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Research Article

Preliminary Pharmacological Screening of *Corchorus olitorius* Extracts for Anti-inflammatory and Wound Healing Activity

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ABSTRACT

Aim of present study was to investigate the preliminary screening of aerial part of *Corchorus olitorius* extracts for anti-inflammatory and wound-healing activity on experimental animals. Different extracts with respective solvents i.e. petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts of *C. olitorius* were obtained by successive solvent extraction. All extracts were screened for anti-inflammatory activity using Xylene-induced ear edema in mice and wound healing effect was studied by using incision wound model in mice. The weight of the ear lobes and biochemical measurements, nitric oxide (NO) levels and MPO in the tissue sample were used to detect the anti-inflammatory effect. The wound healing effect was observed by measurement of tensile strength and protein and hydroxyproline level assessment in the healed tissues. Observations of present study were confirmed that ethanol extract of *C. olitorius* (EECO) was showed significant ($p<0.05$) inhibition in the ear edema of mice. NO and MPO activity was decreased significantly in EECO-treated mice. Wound healing potential was observed by significant improvement in tensile strength as well as protein and hydroxyproline level of healed tissue of EECO-treated mice. In conclusion, ethanol extract of *C. olitorius* was found most effective for anti-inflammatory through reduction of NO and MPO activity as well as wound healing potential in experimental mice.

Introduction

Healing of any wound is a dynamic, complex process. The individual's fluctuating health status causes changes in the wound environment. Knowing the fundamental concepts of wound healing can be framed by knowing the physiology of the typical wound healing pathway throughout the phases of hemostasis, inflammation, granulation, and maturation.[1] The quantity of inflammatory cells in the wound reduces during the proliferative phase. Fibroblasts, endothelial cells, and keratinocytes release additional growth factors necessary to mediate wound-healing. Inflammation is now recognized as a type of nonspecific immune response.[2] Vascular and cellular alterations are the two main categories for inflammation's main ingredients. Vascular changes include a rise in blood flow, temporary constriction of blood vessels, and temporary dilatation of arterioles and venules; a rise in permeability causes the release of chemical mediators, swelling, and a rise in viscosity. Leukocytes migrate from the circulation to the bacterial destruction during cellular changes. These alterations could be observed to investigate any medicinal substance to test for anti-inflammatory efficacy.[3]

Corchorus olitorius Linn (Malvaceae) is an annual herb can reach a height of 2.4 m. It has leaves with alternately slightly incised margins. Small, five-petalled yellow flowers of *C. olitorius* subsequently develop into a brown, multiseed pod.[4] In skin cosmetics, the leaf extracts can serve as moisturizers. The extracts are made up of uronic acid, which contains muco-polysaccharide, calcium, potassium, and other nutrients that work well as moisturizers. Cardenolides, beta-sitosterol, ceryl alcohol, and oligosaccharides are present in the seeds. Corchorusins and triterpenoidal glycosides are present in the aerial portions.[5] Treatments with *C. olitorius* include

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those for tumors, colds, constipation, and fevers. It has long been used for a variety of purposes, such as lowering the incidence of diseases linked to lifestyle choices, obesity, diabetes, cardiovascular disease, atherosclerosis and cancer.[6] Traditionally, in a folklore medicine, it has been reported to treat various kind of illnesses such as pimples, wounds, diabetes, hypertension, aches, pains, infertility, insect bites and swellings.[7] Allegedly stomachic and anti-inflammatory, seeds and aerial portions are utilized in pneumonia. In addition to antidiabetic benefits, pharmacological action has been demonstrated to have antiobesity, anti-inflammatory, antibacterial, and gastroprotective qualities.[8,9] The current study, based on prior research and published literature, was to study preliminary screening of *C. olitorius* (aerial parts) extracts for anti-inflammatory and wound healing effects in the experimental animal models.

Material and Methods

Plan Materials Identification and Collection

The plant material aerial parts of *C. olitorius* were collected from the locality of Raisen (M.P), in the season of August-September of the year. Prepare a herbarium for identification and authentication in Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur. Plant materials were gathered, dried in the shade to make powder, and then put away for further processing, including solvent extraction.

Extraction and Phytochemical Studies

The dried powdered plant material of *C. olitorius* (150 g) was used for the extraction process, and it was extracted using petroleum ether, chloroform, ethyl acetate, ethanol, and water in that order. The appropriate extracts were produced following thorough extraction using each solvent. Until enough extracts were collected, this extraction procedure was repeated. After the extraction procedure was finished, the solvent was entirely evaporated using a vacuum evaporator while operating at reduced pressure, yielding completely dried extract for subsequent use.

Studies of phytochemical screening begin with a qualitative chemical test of various chemical components utilizing various chemical reagents. Different extracts of *C. olitorius* aerial extracts were utilized in qualitative chemical assays to identify the presence of various chemical ingredients. [10-12]

Anti-inflammatory Activity

Experimental animals

Swiss albino mice in good health, weighing between 95-100 g were selected for anti-inflammatory screening of extract. They may be of either sex. Before the experiment, they were kept and maintained in the animal house for a week at a controlled normal temperature of 25°C, relative humidity of 44 to 56%. It should be maintain proper light and dark cycles of 10 and 14 hours, respectively. All animals care and experimental protocols were in accordance with CPCSEA/ IAEC. The animals were grouped and housed in polyacrylic cages individual group wise prior to the experimental process starting of 7 days allowed for acclimatization to the environmental conditions. All experimental Animals were unconstrained to access to food and water. Though, they went 48 hours without eating or drinking before the operation. All selected animals were divided into the seven following groups, each group contains five animals.

Group I was control and saline solution (10 mL/kg p.o) was given to every animal of the group

Groups II was given orally, petroleum ether extract of *C. olitorius* (PEECO) to each animal of group (200 mg/kg; p.o.)

Groups III, was given orally, chloroform extract of *C. olitorius* (CECO) to each animal of group (200 mg/kg; p.o.)

Group IV was given orally, ethyl acetate extract of *C. olitorius* (EAECO) to each animal of group (200 mg/kg, p.o.)

Group V was given orally, ethanol extract of *C. olitorius* (EECO) to each animal of group (200 mg/kg; p.o.)

Group VI was given orally, aqueous extract of *C. olitorius* (AECO) to each animal of group (200 mg/kg; p.o.)

Group VII was given orally, indomethacin (5 mg/kg; p.o.) to each animal of group

By triturating the extracts with 2% Tween-80, a suspension of the extracts was created. The animals were starved before being dosed. After the animals had been fasting, they were weighed and given oral test extracts. Rats were then kept without food for a further 3 to 4 hours after the dose.

Xylene-induced ear edema

As per following CPCSEA guideline and protocol, all experimental Swiss albino mice were fed various extracts orally once a day for two weeks, and xylene (0.05 mL) was administered to the right ear's posterior area at the same time. The left ear is still not being treated. Both ears were cut off, and ear lobe weights were taken, three hours following xylene treatment. Edema was measured by calculating the weight of each treated and control ear of animals and then measure their weight difference for determination of percentage edema.[13] The percentage inhibition (%) of edema of each group of animals was calculated with the help of following formula:

$$\text{Percentage edema} = \frac{\text{Observed weight of right ear} - \text{Observed weight of left ear}}{\text{Observed weight of left ear}} \times 100$$

The percentage edema was calculated by measurement of weight difference between control ear and test ear of each mouse with accurately.

Determination of nitric oxide (NO) level

Nitric oxide (NO) and reactive oxygen species are produced throughout the inflammatory process, and when they combined, and may harm to the tissue. This incidence

causes an inflammatory response that increases release of different types of reactive of radicle species of oxygen and other inflammation mediators, cytokines and prostaglandins E2 (PGE2), arachidonic acid and some derivatives. In addition, NO is a strong vasodilator that contributes to the inflammatory process and causes edema to occur.[14,15] Nitric oxide levels in the treated mouse ears were measured using the Griess reaction indirectly, which gauges nitrite, a consequence of reactions of NO with oxygen.[16] In short, 50 mLof tissue supernatant from each ear were combined with 50 mL of 1% sulfanilamide in the solution of 5% phosphoric acid andthis mixture was incubated at 22°C in the dark for 5 minutes. After that, 50 µL amount of 0.1% solution of naphthylethylenediamine dihydrochloride was added, and a microplate reader was used to measure the absorbance at 540 nm (ELISA, Micro Lab, Ahmedabad, India). Based on a sodium nitrite standard curve, the amount of nitrite was determined. It was represented as nmol of nitrite per ear.

Myeloperoxidase (MPO) enzyme level determination

The reported standard method of Krawisz et al. (1984)[17] with slight modification was used to measure tissue MPO activity 24 hours after applying xylene to the mouse ear. In brief, 10 mL of ice-cold 50 mM potassium phosphate buffer (pH 6) containing solution of 0.5% hexadecyltrimethyl-ammonium bromide was used to punch and mince 6 mm ear tissue. The tissue homogenate was sonicated at 12000 g (Remi Centrifuge, India) and centrifuged at 4°C for 20 minutes. By mixing 0.1 mL of the supernatant with 2.9 mL of 50 mM phosphate buffer in 0.0005% hydrogen peroxide, the MPO activity was measured spectrophotometrically at 460 nm. At room temperature, the change in absorbance per minute was used to measure MPO activity. Rats treated with test and comparision with vehicle showed increased activity as a percentage of MPO level.

In-vivo Wound Healing Activity

Experimental animal protocol

Wound healing activity was assessed using healthy Swiss albino mice (weighing 95–100 g) of either sex as per CPCSEA guidelines. Prior to the experiment, all animals were maintained in the animal house for a week at a controlled room temperature of 25°C, and relative humidity (44–56%) with proper light and dark cycles of 10 and 14 hours, respectively. The animal care and experimental protocols were in accordance with the CPCSEA/IAEC. Experimental animals were selected at random pattern, marked with a marker to enable for individual recognition and place in its separate cage for at least seven days prior to dosing to give it time to get used to the lab environment. For the experiment, the rats were kept in groups in polyacrylic cages individual group wise. During whole experimental process all animals have unlimited access to food and water freely.

Extracts were prepared as a suspension by triturating with 2% Tween-80 and applied topically twice in a day.
All experimental animals were distributed in to seven groups containing 5 animals each group as given followings:
Group I was represent control and treated with vehicle given topically twice in a day to each animal of that group.
Groups II was applied topically, petroleum ether extract of *C. olitorius* (PEECO) twice in a day to each animal of group
Groups III, was applied topically, chloroform extract of *C. olitorius* (CECO) twice in a day to each animal of group
Group IV was given topically, ethyl acetate extract of *C. olitorius* (EAECO) twice in a day to each animal of group
Group V was given topically, ethanol extract of *C. olitorius* (EECO) twice in a day to each animal of group
Group VI was given topically, aqueous extract of *C. olitorius* (AECO) twice in a day to each animal of group
Group VII was given topically, povidine iodine ointment twice in a day to each animal of group

Incision wound model

Selected animals were anesthetized using standard protocol before creating incision wounds. On each side of the rat's depilated back, two para vertebral long incisions were cut through the skin, about 1.5 cm from the midline. Throughout the experiment, no local nor systemic antimicrobials were applied. Sutures were applied with the use of black silk surgical thread (No. 000). A surgical stainless steel curved needle (No. 11), the two edges were maintained together and sewn together. To ensure a complete shutting of the wound, the continuous threads were applied on both wound edges close to each other tightened. Following stitching, the incision was left uncovered while simple ointment base, a sample medicine, and a normal drug ointment were applied every day for up to nine days. On the ninth day, sutures were removed once the wounds had fully healed, and a tensiometer was used to evaluate the skin's tensile strength.[18]

Tensile strength determination

Healed skin tissue is required to check their resistance that representing to tensile strength of tissue. The resistance to breaking under tension is known as tensile strength. It can partially identify the treated tissue and shows how much the mended tissue resists breaking under tension. To evaluate the tensile strength, the recently healed tissue, including the scar, was removed. Before testing, the animal was anaesthetized with diethyl ether using an open mask method. The sutures are taken out of the stitched wounds on the rats a day before the tensile strength test is measured. Then, in the middle of the board, a stack of paper towels was placed on top of the animal. The instrument used for measurement is called 'Tensiometer'.[19]

Protein content determination

Standard reported method of Lowry (1951) with slight modification, the protein content of skin tissues was

assessed.[20] To quantify the amount of tissue protein, sodium tartrate, copper sulphate, and sodium carbonate were added to a solution of tissue lysate. Folin-Ciocalteau reagent was applied after the mixture had stood for 10 minutes, and the mixture turned bluish in 20 to 30 minutes. The absorbance was taken at 650 nm using spectrophotometer. Protein content was calculated with the help of standard curve of protein.

Hydroxyproline measurement

A portion of dry, defatted tissue weighing 10 to 20 mg was put into glass tubes with a stopper or a screw cap, and added 4 mL of distilled water at the end. The tubes were then heated for six hours at 15 lb of pressure in an autoclave. The distilled water containing gelatin was then put into a small beaker (20–30 mL) after the tubes were taken out and given time to decrease temperature. A mixture of aqueous, including gelatin, was placed into a small beaker (20–30 mL) after the tubes were taken out and given time to cool. In two separate portions, taken 1-mL portions of 6N hydrochloric acid, the solution in the residual was absorbed and then transferred to stoppered tubes. The autoclave was heated for 6 to 12 hours at 15 lb of pressure to achieve hydrolysis. After chilling, the tissue hydrolysate was diluted with 10 mL of distilled water and filtered. The diluted hydrolysate was further diluted with 5.0 mL of assay buffer 2.5 mL of chloramine T reagent and let to stand for 20 minutes. at room temperature in order to prepare the sample for the colorimetric analysis. This solution was mixed thoroughly, 2.5 mL of freshly made dimethylamino-benzaldehyde reagent was added, and the tubes were heated up to 60˚C for 15 minutes before cooling in tap water for 5 minutes.[21] The absorbance was measured at 557 nm within 45 minutes with the heal of UV visible spectrophotometer (Shimadzu).

Statistical Analysis

All tabulated data were represented as mean ± SD for all statistical analyses. Each treatment group contained six animals. The variation between mean values was determined by one way variance analysis (ANOVA) and the multiple comparisons test (Tukey's). GraphPad Instat Software executed a statistical analysis of the results. If $p < 0.05$, then all collected data were considered statistically significant.

Results and Discussion

Phytochemical Studies

The dried powder of aerial parts of *C. olitorius* were subjected to extraction with petroleum ether, chloroform, ethyl acetate, ethanol and water to get respective extracts. The phytochemical analysis of all extracts of *C. olitorius* was showed that different category of chemical components were present in different extracts. Sterols, terpenoids, and fatty oils were present in the *C. olitorius* petroleum ether extract, while flavonoids, glycosides, phenolic compounds, and amino acids were present in ethanol extract of *C. olitorius.* The screening of phytochemicals' outcomes showed good content.

Anti-inflammatory Activity

Different models used for the in vivo screening for anti-inflammatory activity and each model have specific importance and by releasing inflammatory mediators, several inducers have caused inflammation. Present study consists of preliminary screening of various extracts of *C. olitorius* (aerial parts) for anti-inflammatory using xylene-induced edema on experimental animals.

Different extracts i.e petroleum ether extract, chloroform extract; ethyl acetate extract; ethanol extract and aqueous extracts of *C. olitorius* were used for anti-inflammatory and wound healing potential. Xylene-induced ear edema model is a convenient animal model of screening for anti-inflammatory effectiveness, especially in cases of fluid retention and edema, which in mice are signs of an early inflammatory reaction.

In present study, different extracts i.e. petroleum ether extract, chloroform extract; ethyl acetate extract; ethanol extract and aqueous extracts of *C. olitorius* were given orally to experimental mice as per given procedure to measure the percent inhibition to which xylene causes mouse ear edema (Table 1 and Fig. 1). Effect of ethanol extract (EECO) at dose of 200 mg/kg; p.o. detected a high percentage of edema inhibition (60.21%), which was near to the standard group receiving indomethacin (5 mg/kg; p.o.) treatment showed 62.36% inhibition. The percentage of inhibition was determined by measuring the ear weight following exposure to xylene. Other extracts treated mice were not observed significant percent inhibition of edema. In present study result indicate that ethanol

Table 1: Effect of different extracts of *C. olitorius* on ear weight in xylene induced ear edema model

Animal groups	*Observed weight of ear lobe (g) Mean ± SD*	*Percentage inhibition of ear edema (%)*
Control (Saline solution)	0.93 ± 0.03	-
PEECO (200 mg/kg; p.o.)	0.82 ± 0.11	4.8
CECO (200 mg/kg; p.o.)	0.84 ± 0.07	9.6
EAECO (200 mg/kg; p.o.)	0.76 ± 0.04	18.27
EECO (200 mg/kg; p.o.)	0.37 ± 0.07	60.21
AECO (200 mg/kg; p.o.)	0.75 ± 0.08	19.35
Indomethacin (5 mg/kg; p.o.)	0.35 ± 0.04	62.36

Each tabulated value represent as mean ± S.D. (n=5), each value considered significant if *p < 0.05 when compared with control and standard group. PEECO: petroleum ether extract of *C. olitorius*; CECO: Chloroform extract of *C. olitorius*; EAECO: Ethyl acetate extract of *C. olitorius*; EECO: ethanol extract of *C. olitorius*; AECO: Aqueous extract of *C. olitorius*

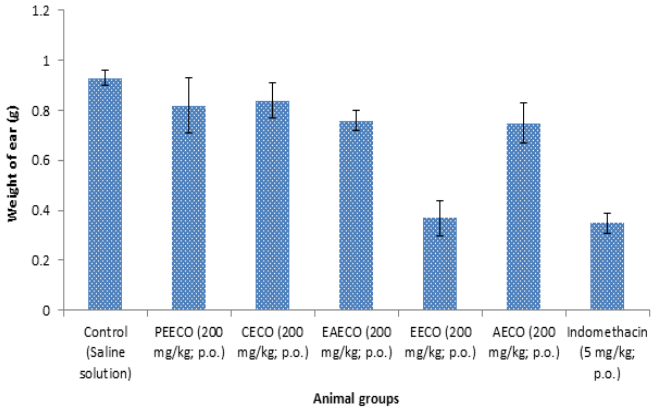

Fig. 1: Effect of different extracts of *C. olitorius* on weight of ear in xylene induced edema in mice. PEECO: petroleum ether extract of *C. olitorius*; CECO: Chloroform extract of *C. olitorius*; EAECO: Ethyl acetate extract of *C. olitorius*; EECO: ethanol extract of *C. olitorius*; AECO: Aqueous extract of *C. olitorius*

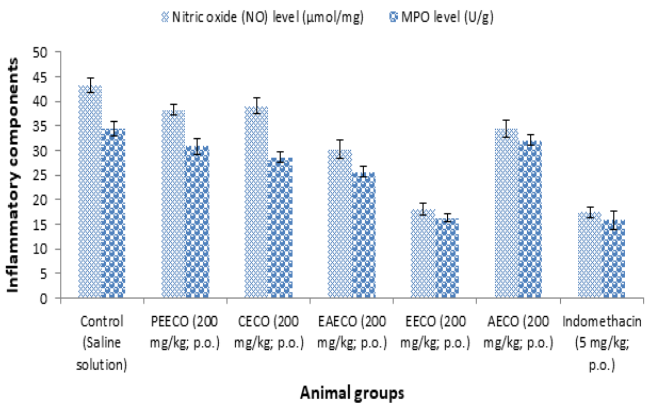

Fig. 2: Effect of different extracts of *C. olitorius* on inflammatory components nitric oxide (NO) and myeloperoxidase (MPO) levels of ear tissue after application of xylene. Data was compared with control group by oneway ANOVA test. All tabulated data are showed as mean ± SD. PEECO: petroleum ether extract of *C. olitorius*; CECO: Chloroform extract of *C. olitorius*; EAECO: Ethyl acetate extract of *C. olitorius*; EECO: ethanol extract of *C. olitorius*; AECO: Aqueous extract of *C. olitorius*

extract of *C. olitorius* possess inhibitory effects against acute inflammation in mice.

Other extract petroleum ether extract, chloroform extract; ethyl acetate extract and aqueous extracts of *C. olitorius* does not showed a significant percent of inhibition.

The administration of xylene causes neurogenous edema in the xylene-induced ear edema model. It has a tenuous connection to substance P. This substance is an undecapeptide in both the central and peripheral nervous systems and plays several physiological roles as a neurotransmitter or neuromodulator. In response to stress, this chemical is released by midbrain neurons, which allows dopaminergic neurotransmission from sensory spinal cord neurons to occur. This neurotransmission activates dorsal neurons. Substance P's participation in neurogenous inflammation is suggested by the peripheral vasodilatation and plasma extravasations caused by its release from sensory neurons. Swelling of ear in the mice is may be the major cause.[22]

Table 2: Effect of different extracts of *C. olitorius* on inflammatory markers in xylene induced ear edema method

Animal groups	*Inflammatory components*	
	Nitric oxide (NO) level (µmol/mg)	*MPO level (U/g)*
Control (Saline solution)	43.25 ± 1.40	34.51 ± 1.44
PEECO (200 mg/kg; p.o.)	38.27 ± 1.05	30.88 ± 1.62
CECO (200 mg/kg; p.o.)	39.08 ± 1.62	28.61 ± 1.08
EAECO (200 mg/kg; p.o.)	30.27 ± 1.77	25.75 ± 1.17
EECO (200 mg/kg; p.o.)	18.13 ± 1.28*	16.33 ± 0.89*
AECO (200 mg/kg; p.o.)	34.55 ± 1.75	32.14 ± 1.02
Indomethacin (5 mg/kg; p.o.)	17.46 ± 1.07	15.85 ± 1.97

Each value is the mean ± S.D. (n = 5), *p<0.05, PEECO: petroleum ether extract of *C. olitorius*; CECO: Chloroform extract of *C. olitorius*; EAECO: Ethyl acetate extract of *C. olitorius*; EECO: ethanol extract of *C. olitorius*; AECO: Aqueous extract of *C. olitorius*

Results of nitric oxide (NO) level and myeloperoxidase (MPO) level were observed in Table 2 and Fig. 2). Comparing the inflammatory control group (the mice with inflamed ears that were only given saline solution) to the non-inflammatory control group, a rise in nitric oxide levels was noted.

Different extracts i.e petroleum ether extract, chloroform extract; ethyl acetate extract; ethanol extract and aqueous extracts of *C. olitorius* were given orally to the respective animal groups along with xylene treatment. Results showed that ethanol extract of *C. olitorius* showed significant reduction in the NO level (18.13 ± 1.28 µmol/mg and MPO level (16.33 ± 0.89 U/g). This was comparable to the standard group of animal treatment. Indomethacin (5 mg/kg; p.o.) group of animals were showed reduced NO level (17.46 ± 1.07) and MPO level (15.85 ± 1.97).

These findings imply that *C. olitorius* showed acute anti-inflammatory properties may be connected, at least in part, to their capacity to obstruct the pathways involved in NO formation.

These observations are suggests that acute inflammation was reduced with the treatment of ethanol extract of *C. olitorius, which* may be connected, at least in part, to their capacity to obstruct the pathways involved in NO formation. Since xylene plays a crucial part in the regulation of the skin's inflammatory response through the activation of intracellular signaling pathways dependent on the iNOS enzyme, where the production of NO encourages vasodilatation, which directly contributes to the development and initiation of inflammation and inflammatory process.[23]

Another enzyme, called MPO, is found in neutrophils, while it is found in much lower concentrations in monocytes and macrophages, are major component of innate immunity. It is well reported that the quantity of neutrophils on the inflamed tissue directly relates to the amount of MPO activity.[24] The drug's inhibition of MPO activity, which stops the production of oxidants such as hypochlorous acid, is directly related to plant extracts' ability to reduce inflammation.[25, 26]

According to preliminary phytochemical analyses, the aqueous extract of *C. olitorius* leaves included phenols and flavonoids. In a previous report,[27] The phenolics and flavonoids are present in the aqueous extract of *C. olitorius* leaves are primarily responsible for the plant's antiviral effect. The current study's findings demonstrated that the myeloperoxidase level of inflamed ears (inflamed controls, treated simply with saline solution) was substantially greater than that of non-inflamed controls (without treated ear)($p < 0.05$), and the plant extract treatment group. The findings demonstrate that xylene can cause neutrophil infiltration into mouse ear tissue. Treatment with plant extract and indomethacin greatly decreased the MPO levels in mouse ears, which may help to mitigate this.

Wound Healing Activity

Wound healing activity of different extracts of *C. olitorius* (petroleum ether extract, chloroform extract, ethyl acetate extract, ethanol extract, and aqueous extract) was investigated using incision wound model in mice. Povidone iodine ointment was used to compare the healing effect. Tensile strength and biochemical parameter measurements allowed for the observation of wound healing effects. Biochemical parameters (protein estimation, hydroxyproline level) was measured after 9th day of treatment in incision wound of mice. Observation of present study was showed that ethanol extract out of all extracts was showed significant increase in tensile strength of wound tissue, and it was near to the standard group of animals and comparable (Table 3, Fig. 3) after 9th day of treatment. As per previous report, leaves of *C. olitorius* are rich source of vitamins (such as B1, B2, folic acid C and E) and minerals such as calcium, iron.[28]

Table 3: Effect of different extracts of *C. olitorius* on tensile strength of wound tissue collected after healing in incision wound model

Animal Groups	*Measured tensile strength (gm/cm^2)*
Control	324 ± 10.25
PEECO	375 ± 10.4
CECO	402 ± 12.42
EAECO	452 ± 12.67
EECO	782 ± 14.52*
AECO	426 ± 12.84
Povidone iodine ointment	802 ± 24.53

Data considered significant if *$p < 0.05$ when compared to the control group. These data analysed by using oneway ANOVA followed by Tukey's test. Value represent as mean ± SD.

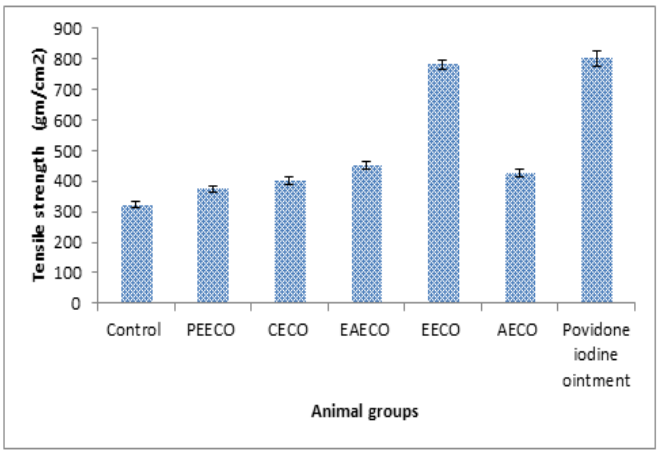

Fig. 3: Effect of different extracts of *C. olitorius* on tensile strength of wound tissue in incision wound model

Table 4: Effect of different extracts of *C. olitorius* on various wound parameters of incision wound model

Animal Groups	*Hydroxyproline content (mg/ g tissue)*	*Protein content (mg/g tissue)*
Control	23.51 ± 0.85	38.75 ± 1.20
PEECO	27.78 ± 1.05	40.25 ± 2.14
CECO	34.62 ± 1.62	42.88 ± 2.50
EAECO	38.07 ± 1.24	51.20 ± 2.64
EECO	73.28 ± 3.26*	86.17 ± 3.57*
AECO	32.41 ± 1.42	42.11 ± 2.61
Povidone iodine ointment	72.15 ± 3.85*	83.34 ± 3.75*

n=6; data represent Mean ± S.D. Tabular values considered significant

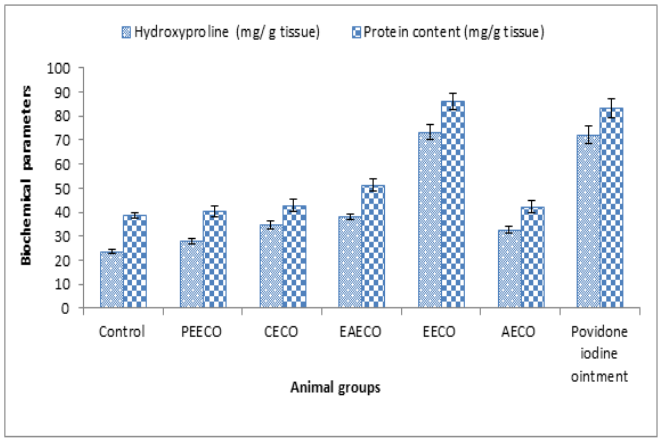

Fig. 4: Effect of different extracts of *C. olitorius* on hydroxyproline and protein content of incision wound tissue

These components may have important role in the improve healing of wounds. Reports of anti-inflammatory effect, antioxidant and antibacterial effects are major contribution in the present study to support wound healing potential of ethanol extract of *C. olitorius* leaves.[29-31] The leaves of *C. olitorius* contain a number of phenolic and flavonoid compounds already reported for antioxidant, antibacterial and antidiabetic effects.[32]

Results of protein content and hydroxyproline content were shown in Table 4 and Fig.4. Ethanol extract of *C. olitorius* showed a significant increase in protein content (86.17 ± 3.57 mg/g tissue) as well as hydroxyproline content (73.28 ± 3.26 mg/g tissue) when compared with control group of animals. These observed results were also comparable to standard group povidone iodine ointment. Other extracts of petroleum ether, chloroform, ethyl acetate and aqueous extracts of *C. olitorius* does not showed a significant increase in proteit and hydroxyproline content.

Conclusion

In conclusion, results of the present study was confirmed that ethanol extract of *C. olitorius* showed significant anti-inflammatory and wound healing potential on test animals may be due to the abundant of flavonoid constituents. Results were showing that ethanol extract of *C. olitorius* may inhibit nitric oxide release in acute inflammation caused by xylene application on the ear. Inhibition of MPO activity by the *C. olitorius* extract may preventing the generation of oxidants directly correlated to the anti-inflammatory potential of *C. olitorius*.

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