BRHAM KAMAL (Epiphyllum oxypetalum): WOUND HEALING ACTIVITY STUDY

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INTRODUCTION:

Brahma Kamal and few other related species of the thistle tribe of plants are native to the Himalayas. Their flowers bloom during mid-monsoon months amongst the rocks and grasses of alpine meadows and gorges. These extremely rare plants are not only famous for their beautiful flowers, but also for their significant importance in traditional medicine. The Indian Himalayan Region is rich in plant diversity, comprising numerous endemic and rare plants. Brahma Kamal, a species of flowering plant named after Brahma – the Hindu god of creation, is considered the 'king of Himalayan flowers'. Brahma Kamal usually blooms in the alpine Himalayan habitats – at the upper reaches of the mountain ranges between 4500–5500 m. Outside India, it has been reported from Myanmar and South-West China. The flower is considered sacred by many, and a person who has seen it bloom is believed to be very lucky. The citation of the name 'Brahma Kamal' is found in the Puranas. According to Hindu mythology, Brahma was born from a huge white lotus called the 'Brahma Kamal'. This flower is also associated with Keywords Brahma Kamal, Saussurea, medicinal plant, Indian Himalayan, endemic. Even, in our epics like Ramayana and Mahabharata, we find references to Brahma Kamal as a holy flower.

DISTRIBUTION:

collection of bracts or leaves surrounding a flower or flower head. All the species mentioned above are restricted to high mountain ranges (3000–5700 m) and are habituated to strong winds, intense cold, and perpetual snow [4]. Brahma Kamal mainly grows 6Soft, weak, and short hairy coverage of a plant organ. in the alpine meadows, glacier slopes, along the sides of lakes and streams, alpine screes, rocky slopes, and some other high mountain habitats (Figures 2a and 2b). In India, it is found in the Himalayan ranges of Kashmir, Sikkim, Garhwal, Chamoli, and Hemkund with a prominence in the Valley of Flowers, at a height of about 3600–4500 m. Outside India, Brahma Kamal also grows in Myanmar, Bhutan, Nepal, East Tibet, and Pakistan. Kasturi Kamal prevails mostly on shady moist rocky slopes and alpine screes at an elevation of 4300–5600 m in Ladakh, Himachal Pradesh, Lahaul and Spiti Valley, Garhwal, and Sikkim in India, and some other neighbouring countries such as Nepal, Bhutan, Southern Tibet, and South-west China. Phen Kamal prefers to grow in drier areas, alpine meadows, screes, and stony slopes at an altitude of 4400–5600 m. Geographical distribution of this plant is limited to the high mountain ranges of Kashmir, Himachal Pradesh, Garhwal, Chamoli, Hemkund, and Sikkim in India, and in Pakistan, Nepal, Bhutan, Tibet, and China. Snow Lotus is restricted to high alpine reaches (up to 5100 m)– Brahma Kamal mainly grows in the alpine meadows, glacier slopes, along the sides of lakes and streams, alpine screes, rocky slopes, and some other high mountain habitats. preferably alpine meadows, screes, and dry rocky slopes. It is nearly endemic to Sikkim Himalayas. Other than India, it is very sparsely distributed in Nepal, Bhutan, and Tibet. Lastly, the Grass-leaved Saw-wort grows in alpine meadows, agricultural fields, and rocky slopes at an altitude of 3500–5600 m, and is restricted to Kashmir, Pindari, Phurkia, and Kumaon in India, and Nepal, Bhutan.

BOTANICAL FEATURES:

Brahma Kamal (**Epiphyllum oxypetalum**) is a perennial plant with stout stem, 15–45 cm long. Leaves are oblong to blunt lanceolate in shape, and leaf margins are toothed. The lower part of the leaf is stalked, and the upper part is half-clasping with the blade 7 7 The expanded flattened part of a leaf. . continuing in a wing down stem. Several purple flower heads8 occur in a dense umbel-like cluster9, each 1.5–2.5 cm long, 8A densely packed cluster of flowers or florets. and is supplemented with involucral-bracts with black margins and tips. The entire flower head is covered by large, pale yellow, boat-shaped papery bracts. 9A cluster of flowers whose spreading stalks or rays arise from the apex of the stem, resembling the spokes of an umbrella. Flowers bloom usually in July– August, and the flowers can be seen till mid-October, after which the plant perishes, becoming visible again in April. Flowers look gorgeous but they smell awful, perhaps that explains why people do not bring them home.

USES IN TRADITIONAL MEDICINE AND FOLK PRACTICE:

Their value in traditional medicine is quite noteworthy. However, the medicinal properties of Brahma Kamal and others are not clinically proven, though such practices may one day be considered for therapeutic uses. The villagers of the upper Himalayan region, who are deeply influenced either by the Indian or by the Tibetan culture, are quite habituated to the many uses of Brahma Kamal and other associated species. Their value in traditional medicine is quite noteworthy. However, the medicinal properties of Brahma Kamal and others are not clinically proven, though such practices may one day be considered for therapeutic uses. The entire plant of Brahma Kamal can be employed as a remedy for a large number of human diseases [7]. Due to its bitter nature, it is an excellent liver tonic and a great appetiser. Soup made from this plant helps soothe liver inflammations and also increases blood volume in the body. Plant juice is useful to treat urinary tract disorders. It clears recurrent urinary tract infections, and can be used as an excellent medicine for sexually transmitted diseases. Brahma Kamal is a helpful medicine to treat fevers. The flowers, rhizomes, and leaves are used for the treatment of bone ache, intestinal ailments, cough, and cold. The rhizomes in particular are used as antiseptic and for healing cuts and bruises. In the Tibetan system of medicine (known as 'Amchi' system), the plant is used in the treatment of paralysis of limbs and cerebral ischemia [8]. Locally, the wool of Kasturi Kamal flower is used for the treatment of cuts where it sticks compactly, seals the wound, and stops bleeding. The plant is also used for the treatment of asthma, bronchitis, rheumatism, menstrual problems, hysteria, and skin diseases. Phen Kamal plant is used as a remedy for cough, leucorrhoea, sexual problems, all kinds of nervous debility, and for blood purification. The root extract is also useful in treating plague, painful periods, and snake bites. In case of Snow Lotus, the whole plant is used to yield a tonic (contains 'acacetin' – a natural flavone related to potassium supply to the heart) for weakness, as therapy for menstrual disorders, and a remedy for arthritis. In the Tibetan system of medicine [8], the entire plant of Grass-leaved Saw-wort or Ghoogee is used as antitussive, aphrodisiac, it suppresses coughing. Also act as blood purifier, and that stimulates menstruation. . It is also applied in the treatment of irregular menstruation, seminal or vaginal discharge, excessive bleeding from the womb, and pain in the waist due to a loss of renal potency. Brahma Kamal, the state flower of Uttarakhand, holds immense sacred value in the Indian Himalayan Region where it is mostly found . This God's own lotus is used as offering in the hill temples of Uttarakhand, like the shrines of Kedarnath, Badrinath, and Tunganath. In September–October, during the festival of 'Nanda Asthami', Brahma Kamal is offered in temples, and is also distributed as 'prasada' [9]. Kasturi Kamal is also believed to guard against evil spirits. The oil extracted from the roots is used in high-grade perfumes, and in the preparation of hair oil. The root of Phen Kamal is used as an insecticide to protect shawls and woollen fabrics, and as incense.

MATERIAL AND METHODS:

Preparation of Plant Extract:

The plant samples collected from the botanical dept. of research of SWAMINATHAN RESEARCH CENTER. Jeypore.Odisha.which is a cultivated plant, actually its origine is uttrakhand, Himalaya. The sample was identified by Botanist of SWAMINATHAN RESEARCH CENTER. The sample was store in ice until being transported to the laboratory.

Extraction Methods:

The extract was prepared by the methods to describe in [10,11] with slight modification. The flower sample and leaf sample were washed in tap water, dried, and placed into a blender to be ground into powder. For solvent ethanol is used for the Soxhlet extraction procedure in different ratio. After 6 to 8 h of extract collected, filter with the muslin cloth and transferred to 50 ml tubes and centrifuged for 15 min at 4,000 rpm at 25ºC. The supernatant was collected and kept for drying.

Phytochemical Analysis: Chemical tests for the screening and identification of active components in the flower extract using standard protocols as described [10,11]. For each test, 100 µl of each solvent extract was used for analysis.

Test for Saponins: The extract was taken in a test tube and shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for Phenols: Extract mixed with 2 ml of 2% solution of $FeCl_3$. Blue/green color indicated the presence of phenols.

Test for Tannins: Extract mixed with 2 ml of 2% solution of $FeCl_3$. Black color indicated the presence of tannins.

Test for Terpenoids: The extract was mixed with 2 ml of chloroform. Then 2 ml of concentrated Sulfuric acid was added carefully and shaken gently. Reddish brown colors observed in the inter-phase indicate the presence of terpenoids.

Test for Flavonoids: Extract was treated with few drops of sodium hydroxide solution, the formation of intense yellow color. Which becomes colorless on the addition of dilute acid indicate the presence of flavonoids.

Test for Glycosides: The extract was mixed with 2 ml of glacial acetic acid containing few drops of 2% $FeCl_3$; mixture poured into another tube containing 2 ml of concentrated sulfuric acids. A brown ring at the inter-phase indicates the presence of glycosides.

Test for Protein: The extract treated with few drops of concentrated nitric acid, the formation of yellow color indicates the presence of proteins.

Test for Alkaloids: The extract was dissolved individually in diluted HCl and filter was treated with saturated picric acids and formation of brown precipitate indicates the presence of alkaloids.

TABLE 1: PHYTOCHEMICAL CONSTITUENTS OF *EPIPHYLLUM OXYPETALUM* EXTRACT

Extracts	Saponins	Phenol	Tannins	Terpenoids	Flavonoids	Glycosides	Proteins	Alkaloids
Chloroform	+	_	+	+	+	_	+	+
Methanol	+	+	_	+	+	+	+	+
Ethanol	+	_	_	+	+	+	_	+
DistilledWater	+	+	_	+	+	+	+	+

Positive (+) show the presence of constituents; whenever negative (-) show the absence of constituents in the flower extract

METHOD OF EVALUATION:

EXCISION WOUND MODEL:- In this Excision Wound Model the Animals were grouped as follows:-

GROUP I: Was served with only ointment base i.e.1 g/kg topically for 16 days.

GROUP II: Received topically application of 10 % Betadine Iodine ointment twice a day for Excision wound model for 16 days.

GROUP-III: Received topical application of 10 % $^{w}/_{w}$ of the extract in simple ointment base twice a day for Excision wound model for 16 days.

First rats were taken and they were grouped into different categories and anaesthetized under Ketamine HCl injection. Excision wounds were inflected on the dorsal thoracic region 1 - 1.5 cm away from the vertebral column on either and 5 cm away from the area. The wounded area preparation was prepared with 70% alcohol, using a sterile round seal of 2.5 cm diameter or a surgical blade or 5-8 mm biopsy punch. The circular skin from the predetermined area on the back of the animal was excised to its full thickness to obtain a wound area of 200-500 mm^2diameter and 2 mm depth. Homeostasis was achieved by blotting the wound with a cotton swab soaked in normal saline. The drug was topically applied twice a day until complete epithelization. The parameters studies of wound were measured at Regular interval of time to determine percentage of wound and epithelization time (indicated by the formation of new epithelial tissue to cover the wound). The wounded areas were later evaluated and wound contraction was calculated as a percentage of the reduction in wounded are on 4^{th}, 8^{th}, 12^{th} and 16^{th} days until complete re-epithelization was achieved. (The day the scar peeled off without leaving any residual drug wound was considered the day complete epithelization was attained). The following parameters were then studied as follows:

EPITHELIZATION PERIOD:-

It was monitored by observing the number of days required for Escher to fall away, leaving no raw wound behind.[19]

WOUND CONTRACTION:-[14]

To monitor this, progressive changes in wound area were followed planimetrically. Leaving the wounding day, wounds were traced on a transparent paper on alternate days. The animal was restrained in proper position during tracing. The tracings were then transferred to 1 mm^2 graph paper. From, this, wound areas were read and the percent of wound contraction was calculated taking the initial size of wound (100 mm^2) as 100%. Percentage wound closure can be calculated using the formula:

$$\textbf{Percentage of wound closure} = \frac{\textit{Initial area of Wound} - \textit{nth day area of wound}}{\textit{Initial area of wound}} X\ 100$$

Evaluation of wound healing:-

Wound contraction, which contributes to wound closure, is expressed as a reduction in percentage of the original wound size is studied starting from the day of operation until the day of complete Epithelialization and evaluated to calculate the degree of wound healing. Wound tissues are analyzed for hydroxyproline content; the collagen composed of amino acid (hydroxyproline) is the major component of extracellular tissue, which gives strength and support. Breakdown of collagen liberates free hydroxyproline and its peptides. Measurement of hydroxyproline, hence, can be used as a biochemical marker for tissue collagen and an index for collagen turnover. The biochemical marker hexosamine, a component of the ground substance for the synthesis of the extracellular matrix is evaluated in granulation tissues of excision wounds in

order to monitor the wound healing process. Since the level of hexosamine is increased between 7th-12th post wounding day and then decreases slowly; the granulation tissue is obtained from wound are on 11th post wounding day.[15]

One of the most crucial phases in dermal wound healing is the progressive increase in biomechanical strength of the tissue; the mechanical properties of the skin are mainly attributed to the function of the dermis in relation to the structure of collagen and elastic fiber networks. Breaking strength of the healed wound is measured as the minimum force required breaking the incision apart. Skin breaking strength gives an indication of the tensile strength of wound tissues and represents the degree of wound healing. Tensile strength has commonly been associated with the organization, content, and physical properties of the collagen fibril network. Tensile strength is the resistance to breaking under tension; it indicates how much, the repaired tissue resists breaking under tension and may indicate in part the quality of the repaired tissue. The sutures were removed on the 7th–9th post wounding day, and the tensile strength was measured on the 8th–10th day. The mean tensile strength on the two par vertebral incisions on both sides of the animal is taken as the measures of the tensile strength of the wound for an individual animal.

EXCISION WOUND MODEL: [19]

In excision wound model, rats were depilated by removing hairs at the dorsal thoracic region before wounding. Rats were anaesthetized by ketamine HCl injection prior to excision. Circular wound of about 2.5 cm diameter was made on depilated dorsal thoracic region of rats under aseptic conditions and were observed throughout the study. The areas of the wounds were measured(in mm^2) immediately by placing a transparent polythene graph paper over the wound and then tracing the area of the wound on it (approx. area 500 mm^2)[16]. This was taken as the initial wound area reading. The rates are categorized into three groups (n=6). The animal of group I treated as control and only ointment base applied topically. The animal of group II treated as test I and received ointment of *Epiphyllum oxypetalum*extract, group III received standard drug Povidone iodine ointment. All samples were applied once daily for 16 days, starting from the day of wounding. The observations of percentage wound closure were made on 4th, 8th, 12th and 16th, post wounding days. The wound area of each animal was measured by using tracing paper method. The percentage of wound contraction was calculated from the days of measurements of wound area. The parameters like wound contraction and epithelization time were evaluated by the excision wound model.[17]

Wound contraction and Epithelization time in excision wound model

The wound contraction was calculated as percentage reduction in wound area with respect to initial wound area while the epithelization time was noted as the number of days after wounding required for scar to fall off leaving no raw wound behind. The percentage wound contraction was determined by using following formula:

$$\textbf{Percentage of wound closure} = \frac{\textit{Initial area of Wound} - \textit{nth day area of wound}}{\textit{Initial area of wound}} X\ 100$$

Effect of control test drug, standard drug (Povidone Iodine) was observed on percentage wound concentration in excision wound model on initial, 4th, 8th, 12th and 16th day interval which is shown in table no.5 it has been seen that significant wound healing took place in case of animals treated with a *Epiphyllum oxypetalum*extract which is 16 days as compared to control & the standard drug which took 23 and 15 days respectively for complete wound healing. The least rate of wound healing was seen in control group which received no treatment and fastest rate of wound healing was seen in standard drug group where animals received standard drug which is Povidone iodine.[19]

RESULT AND DISCUSSION: In the present study ethanolic extract were prepared from leaves of Epiphyllum oxypetalum and its wound healing activity was studied by using established models in rats. To study the wound healing activity of ethanolic extract, the excision wound model is selected because the technique is simple to be used for routine screening of wound healing activity.

The ethanolic extract of *Epiphyllum oxypetalum*ointment exhibited significant wound healing activity in excision wound model as comparable to the marketed 10% $^{w}/_{w}$ Povidone- iodine ointment.

Thus, it is concluded that *Epiphyllum oxypetalum*is beneficial for healing of wound & it has amazing wound healing properties.

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