sterilized razor. 70% ethanol was used to sterilize the shaved area. The method by Rozaini, *et al*. 2004, was used to create burn wounds with little modifications[32]. Under light diethyl ether anesthesia, a cylindrical metal rod (10 mm diameter) that was heated for 30 seconds over an open flame was pressed to the disinfected surface of mice for 20 seconds. The experiment was conducted according to IAEC. The burn wound animals were grouped into 4 (n=12).

Group-1: Negative Control
Group-2: Positive Control (Silver sulphadiazine and chlorhexidine gluconate cream; (1 % w/w)
Group-3: Methanolic leaves extract Ointment of *C. depressus* (MOCD)
Group-4: Aqueous leaves extract Ointment of *C. depressus* (AOCD)

All the groups received their respective ointments once daily topically starting from the initiation of the burn till healed completely. Percentage wound contraction, epithelialization time and hydroxyl proline content were determined and tabulated.

3.7.8.1 Measurement of Wound Area

On each four-day interval, the changes in the wound area were measured (mm^2) by tracing on transparent paper. Wound contraction was measured as a percentage reduction in the initial wound size.

3.7.8.2 Determination of Hydroxyproline Content

The techniques as described by Neuman and Logan, 1950, were utilized to determine the hydroxyproline content[33].

3.7.8.3 Statistical Analysis

One-way ANOVA followed by Dunnett's test was used for all data. The significance threshold was set at $P<0.05$.

4. Results

In order to standardize, the physiochemical parameters of the Plant were investigated, and found that the foaming index for the leaf, stem, and root are less than 100; the swelling index for the leaf is higher in comparison to the stem and root. The total (mean) ash for leaf, stem, and root are 5.08, 5.41, and 6.02 respectively. The findings were depicted in Table 1.

4.1 Fluorescence Analysis

The analysis of the powder leaves with methanol resulted in Dull straw green (Dsg) colour under Visible rays and bright yellowish green (Byg) under UV light at 254 nm. The result of the reaction with other chemicals for stem, root, and leaf were recorded in Table 2.

4.2 Extractive Value of Extracts

The extractive value of the methanolic leaf extracts was highest at 12.15%, followed by aqueous leaf extracts

Table 1. Physio-chemical parameters of *Corchorus depressus*

Sl. No	Parameters	Leaf (mean*±*SD*)	Stem (mean*±*SD*)	Root (mean*±*SD*)
1	Total ash	5.08±0.02	5.41±0.02	6.02±0.12
2	Acid-insoluble ash	0.53±0.03	0.43±0.03	0.34±0.02
3	Water-soluble ash	1.18±0.15	0.99±0.06	0.95±0.02
4	Sulphated ash	7.05±0.12	6.35±0.2	8.23±0.23
5	Loss on drying	0.57±0.05	0.86±0.04	0.99±0.03
6	Swelling index	13.5±0.05	7.58±0.01	9.0±0.1
7	Foaming index	Less than 100 (<100)	Less than 100 (<100)	Less than 100 (<100)

*Mean value (six readings). The values are presented as (mean ± SD)

Table 2. Fluorescence analysis of *Corchorus depressus*

Sl. No	Treatment	Light ray	Leaf	Stem	Root	Light ray	Leaf	Stem	Root
1	Powder	Visible ray	Dsg	Dyg	Lb	Ultraviolet ray at 254nm	Byg	Yg	Bgg
2	In methanol		Dsg	Dyg	Dbb		Byg	Byg	Byg
3	In ethanol		Syg	Syg	Dbb		Byg	Byg	Bgg
4	In HCl		Byg	Dgg	Dgb		Byg	Yg	Bb
5	In HNO3		Bgg	Dgg	Dgb		Byg	Yg	Bb
6	In H2SO4		Bg	Dbg	Db		Bgb	Bg	Bb
7	Ammonia Solution		Dyg	Dyg	Dgb		Byg	Dyg	Bgg

Abbreviations: Bb: Blackish Brown; Bg: Bright Green; Bgg: Bright greyish green; Byg: Bright yellowish green; Bgb: Bright greenish brown; Dbb: Dull blackish brown; Dyg: Dull Yellowish green; Dsg: Dull straw green; Dgg: Dull greyish green. Dbg: Dull blackish green; Dgb: Dull greenish brown; Lb: Light brown; Db Dark brown; Yg: Yellowish green; Syg: Straw yellowish green

Table 3. Evaluation of the successive extractive values of all the extracts

Sl. No.	Type of extract	Stem	Leaf	Root
1	Pet. Ether	0.81 %	1.22%	0.47%
2	Chloroform	2.34 %	2.05%	0.79%
3	Ethyl acetate	1.08 %	1.85%	1.74%
4	Methanol	2.98 %	12.15%	6.12%
5	Aqueous	3.06 %	9.0%	2.34%

(9.0%). The other extractive values are presented in Table 3.

4.3 The Colour and Consistency of Extracts

The observer parameters for the leaf, stem, and root were recorded in Table 4. The colour of the methanolic leaf extract was greenish-black with a semisolid appearance, whereas for the aqueous leaf extract the colour and appearance were greenish-brown and semisolid (SS) respectively.

From the Qualitative Phytochemical analysis of *C. depressus*, the methanolic and the aqueous leaf extracts showed the presence of different Phytochemicals. The maximum number of phytochemicals like the alkaloids, glycosides, steroids, flavonoids, Saponins, tannins, and phenolic compounds were detected from the methanolic leaf extract, followed by the detection of saponins, flavonoids, tannins, and phenolic compounds from the aqueous extract.

4.4 HPLC Chromatogram Result Analysis

The HPLC chromatogram (Figure 2) resulted in a single large peak having a retention time of 6.451 min. The chromatogram of standard Luteolin (Figure 2a) also depicts similar results.

Table 4. Evaluation of Colour and Consistency of different extracts

Sl. No.	Type of extract	Colour			Appearance		
		Stem	Leaf	Root	Stem	Leaf	Root
1	Pet. Ether	Light brown	Yellowish green	Light brown	Solid	Solid	Solid
2	Chloroform	Light brown	Greenish black	Light brown	Semi-solid	Semi-solid	Solid
3	Ethyl acetate	Blackish brown	Straw green	Blackish brown	Semi-solid	Semi-solid	Solid
4	Methanol	Blackish brown	Greenish Black	Blackish brown	Semi-solid (SS)	Semi-solid (SS)	Semi-solid (SS)
5	Aqueous	Light brown	Greenish brown	Brown	Semi-solid (SS)	Semi-solid (SS)	Semi-solid (SS)

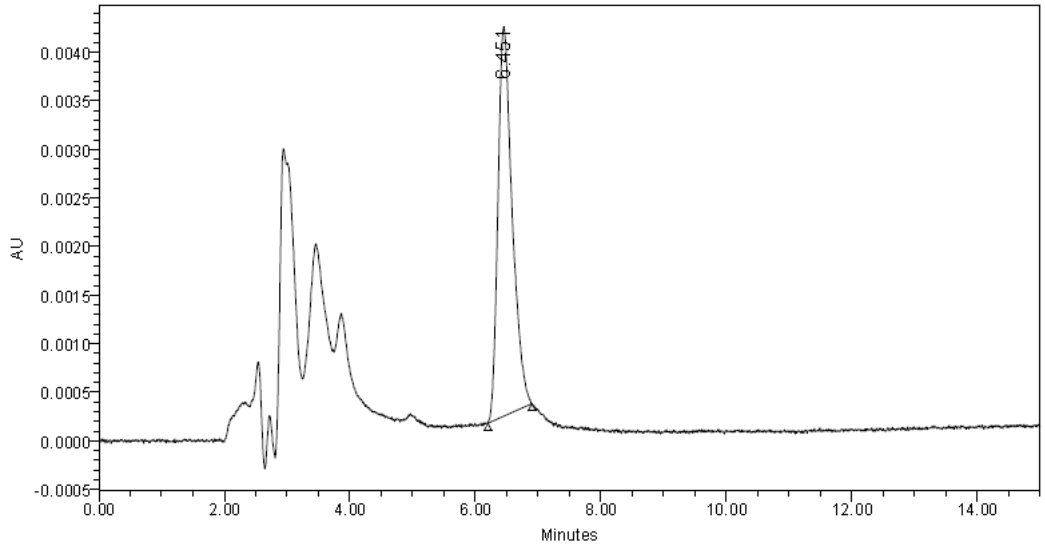


Figure 2. The HPLC chromatogram results in a single large peak with retention time 6.451 min.

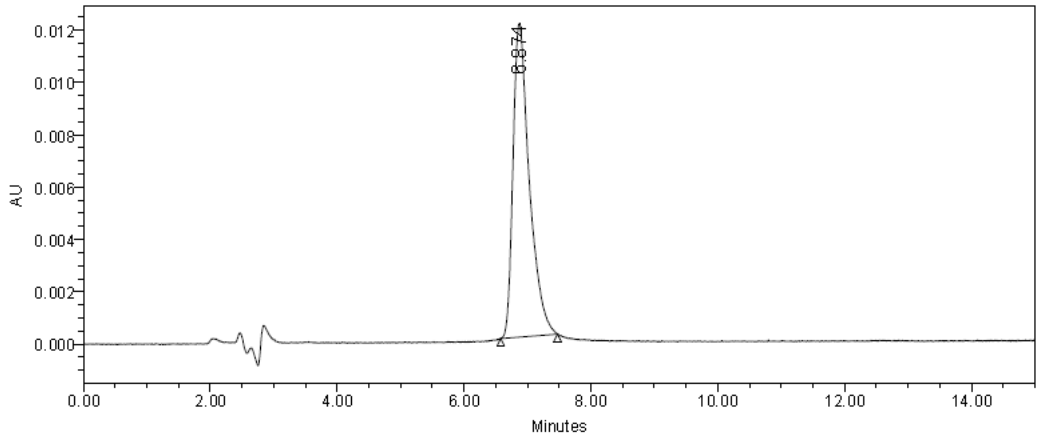


Figure 2a. The chromatogram of standard luteolin.

4.5 HPTLC Chromatogram Result

Nine peaks at R*f* value 0.05, 0.16, 0.47, 0.48, 0.51, 0.66, and 0.68 were present in the Chromatogram of methanolic extract of *Corchorus depressus* (Figure 2b) along with a peak at R*f*: 0.34. The identity of luteolin spot was confirmed by a comparison of its R*f* with the spectrum from a standard luteolin (Figure 2c).

4.6 FT-IR Analysis

FT-IR spectrum of methanolic extract of *Corchorus depressus* presented as F-9, showed an absorption band at 3417.30 cm^{-1} for OH- stretching, at 1614.20 cm^{-1} for C=O group, intense band at 1454.44 cm^{-1} for the aromatic group and 2935.98 cm^{-1} for C-H stretching (Figure 3).

The IR spectrum of Quercetin (Figure 3a) showed the absorption band at 3417 cm^{-1} is for the hydroxyl group and the presence of (C=O) is depicted with an intense absorption at 1644 cm^{-1}. As there is the presence of an aromatic group in the compound, an intense absorption band at 1458 cm^{-1} is present.

The FT-IR spectrum of Kaempferol depicted in (Figure 3b) revealed an absorption band at 1664 cm^{-1} for (C=O) group, 3391 cm^{-1} for OH group stretching. The presence

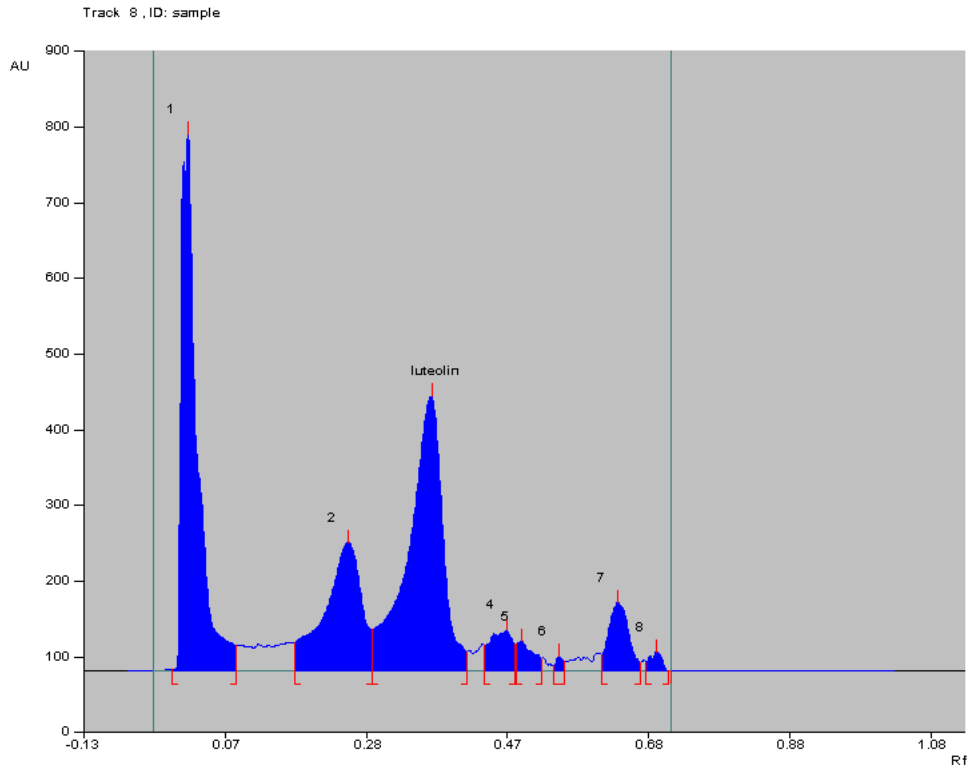


Figure 2b. HPTLC Chromatogram of methanolic extract *of Corchorus depressus*.

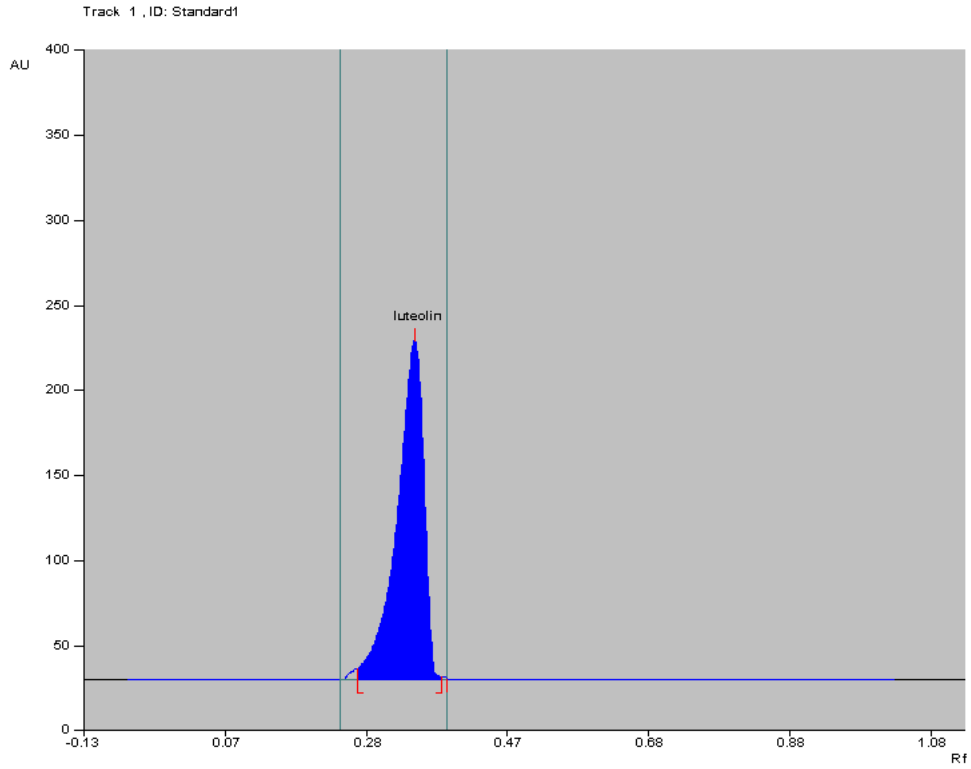


Figure 2c. HPTLC of standard luteolin.

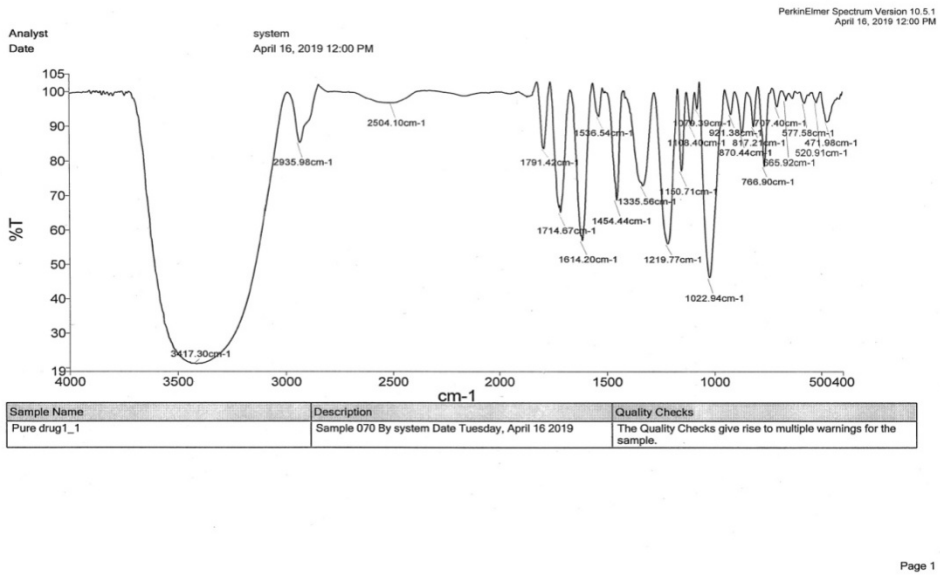


Figure 3. FTIR fingerprint spectrum of methanolic extract of *Corchorus depressus*.

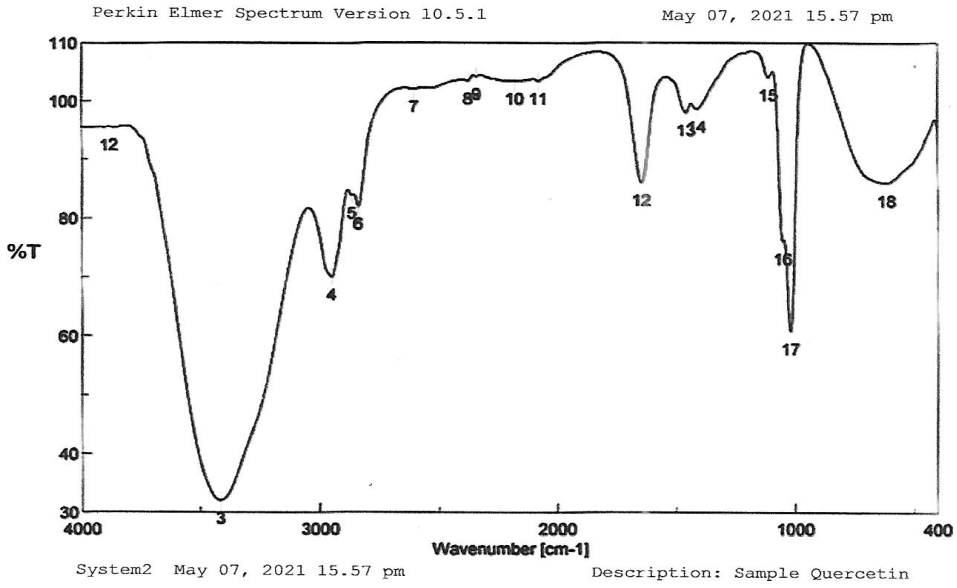


Figure 3a. FTIR fingerprint spectrum of Quercetin.

of an aromatic ring is denoted by the absorption at 1567 cm^{-1}. The band at 2900 cm^{-1} is for C-H stretching. The characteristic absorption band IR (KBr) cm^{-1}: 3422, 1659, 1369, and 3100 observed are for the presence of OH group stretching, (C=O) group, for the presence of aromatic ring and for C-H stretching respectively for Luteolin. In luteolin, the absorption band at 1164 cm^{-1}, this denotes the (C-O-C) stretching vibration mode (Figure 3c). The results (Table 5) are in good agreement with the previously studied literatures[34-38].

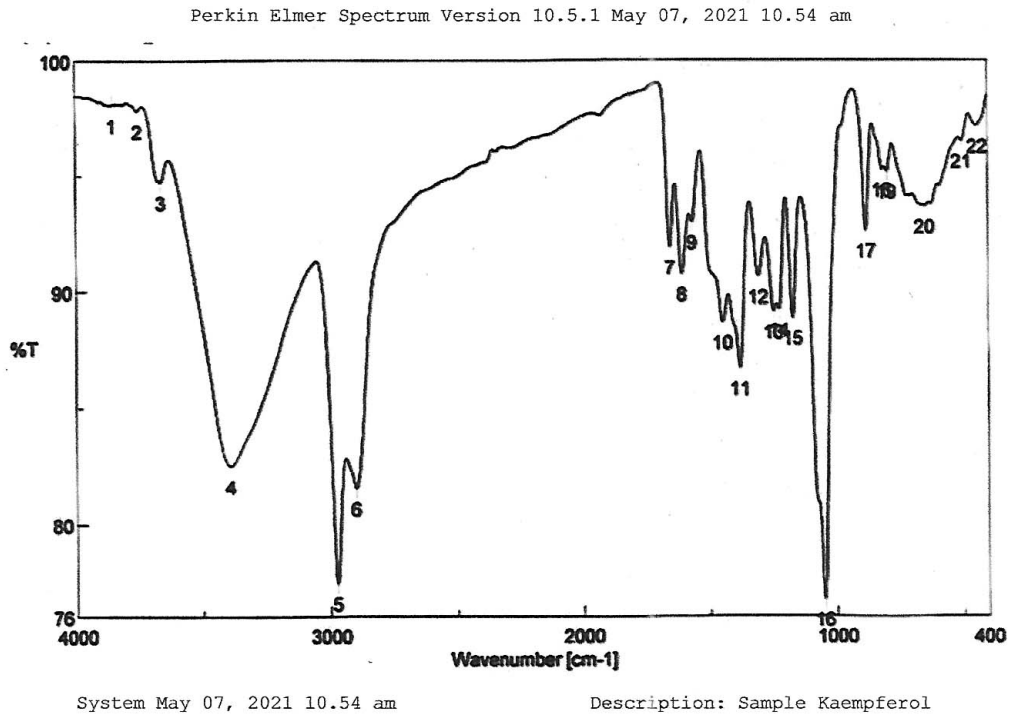


Figure 3b. FTIR fingerprint spectrum of Kaempferol.

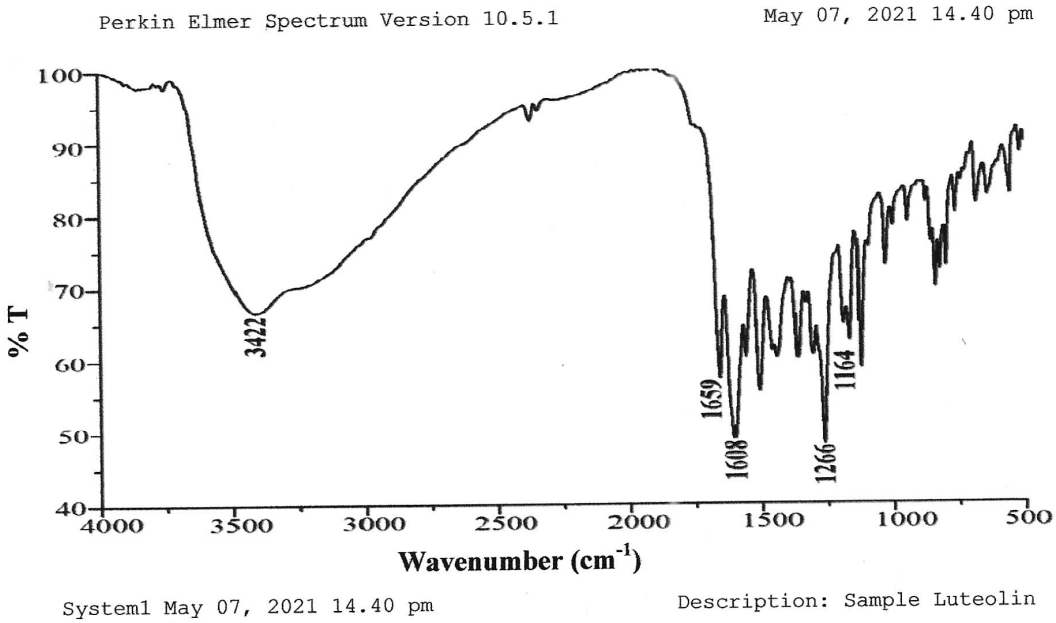


Figure 3c. FTIR fingerprint spectrum of Luteolin.

Table 5. FTIR Spectrum comparative fingerprint analysis for quercetin, kaempferol, luteolin, and methanolic extract of *Corchorus depressus*

Sl. No.	IR Spectrum Methanolic Extract of *C. depressus* (cm^{-1})	IR Spectrum of Quercetin (cm^{-1})	IR Spectrum of Kaempferol (cm^{-1})	IR Spectrum of Luteolin (cm^{-1})	INFERENCE
01	3417.30	3417	3391	3422	-OH group stretching
02	1614.20	1644	1664	1659	C=O group
03	1454.44	1450	1567	1369	Aromatic group
04	2935.98	2940	2900	3100	C-H stretching

4.7 *In-vitro* Stimulation of Interleukin-10 (IL-10)

Among all extracts, the methanolic extract (MeoH fraction) of *C. depressus* (Table 6).

showed a higher percentage (57.2%) stimulation of 'IL-10' production followed by aqueous extract (40.6%); hence MeoH and aqueous fractions were taken for further studies on animal models.

4.8 Acute Dermal Toxicity Study

The result obtained from the dermal toxicity (acute) with both extracts of *C. depressus* revealed no adverse skin reaction. During the initial 24 hrs of the observational period, all rats did not show significant changes or impairment in behavior, posture, skin, fur, and breathing pattern. In all rats, mortality and toxic effects were not observed throughout the study period.

Table 6. Percentage stimulation of interleukin-10 (IL-10) production

Test substance	Concentration (mg/ml)	% Stimulation
Control	-	-
Dexamethasone	0.001	58.1%
Pet ether Fraction	10	16.3%
Chloroform fraction	10	27.0%
Eth. Ac. fraction	10	35.5%
MeoH fraction	10	57.2%
Aqueous fraction	10	40.6%

All the hematology and serum biochemical results were within normal ranges (Tables 7 and 8). In between the methanolic, aqueous, and negative control groups, no significant ($p<0.05$) difference was observed.

Table 7. Blood parameters in Acute Dermal Toxicity for MOCD, AOCD, and negative control

Sl. No	Blood Parameters	MOCD	AOCD	Negative Control
01	RBC ($\times 10^{12}$ /L)	7.38 ± 0.11	7.56 ± 0.06	7.74 ± 0.06
02	Hb (g/L)	152.1 ± 0.65	154.2 ± 0.68	154.1 ± 0.66
03	Platelets ($\times 10^{9}$/L)	1451.31 ± 2.28	1429.7 ± 1.48	1435.2 ± 2.92
04	MCHC	307.88 ± 0.74	308.7 ± 0.64	315.36 ± 0.6
05	WBC ($\times 10^{9}$/L)	6.73 ± 0.11	6.79 ± 0.08	7.08 ± 0.4
06	Monocytes ($\times 10^{9}$/L)	0.42 ± 0.03	0.49 ± 0.04	0.46 ± 0.03
07	Lymphocytes ($\times 10^{9}$/L)	4.93 ± 0.04	4.89 ± 0.13	5.04 ± 0.22
08	Eosinophils ($\times 10^{9}$/L)	0.17 ± 0.02	0.18 ± 0.01	0.15 ± 0.02
09	Basophils ($\times 10^{9}$/L)	0.07 ± 0.03	0.08 ± 0.02	0.06 ± 0.02
10	Neutrophils ($\times 10^{9}$/L)	1.29 ± 0.16	1.32 ± 0.13	1.26 ± 0.09

MCHC: Mean corpuscular hemoglobin concentration, WBC: White Blood Corpuscle, RBC: Red Blood Corpuscle, Hb: Hemoglobin. Values are in (mean ± SD); Showed no significant difference ($p<0.05$) between the groups.

Table 8. Serum Biochemical parameters in Acute Dermal Toxicity for MOCD, AOCD, and negative control

Sl. No	Serum Parameters	MOCD	AOCD	Negative Control
01	Urea (mmol/L)	6.12 ± 0.33	6.05 ± 0.13	5.96 ± 0.22
02	Creatinine (µmol/L)	63.16 ± 0.45	62.5 ± 0.92	61.46 ± 0.71
03	Total Protein (g/L)	64.78 ± 0.42	63.28 ± 0.72	62.77 ± 0.38
03	Albumin (g/L)	44.31 ± 0.72	43.33 ± 0.55	43.06 ± 1.07
04	Globulin (g/L)	20.48 ± 0.88	19.95 ± 0.74	19.7 ± 0.88

Values are in (mean ± SD); Showed no significant difference ($p<0.05$) in between the groups.

4.9 Stability Study of the Formulation

The physical evaluation of samples stored at accelerated conditions showed no phase separation, unpleasant odor, and physical instability. The spreadability of the ointment was tested and found that there is no change in spreading capability (Table 9). Thus, the formulations are stable at accelerated stability conditions.

4.10 Excision Wound Model

Wound healing is a dynamic and complex process with the wound environment altering in response to the individual's changing health status. The basic concepts of wound healing can be understood by studying the physiology of the typical wound healing trajectory through the phases of haemostasis, granulation, inflammation and maturation.

We compared the wound contraction between the treatment groups with Placebo control (Figure 4) and the MOCD treatment (Figure 4a) resulted that excision wounds have been steadily contracting over time which was statistically significant when compared to placebo control. A similar kind of effect resulted with 5% w/w providone iodine ointment (Figure 4b). However, the

Table 9. Spreadability of ointment formulations at accelerated stability conditions

Day	Spreadability (g/s)		
	Placebo Ointment	5% w/w MOCD	5% w/w AOCD
Initial period	5.50	5.89	5.87
7th day	5.56	5.87	5.88
14th Day	5.55	5.87	5.89
30th Day	5.60	5.88	5.87
45th Day	5.53	5.73	5.85

Photographs of the Excision Wound model rats

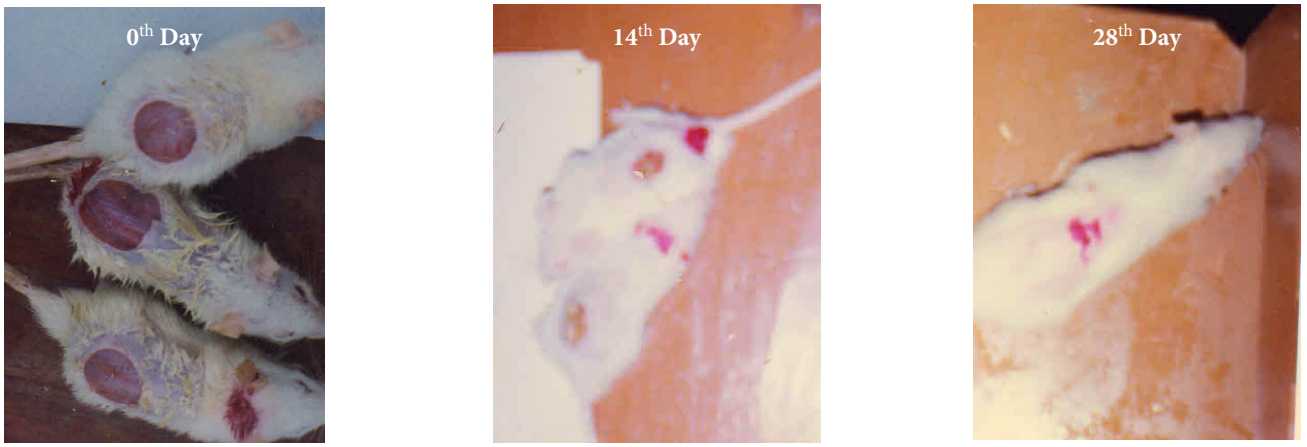


Figure 4. Placebo Control Groups in Excision-wound model rats.

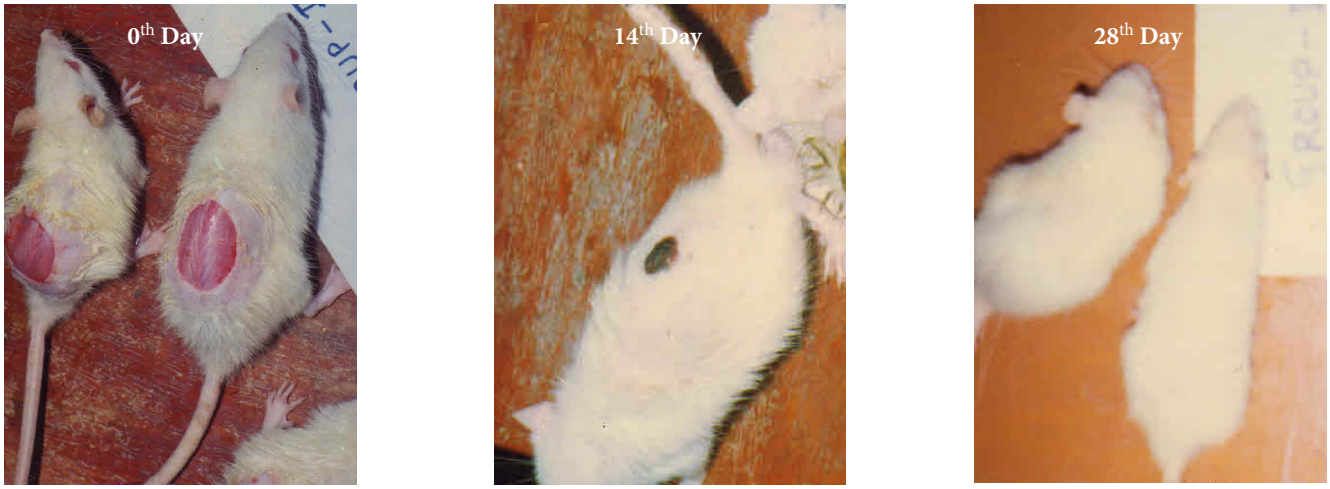


Figure 4a. Test (MOCD) treated Groups in Excision-wound model rats.

Figure 4b. Positive Control Groups in Excision-wound model rats.

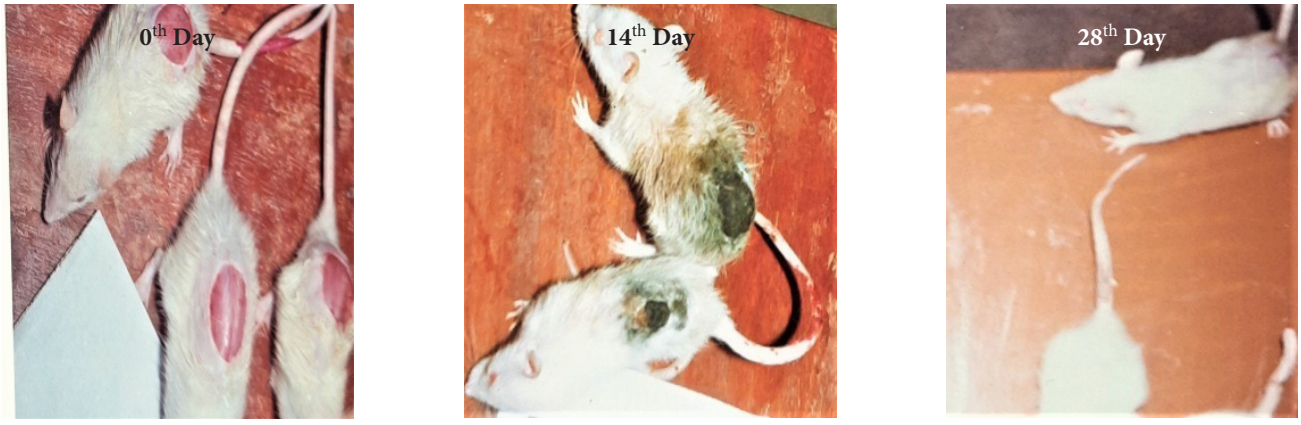


Figure 4c. Test (AOCD) treated Groups in Excision-wound model rats.

AOCD produced moderate activity (Figure 4c). The mean wound (mm^2) contraction graph of MOCD, AOCD of *C. depressus* and Positive control was depicted (Figure 5).

The mean wound healing time (MHT) reduced by the application of MOCD was approximately 7 days faster when compared to the control (Figure 6).

4.11 Histopathology

The histopathological examinations for control, positive control, and test groups show promising results as depicted in their respective figures (Figure 7a to 7d).

The water flow technique was utilised to determine the tensile strengths of the granuloma tissue[39,40]. The MOCD

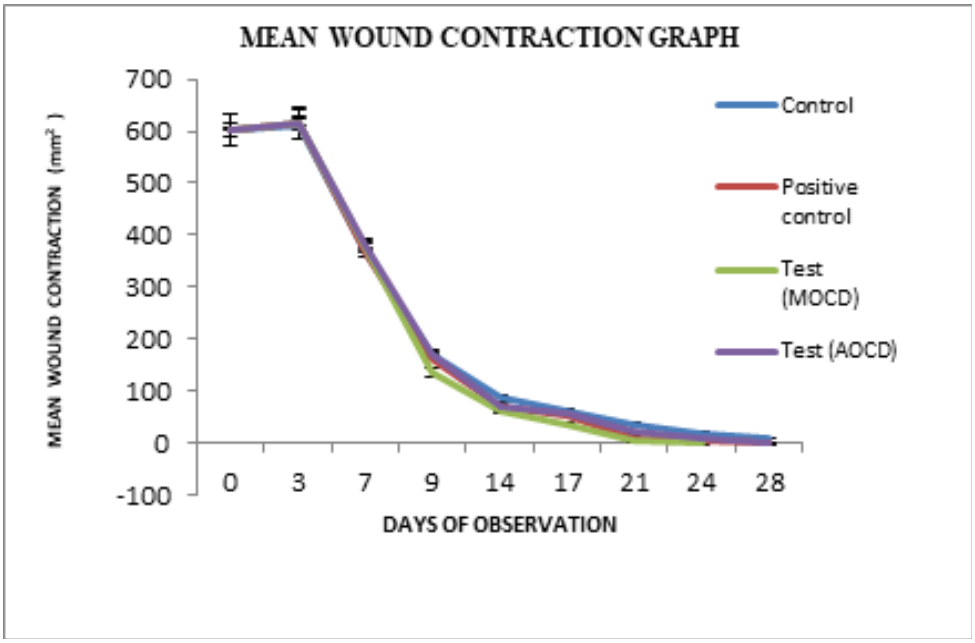


Figure 5. Mean wound contraction graph for MOCD, AOCD of *C. depressus* and Positive control. All the values are expressed as (Mean± SD).

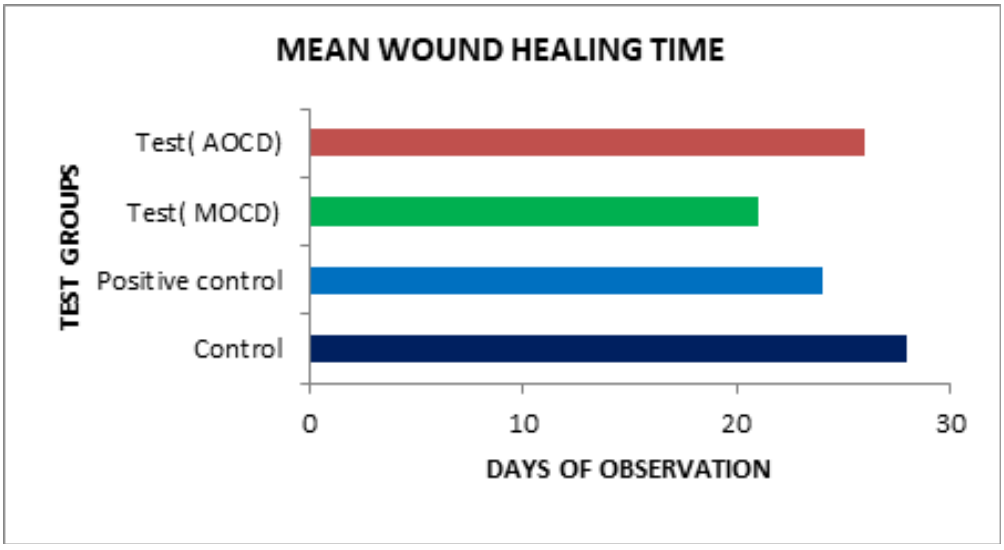


Figure 6. Comparison of the Mean Wound Healing Time (Days) in Excision wound model.

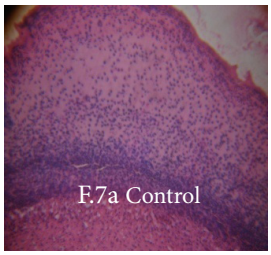


Figure 7a. The Normal control group rats showing moderate aggregation of macrophages.

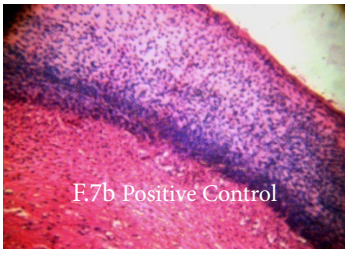


Figure 7b. Positive control animals showing decreased macrophage aggregation.

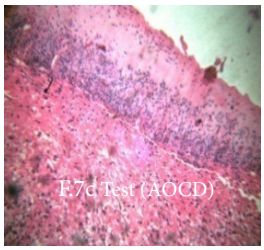


Figure 7c. The AOCD test rats showing aggregation of macrophages.

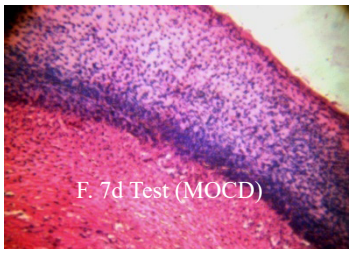


Figure 7d. The MOCD test rats showing aggregation of macrophages.

showed a highly significant increase in breaking strength (381.33 ± 4.58, P<0.05), when related to control (156.5 ± 5.75). Positive Control depicted a significant increase in breaking strength (286.83 ± 7.90) (Table 10).

4.12 Nitric oxide Estimation

Nitric oxide has a role in a variety of physiological processes including smooth muscle relaxation, neuronal signaling, platelet aggregation inhibition, and wound healing process. The MOCD showed a significant inhibition of nitric oxide levels in the scar tissue when related to the control and positive control on the 14th day (Figure 8).

Table 10. Influence of *C. depressus* on tensile strength of granuloma tissue

GROUP	Breaking Strength (g)
Control	156.5 ± 5.75
Test (*C. depressus*) MOCD	381.33 ± 4.58*
Test (*C. depressus*) AOCD	252.18±3.24*
Positive control (Betadine)	286.83 ± 7.90*

*shows a significant difference at P<0.05 when compared to the control. Values are Mean± SD. Data analyzed by One-way ANOVA followed by Dennett's test.

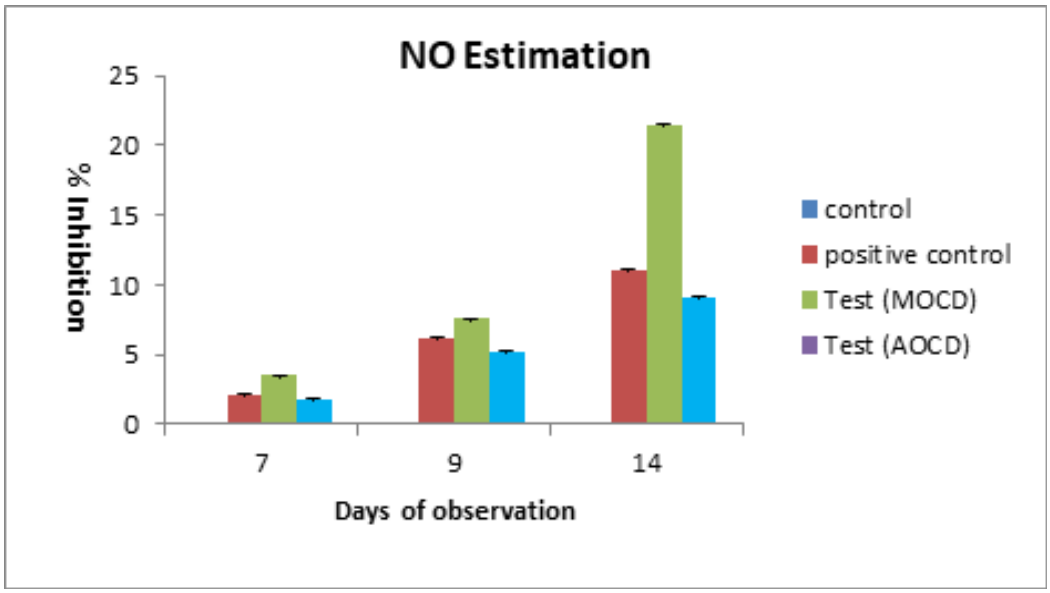


Figure 8. Nitric-oxide Estimation Graph for Excision wound model. Values are Mean ±SD. *indicates significant difference at P<0.05 when compared to control.

4.13 Linear Incision Wound Model

There was a significant increase in the tensile strength of the Extract ointment group whsen compared with the vehicle group. This may have resulted because of an increase in remodeling of collagen (Table 11 and Figure 9).

Figure 9. Measurement of Tensile Strength by using a Tensiometer.

4.14 Dead space wound model

This model demonstrated that the MOCD increased the hydroxyproline content (75.43 ± 2.97, p<0.001) significantly when compared to the control. The hydroxyproline content may be correlated with collagen production (Table 12).

4.15 Hydroxyproline Content for *in-vivo* Mice-burn Model

Our study resulted that the hydroxyproline content (Figure 10a) significantly increased in Gr-2 (Positive control) and Gr-3(MOCD) groups (p<0.05) as related to the Gr-1(Negative control).

4.16 Wound Contraction for Mice-burn Model

Significant (p<0.05) wound contraction was found from the 12th day in Gr-3 (MOCD group) till the 24th day. The positive control group Gr-2 also produced a significant

Table 11. Comparison of Tensile strength (N) values of the extract of MOCD, AOCD, vehicle, negative control and reference drug on Linear incision wound model

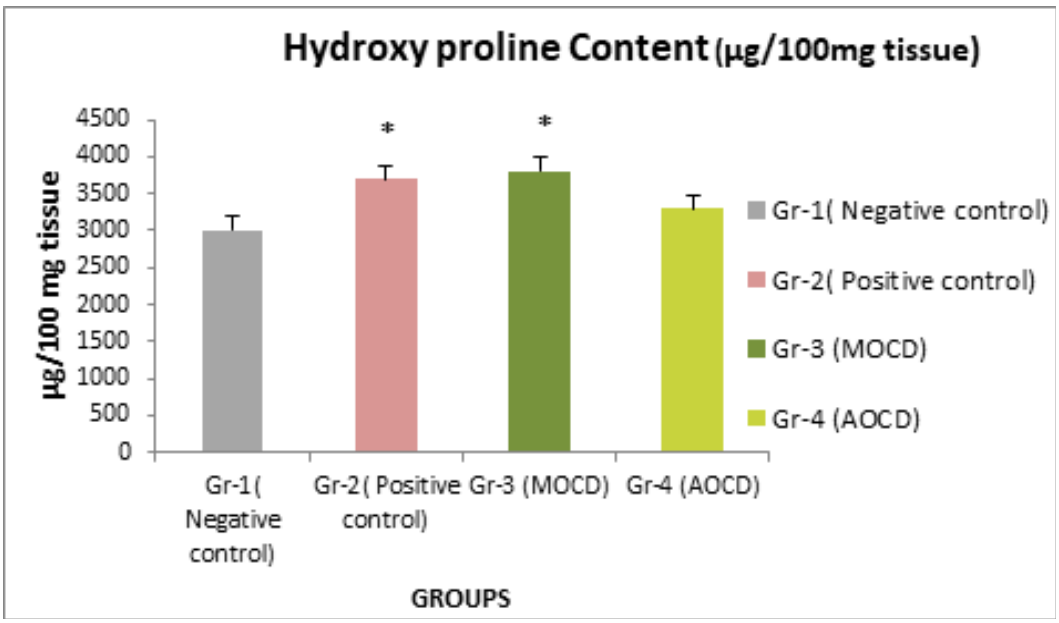
GROUP	Tensile strength (N) Mean ± S.E.M	% Tensile strength
Vehicle	18.91 ± 1.23	25.14
Negative control	15.11 ± 1.62	-
Extract ointment (5% w/w) MOCD	25.30± 2.16	33.79**
Extract ointment (5% w/w) AOCD	21.11±1.35	11.63
Positive control	29.21 ± 1.1	54.46***

**indicates significant difference at P<0.01, ***p<0.001. Percentage of contraction values: The vehicle group is compared to the negative control group. The extract and positive control are compared to the vehicle group. Mean±S.E.M, S.E.M is the Standard Error of Mean.

Table 12. Hydroxyproline Estimation in Dead Space Wound Model

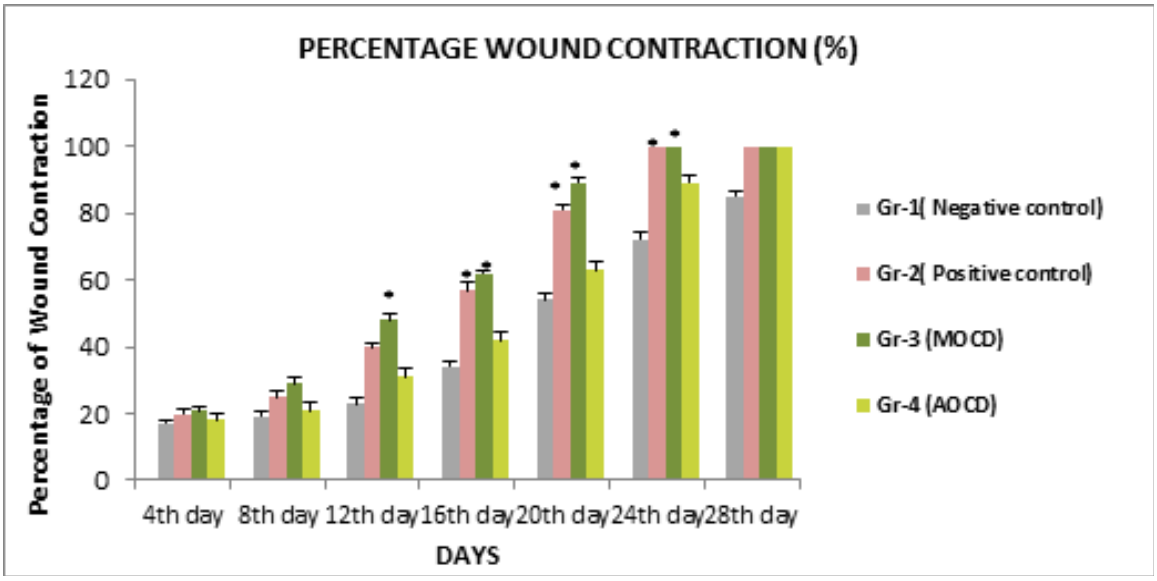
GROUP	Hydroxy Proline Content (µg/mg tissue)
Control	43 ± 2.34
Positive control	70.2 ± 3.42**
Test extract (*C. depressus*) MOCD	75.43 ± 2.97***
Test extract (*C. depressus*) AOCD	55.26 ± 1.53*

**indicates significant difference at P<0.01, *p<0.05, ***p<0.001 Vs Control; Mean± S.E.M, S.E.M is Standard Error of Mean.



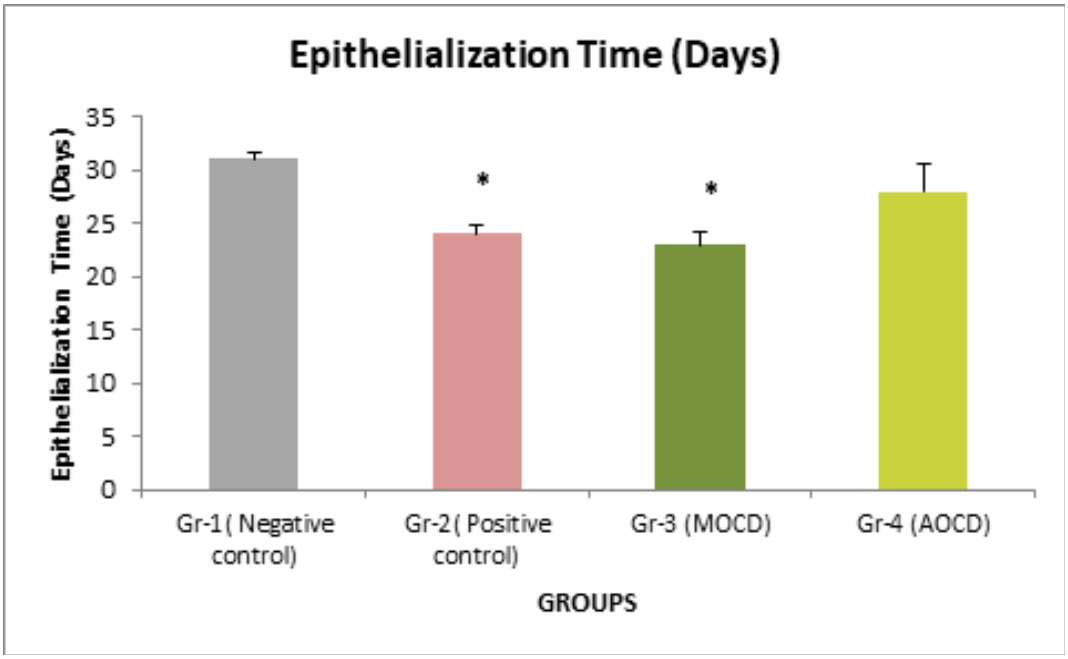
N=6, Values are mean± SEM. *indicates significant difference at P<0.05 when compared to Gr-1 control.

Figure 10a. Effect of topical application of MOCD and AOCD on hydroxyproline content in Mice burn model.



N=6, Values are mean± SEM. *indicates significant difference at P<0.05 when compared to Gr-1 control.

Figure 10b. Effect of topical application of MOCD, AOCD and the standard on percentage of wound contraction in Mice burn model.



N=6, Values are mean± SEM. *indicates significant difference at P<0.05 when compared to Gr-1 control.

Figure 10c. Effect of topical application of MOCD, AOCD and the standard drug on Epithelialization time (Days) in Mice burn model.

percentage of wound contraction when related to the Gr-1 Negative control (Figure 10b).

4.17 Epithelialization Time for Mice-burn Model

The epithelialization time (Figure 10c) was significantly reduced in Gr-2 (Positive control) and Gr-3 (MOCD treatment group).

5. Discussion

Wound healing of the skin is a complex biologic phenomenon that occurs when local epithelial cells and fibroblasts interact with inflammatory cells such as macrophages, neutrophils along with mast cells. Mast cells are found in great numbers in wounded skin in comparison with noninjured skin[41].

Wound repair consists of a long-term remodelling phase where neutrophils form a first-line defense against infection and also a source of pro-inflammatory cytokines. Wound healing is also regulated by macrophages by exerting antimicrobial function; wound debridement and the production of various growth factors such as PGDF, and TGF-α[42].

Major source of IL-10 in the process of wound healing remains to be found out. B-cells, Macrophages, regulatory T-cells, and keratinocytes are reported to produce the cytokines. Mast cell count was, however, favorably linked with IL-10 expression levels. Mast cells have been found to take a crucial role in wound healing, thereby promoting fibroblast proliferation, collagen synthesis, and angiogenesis[43,44].

The present investigation depicts that the methanolic extract of *C. depressus* shows a higher percentage (57.2%) of increase in Interleukin 10 (IL-10) production, followed by aqueous extract (40.6%). Hence, the methanolic and aqueous fractions were taken for initial acute dermal toxicity and then for the formulation of ointment and screening on animal models for wound healing activity.

During the acute dermal toxicity study, we proceeded with blood and serum analysis among the study group of animals, since blood serves as the major medium for carrier of drugs. Toxicants can damage mature cells directly or indirectly in the bone marrow, resulting in a decrease in cell counts in the haemopoetic system. Serum biochemical markers can also be used to assess medicinal plant toxicity[45,46].

The dermal toxicity study resulted in non-significant alteration in haemological and serum biochemical studies, suggesting that herbal extracts did not induce any allergic skin and systemic toxic reaction in rats.

The wound and its repair are aided by the genetic response that regulates the body's own cellular resistance systems[47]. Hence in our present study, incision and excision wound models were used to find the effect of *Corchorus depressus* Linn. extract on different wound healing phases.

Hydroxy proline is a component of the triple helix collagen fibre. Because hydroxyproline concentration is proportional to that collagen content, it has been employed as a marker to determine collagen content[48,49]. The estimation of hydroxyproline content showed that MOCD enhanced the collagen production. So increased collagen synthesis by the plant extract on rats may result significantly to healing and to provide the required strength to repair[50]. Collagen not only gives the tissue matrix strength, but it also helps with haemostasis and epithelization in the later stages of wound healing[51]. The improvement in tensile strength of the wounds in incision wound model could be related to increased collagen remodelling and the creation of stable inter and intramolecular cross linkages. The collagens are generated and laid down at the wound site, where they are cross linked and form fibres[52]. As the MOCD treated group showed greater tensile strength, hence it may have increased in collagen synthesis per cell, and also helped in the cross linking of the protein.

In the mice burn model significant burn wound healing was exhibited by methanolic extract ointment group (MOCD) from the 12th day till the 24th day and there was also a significant reduction in epithelialization time by MOCD in comparison to the negative control group. The activities may be correlated to the presence of Luteolin in the extract.

Infection is a common cause of postoperative wound complications. Previous research has demonstrated that the antimicrobial activity of several plants authenticates the healing of wounds. The past review article[53] describes that the plant has been used as an antibacterial, antifungal, and anthelmintic drug in Folklore medicine, and from our previous study[54] on *C. depressus*, it was verified that the plant has antibacterial action against *E. coli*, *B. subtilis* and *S. aureus*, antifungal against *C. albicans* and *A. niger* and anthelmintic activity. Hence, the present wound healing activity may have resulted due to its antimicrobial action.

The phytochemical studies (preliminary), showed the detection of alkaloids, Flavonoids, glycosides, carbohydrates, phenols, and Tannins.

Flavonoids are known to aid the process of wound healing, owing to their astringent and anti-microbial properties, which are thought to be accountable for wound contraction and a higher rate of epithelialisation[55,56]. The detection of flavonone (Luteolin) in the methanolic extract was successfully carried out with HPLC and HPTLC studies and confirmed with the IR spectral analysis. The IR spectrum of Flavonol like Quercetin and Kaempferol and their comparison with the IR spectrum of methanolic extract confirms their presence in the methanolic plant extract.

Tannins, which are found in large amounts in many plant extracts, operate as free scavengers[57]. The significance of anti-oxidants derived from plant extracts in wound healing has received a lot of attention today[58]. Hence, the wound-healing activity of the herbal formulation could be because of the presence of Luteolin, Quercetin, kaempferol, and their anti-oxidant activity. The pace of collagen synthesis and most importantly the maturation process affected the wound-breaking strength. In our study there is a significant ($P<0.05$) increase in the breaking strength in MOCD treated group, hence we assume that *C. depressus* might have increased the collagen and also altered the maturation process, by affecting the cross-inking of collagen or by affecting the quality of the collagen fibrils.

6. Conclusion

Identification of Quercetin, Kaempferol, and Luteolin in the methanolic extract and the use of methanolic extract ointment of *Corchorus depressus* (Tiliaceae) for wound healing have been enlightened by this work. All these findings point to the possibility that the considerable wound healing property of the plant may be credited to a variety of mechanisms that include stimulation of collagen deposition, tissue granulation, and increasing tensile strength. The presence of Flavonoids, Tannins and their anti-oxidant, astringent properties may also be the mechanism behind the wound healing activity; the biochemical findings also favored to us in this case of research. The antimicrobial activity of this plant as previously studied by us further strengthens the utilization of this plant when it comes to the management of wound healing. The novelty of the research is best understood

from the fact that a detail scientific study corresponding to the wound healing study of *C. depressus* has not been reported so far and, to the best of our knowledge, we herein report for the first time. The present findings provide scientific evidence and back up Odisha Tribals' assertions about the wound-healing activity of the plant.

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8. Authors Contributions

Khuntia T. K. performed the phytochemical, pharmacological and analytical experiments presented in the manuscript. Khuntia T. K., Mohanty A. K., Senapati A. K. wrote the paper. Nanda U. N., and Senapati A. K., guided each step of the experiment, and reviewed the final manuscript. Khuntia T. K. and Mohanty A. K. involved in carrying out the formulation presented in the manuscript. All the authors have agreed to submit the final manuscript to the esteemed journal.

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