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Phytochemical Screening and Evaluation of Bone Healing activities of *Disporum cantoniense*

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ABSTRACT:

Background: *Disporum cantoniense*, also called bhui harjora is found especially in the subtropical zone of eastern Nepal and has been used traditionally as first aid for bone fractures. **Aim:** The present investigation was designed for the phytochemical screening and evaluation of the bone healing property of *D. cantoniense* on a rabbit. **Method:** Extract of roots was made into a paste in the form of poultice. About 3 groups of rabbits i.e., test, control, and standard were induced closed transverse fractures in the left femur under anesthetic effect, which were then immobilized by a splint. The development of the bone healing process was observed with the help of radiography. All the groups of rabbits were kept in a similar environment system that included diet, water, humidity, and temperature. **Result:** Phytochemical screening using different solvent systems showed the presence of Alkaloids and Flavonoids. The radiographic studies showed a significant soft callus formation at the end of fifteen days in both test and standard groups while not so much in the controlled groups. At the end of a month, both the controlled and test group had almost perfectly joined bones. **Conclusion:** The plant show bone healing property and the phytochemical studies showed the presence of Flavonoids which is an essential anti-oxidant for bone healing along with other phytochemicals.

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INTRODUCTION:

Bone is a dynamic biological tissue composed of metabolically active cells integrated into a rigid framework, which protect various organs of the body, produce red and white blood cells, store minerals, provide structure and support for the body and enable mobility [1,2]. When there is complete or incomplete separation in the continuity of bone, it's called a bone fracture. Bone fracture is accompanied by pain, swelling, bruising and discolored skin around the affected area.

Previously, the absolute majority of fractures and dislocations were treated non-operatively [3]. The development of operative treatment of fractures was influenced by three major inventions: anesthesia in 1846 A.D., antisepsis in 1865 A.D., and x-rays in 1895 A.D [4]. Treatment focuses on providing the injured bone with the best circumstances for optimum healing (immobilization). The fractured bone area is immobilized for 2 to 8 weeks. If a broken bone has been aligned properly and kept immobile, the healing process is usually straightforward. Callus forms on either side of the fracture and grows toward each end until the fracture gap is filled. Eventually, the excess bone smooth's off and the bone is as it was before. The patient's age, which bone is affected, the type of fracture, as well as the patient's general health are all factors that influence how rapidly the bone heals [5].

Many chemical constituents extracted from medicinal plants help in the bone healing process. Scientists' interest in the medicinal plant has grown in recent times as numerous natural products have been isolated with distinct biological properties showing a tendency to inhibit or promote certain biological actions [6]. Despite many being aware of the advantages of the use of various medicinal plants, lack of knowledge about the ways of extraction, actual medicinal value possesses a setback about its intensive use [7]. The medicinal plant *Disporum cantoniense* also called bhui harjora has been used for many years by local people as first aid for bone fractures [8,9]. The conservation of this plant has received relatively very little attention due to a lack of management and research support.

D. cantoniense is a glabrous, erect herb with creeping rootstocks, simple, alternate, sessile, and long–pointed leaves as shown in Fig 1 and 2.

Fig 1. The roots of the *D. cantoniense*.

Fig 2. The leaves of the *D. cantoniense*.

It is cultivated by both seed sawing and vegetative methods (root) in April and May. The roots of the plant are collected especially in the time of august and November to use it on fracture and dislocation of bones by the local people. Local people in eastern Nepal use it by making a paste of the roots in the form of a poultice with the splint [10,11]. However, it has not been scientifically defined and identified because of insufficient plant samples and vivid lines of the usage for healing. Thus, the study was designed to evaluate the bone healing potency of root extract of *D. cantoniense* concerning spontaneous radiological study and also to relate about the phytochemicals present in the extract which might be responsible for the activity.

MATERIALS AND METHOD:

Reagents and Chemicals:

Ketamine injection was gifted by Jawa Pharmaceuticals Pvt. Ltd., India. Xylazine 2 % injection was purchased from Interchemie werken "De Adelaar" BV, Netherlands. Petroleum ether, acetone, benzene, pyridine, ferric chloride, chloroform, and potassium dichromate were purchased from Fisher Scientific India Pvt. Ltd. Ethanol was purchased from Merck India. All the chemicals and reagents used were of analytical grades.

Collection and authentication of plant material:

D. cantoniense was collected from Wsingtharpu, Bhojpur in the time of august and November. The plant after its collection was authenticated by National Herbarium & Plant Laboratories, Godawari, Lalitpur (Ref. no.: 2075/08/17).

Experimental animals:

Rabbit *(Oryctolagus cuniculus)* of both sexes, weighing 1 to 1.5 kg were obtained from Himalayan rabbit house, Thankot. The rabbit was selected as the anatomy of the rabbit's hind limbs are structurally similar to that of other land mammals and contribute to their specialized form of locomotion. The Bones of the hind limbs consist of long bones (the femur, tibia, fibula, and phalanges) as well as short bones (the tarsals) [12]. The animals were maintained under the standard condition in an animal house i.e., stainless steel cage with polypropylene bottom for waste management. Greenly vegetables, chickpeas, and carrots were provided as a major diet and filtered water was supplied in polypropylene bottles with stainless steel sipper tubes. The study was ethically approved by the Nepal Veterinary Council, Government of Nepal with reference number 184/75-76.

Preparation of Plant extract:

Fresh roots were separated from the whole plant and were washed thoroughly with water to remove any dirt particles on the surface of the roots. The infected roots were removed. They were forwarded for shade drying for almost 20 days.

The dried roots of the plant were taken and crushed into a fine powder with the help of a grinder and were kept in air-tight polyethylene bags in the dark until the extraction process. Later the roots were ground for making it a paste to apply topically and also for the different phytochemical tests for different solvent extraction.

Extraction by Soxhlet process:

Extraction was carried out using a solvent system with increasing order of polarity viz. Petroleum ether, benzene, chloroform, acetone, and ethanol for 3 h at 60 °C. About 50 g, of the powder sample of *D. cantoniense* was loaded in the thimble. The amount of solvent that was used to extract was about 300 ml each time. After extraction, the extract was filtered using a motor filter. The filtrate then went under various phytochemical tests [13,14].

Phytochemical screening:

The phytochemical examination was carried out for root extract of *D. cantoniense* as per standard methods [15,16].

Radiological studies:

Rabbits weighing 1 to 1.5 kg were divided into 3 groups with three rabbits each – the control (C/no. = 3), the test (T/no. = 3), and the standard (S/no. =3). Surgery was carried out under intramuscular general anesthesia with ketamine HCl 10 to 20 mg/kg body weight and xylazine 1 to 3 mg/10 kg body weight by a professional veterinary physician. Closed transverse fractures were induced in the midshaft of the left femur. The control group of animals were left with bandages around the fractured area and were noted as a Control group (C_1, C_2, and C_3) as given in Table 1. The poultice of the root was applied to the test group (T_1, T_2, and T_3) and then made immobilized with a bamboo splint. The leg of the standard group (S_1, S_2, and S_3) was immobilized with the help of a standard Thomas splint. All rabbits were housed in cages and were allowed to move freely without external support. Consequently, they wore fed a normal diet. All subjects were observed daily with radiological studies carried out every 2 weeks [13].

Table 1. Treatment given to each group.

Name of group	Treatment
Group I- Control (C)	No treatment
Group II- Test(T)	Root poultice with a bamboo splint
Group III- Standard(S)	Thomas splint

RESULTS:

PhytochemicalScreening:

Phytochemical screening of different solvent extracts showed the presence of most targeted phytochemicals as given in Table 2.

Table 2. Phytochemical tests for different extracts.

Tests	PEE	BE	CE	AE	EE
Alkaloids					
DT:	+	+	+	+	+
WT:	+	+	+	+	+
Flavonoids					
ST:	-	+	+	+	+
ART:	+	+	+	+	+
Glycoside					
LT:	-	-	+	+	+
Tannins					
FCT:	-	-	-	-	+
PDT:	-	-	-	-	-

"+": Present, "-": Absent.DT - Drangendroff's, WT - Wagner's Test, ST - Shinoda Test, ART - Alkaline Reagent Test, LT - Legal's Test, FCT - Ferric Chloride test and PDT - Potassium dichromate test. PEE - Petroleum ether extract, BE - Benzene extract, CE – Chloroform Extract, AE - Acetone extract and EE - Ethanol extract.

Radiological Results:

The x-ray was performed every 2 weeks. The control group of animals left with bandages around the fractured area which were noted as Control group (C1, C2, and C3) on day 1, showed no notable callus formation on an examination performed on day 15. On the radiological examination performed on day 30, it was observed that there was an initiation of callus formation as shown in Fig 3.

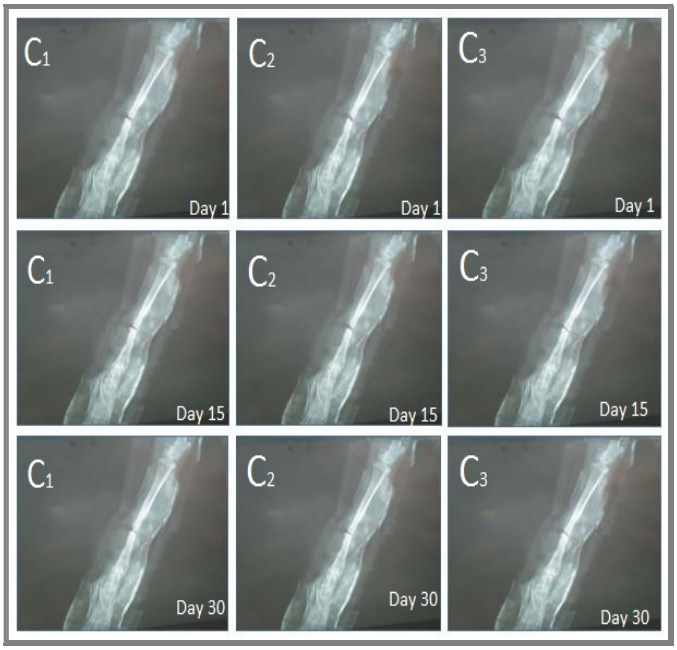

Fig 3. X-rays of transverse fractures in mid-shaft of the left femur three rabbits of Control (C) group (Group-I) on day 1, day 15 and day 30.

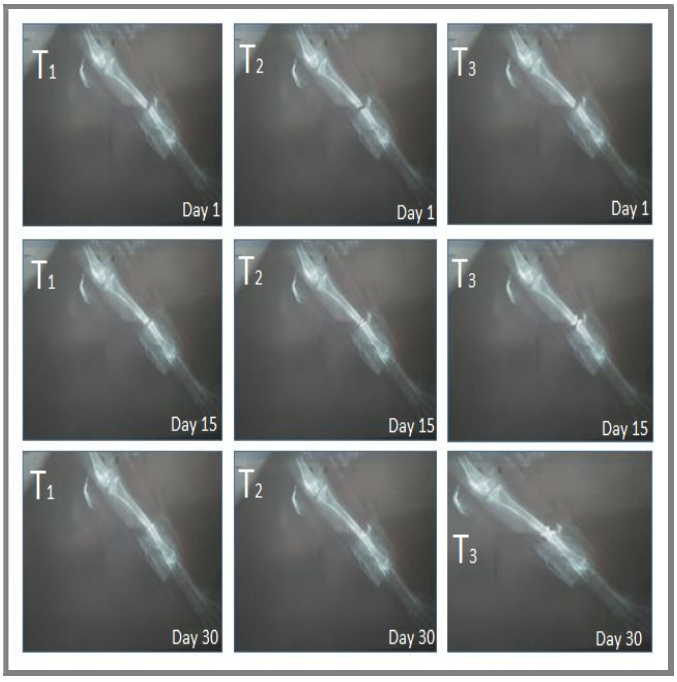

Fig 4. X-rays of transverse fractures in mid-shaft of the left femur three rabbits of Test (T) group (Group-II) on day 1, day 15 and day 30.

The poultice of the root of *D. cantoniense* was applied to the test group (T_1, T_2, and T_3) which was then made immobilized with a bamboo splint after closed transverse fracture on Day 1. Out of three rabbits, T_2 and T_3 showed initiation of soft callus formation on Day 15. On Day 30, there was remarkable soft callus formation around the fracture on all rabbits of this group as shown in Fig 4.

The leg of the standard group (S_1, S_2, and S_3) was immobilized with the help of a standard Thomas splint on Day 1. On radiological examination on Day 15, only S_2 showed initiation of formation of soft callus. On Day 30, examination showed remarkable soft callus formation joining the fracture as shown in Fig 5.

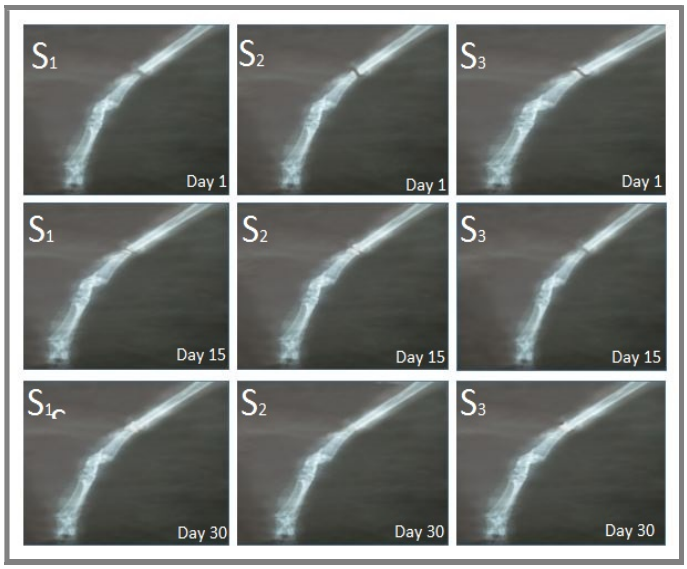

Fig 5. X-rays of transverse fractures in mid-shaft of the left femur three rabbits of Standard (S) group (Group-I) on day 1, day 15 and day 30.

DISCUSSION:

Phytochemical studies on the root extract of *Disporumcantoniense* in the different solvent system was performed to locate the best solvent system helpful for the extraction of phytochemicals having anti-oxidant property, especially flavonoids and alkaloids. Ethanolic extract showed the presence of all targeted phytochemicals viz. Alkaloids, Flavonoids, Tannins, and Glycosides. Flavonoids especially methyl gallate, myricetin, daidzen, geniotein and daucosterol have shown potential medicinal values for mandibular fracture healing [17]. All solvent systems used for the extraction of phytochemicals showed the presence of alkaloids and flavonoids. Studies have also shown the presence of flavonoids as luteolin and apigenin in *D. cantoniense* [18]. Thus, it can be related that the potentiality of bone-healing might be the result of the

presence of a high amount of flavonoids in the root extract of *D. cantoniense.*

Flavonoids have antioxidant properties which increase osteoblastogenesis and decrease osteoclastogenesis as given in Fig 6.

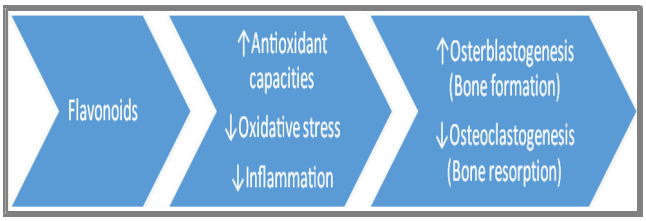

Fig 6. Role of flavonoids in bone formation.

Osteoblastogenesis is associated with an increase in osteoblast number and osteoid deposition thus increasing bone mineral apposition and bone formation rate giving bone higher strength [19]. Hence, it is responsible for the bone healing process. The test group with *D. cantoniense* poultice and the standard group with Thomas splint both had a similar development of the soft callus formation. The test group started showing the formation of the soft callus within 15 days of observation. The progress between the test group and the standard group was quite at a similar rate which can be discussed as both the groups have a similar level of treatment. The bone healing process is to restore the tissue to its original physical and mechanical properties and is influenced by a variety of systemic and local factors. The healing process is mainly determined by the periosteum. The periosteum is one source of precursor cells that develop into chondroblasts and osteoblasts that are essential to the healing of bone. The bone marrow, endosteum, small blood vessels, and fibroblast are other sources of precursor cells. Osteoclasts secrete an antioxidant called glutathione peroxidase which reduces hydrogen peroxide which is another antioxidant hence supplement of antioxidants can strengthen the bone and promote fracture healing in osteoporotic patients [20].

At the end of 30 days, there was a significant formation of soft callus in the test group, unlike the controlled group which showed a very slow formation of callus. *D. cantoniense* poultice must have contributed to the bone healing process of the closed transverse fracture induced in the test groups with the help of flavonoids found in it. Flavonoids show promising result in promoting bone health, both in the primary prevention of bone loss in later life and as a complementary therapy during conditions of high oxidative stress or chronic inflammation. Studies also show their roles in cell signaling, including Wnt-β-catenin and Bone morphogenetic protein pathways (BMP pathway) that stimulate bone formation, in addition to their anti-resorptive roles in inhibiting osteoclast activation [21]. The interaction of dietary factors with these signaling pathways is a rich area for future research.

CONCLUSION:

Disporum cantoniense root poultice which has been used traditionally as treatment of bone fracture in native parts of Nepal showed remarkable bone healing properties in our study. Preservation and conservation of this medicinal plant should be encouraged so that it can be sustainably utilized. This study will lay a foundation for the isolation of active compounds which might be responsible for bone healing activities.

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