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Evaluation of wound healing activity of henna, pomegranate and myrrh herbal ointment blend

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ABSTRACT

This study assessed the wound healing potential and antimicrobial activity of henna, pomegranate and myrrh extract formulations and their blend in excision, and dead space wound models in rats in comparison to a marketed ointment (gentamycin). The natural extracts were used in ointment formulations alone or in a combination of three extracts at a total concentration of 15% w/w in medications. The percent of wound contraction in case of henna, myrrh, pomegranate, the blend and gentamycin (10 mg/kg) were 85.90–98.5%, 88.35–99.52%, 93.55–100%, 97.30–100%, and 90.25–100% from days 16 to 20, respectively. The blended formulation showed the highest increase in the percent of wound contraction and decrease in the epithelisation period compared to other formulations and showed comparable results to the standard ointment. The histological studies of excision biopsy at day 24 showed healed skin structures with normal epithelisation, the restoration of adnexa and fibrosis within the dermis in all of the formulation- and gentamycin-treated groups while the control group lagged behind in the formation of the amount of ground substance in the granulation tissue. The formulations showed antimicrobial activity against *Candida*, *Staphylococcus aureus*, mucous membrane infections and *E. coli* topical infections. The study proved the wound healing potential and antimicrobial activity of the herbal extract.

1. Introduction

A wound is a break formed in the normal continuity of the skin, thereby forming a disruption in its cellular and anatomic structures and affecting its functionality. Wounds may be classified by several methods; their location, aetiology, presenting symptoms or type of injury, wound depth and tissue loss or clinical appearance of the wound (Flanagan, 1994). Wound healing or wound repair is a complicated process in which the tissue repairs itself after being injured. It involves three overlapping phases, namely inflammation, cellular proliferation and remodelling, which are orchestrated in a controlled manner to resume their normal function (Clark, 1993; Sahl and Clever, 1994; Singer and Clark, 1999).

Many efforts have been made to explore new agents that can enhance healing and allow for the speedy recovery of injuries while saving patients from amputation, similar complications and additional problems. Medicinal plants were shown to play an important role in curing skin disorders like cuts and burns (Kokane et al., 2009). Nevertheless, the selection of plants based on their activity is critical and requires care in order to determine the value of the plant (Shivhare et al., 2010).

Traditional medicinal plants are often used to obtain preparations beneficial for wound healing purposes comprising a wide area of different skin-related diseases (Maver et al., 2015) such as *Lawsonia inermis* (Salih et al., 2017) and Myrrh (Walsh et al., 2010). Herbs such as henna, pomegranate and myrrh have been widely used in traditional systems of medicines due to their antiseptic and anti-inflammatory properties (Li et al., 2006; Shen et al., 2012).

Pomegranate is one of the important fruits stated in the Holy Qur'an. *Punica granatum* belongs to the family of Punicaceae, and is more commonly known as pomegranate, granats, grenade and punica apple (Voravuthikunchai et al., 2005). Pomegranate extract is becoming popular in the Western world for the treatment and prevention of arthritis and other inflammatory diseases (Ahmed et al., 2005). Also, of the peel, pulp and seed, pomegranate peel had the highest antioxidant activity (Guo et al., 2003). In folk med-

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icine of many cultures, pomegranates have been used extensively (Longtin, 2003). Large amounts of polyphenols are found in pomegranate peel, for example ellagic tannins, ellagic acid and gallic acid. It has been utilised in the preparation of food recipes, cosmetics, tinctures and therapeutic formulae (Ben Nasr et al., 1996). The effectiveness of the dried fruit peel in the treatment of respiratory and urinary tract infections and diarrhoea has been reported (Li et al., 2006). It has also been reported to have anti-fertility effects (Umadevi et al., 2013) cytotoxic activity (Ampasavate et al., 2010) hepatoprotective activity (Ashoush et al., 2013) and hypoglycemic activity (Hontecillas et al., 2009). The ethanolic extract of pomegranate peel has an ameliorative effect against chlorpyrifos-ethyl-induced oxidative stress in rats (Mahgoub and Nashwah, 2009). It also has a potent nephroprotective action and suppresses ferric nitrilotriacetate-induced renal oxidative damage in rats (Mahgoub and Ali, 2010).

Myrrh is the stem resinous exudates that belong to various Commiphora species depending on their growing area. It is an oleo-gum resin that form emulsion upon mixing with water. Published researches showed different medicinal effects for myrrh. The medical uses including fasciolicidal effect, and for the treatment of schistosomiasis (El Ashry et al., 2003; Massoud et al., 2001). Myrrh, either as a whole or some of its components, appears to have therapeutic effectiveness in the treatment of gynaecological cancer diseases (Su et al., 2011), while proving effective as an aesthetic, antibacterial, antifungal and anti-hyperglycaemic agent (Abdallah et al., 2009). Other medicinal uses include anti-ulcer, antipyretic, analgesic, antioxidant and anti-inflammatory effects (Shen et al., 2012). Myrrh has been employed successfully in treatment of wounds and ulcers (Walsh et al., 2010) and is extremely useful topically to facilitate drying and provide wound cleansing (Nomicos, 2007). Myrrh contains about 2–8% essential oil, 40–60% water-soluble gum and 23–40% alcohol-soluble resins. The essential oil is rich in furanosesquiterpenoids, with about 20 compounds having been isolated (Zhu et al., 2003).

Henna, Laurus nobilis Linn., (Lauraceae) is a strong evergreen tree which breeds in the wild or can be cultivated. It has been reported to have carminative, astringent, diaphoretic, diuretic, digestive, emetic and stomachic properties (Nayak et al., 2006), antirheumatic and antineuralgic properties (Marc et al., 2008). Its oil can be used in liniments for sprains and bruising (Guru Prasad, 2011). Owing to its high content of lauric acid, it provides an insecticidal effect against moths. It has also an activity against ringworm infection (Badoni Semwal et al., 2014) and intestinal amoebiasis (Venkata Subbaiah and Savithramma, 2012). The oil extracted from the leaves contains compounds like linalool (monoterpenoids), which is the major compound (50%), whereas *p*-cymene, α-pinene, limonene and β-pinene are present at levels of around 5–10% each. Also, phenylpropanoids may be found in trace amounts (Nayak et al., 2006).

In light of these traditional uses of plants, cited activities and observations, the present study was performed to assess the synergistic wound healing potential of the aforementioned herb extract formulation blend in excision and dead space wound models in rats when applied topically in comparison to a marketed ointment. Surveying the literature revealed that the synergistic wound healing activity of such blend had not been previously examined. In addition, the study also aimed to evaluate the antibacterial and antifungal activities of the three extracts.

2. Materials and methods

2.1. Materials

Henna, pomegranate and myrrh extracts were obtained from Kuber Impex Limited (New Palasia, India). Yellow soft paraffin, mineral oil and yellow beeswax were purchased from Loba Chemie (Mumbai, India).

2.2. Methods

2.2.1. Ointment preparation

Ointments of henna extract, pomegranate extract, myrrh extract and the three blended extracts were prepared according to the following formula:

Yellow Soft Paraffin	52%
Bees wax	3%
Liquid paraffin	25%
Natural Extract	15%

Weighed amounts of yellow soft paraffin, yellow beeswax, and liquid paraffin were added to a 250 mL beaker. Beaker contents were heated over a water bath to 70 °C and then removed from the heat source. When the temperature of beaker contents lowered to 50 °C, the weighed amounts of natural extract were added to the beaker content and mixed vigorously for 5 min using a hand mixer. The natural extracts used here were 15% henna extract, 15% pomegranate extract, 15% myrrh extract or a blend of 5% of each of the three extracts.

2.2.2. Models for wound healing activity

2.2.2.1. Excision wound model. The excision wound study included the use of male Wistar rats (200–250 g). Animals were chosen and divided into six groups of five animals each. Anaesthetic ether was used to anaesthetise the rats, which were then depilated before wounding at the predetermined site. An excision wound was perpetrated by removing away approximately 500 mm^2 full thickness of the assigned area on the anterior-dorsal side of each rat (Dash et al., 2001). The treatment of animals involved the external application of various formulations at a concentration of 10 mg/kg body weight. The assigned groups of animals including group I, group II, group III, group IV, group V and group VI were treated with methanolic extract of myrrh (M) paste (w/w), pomegranate (P) paste (w/w), henna (H) paste (w/w), blend (B) paste (w/w), base ointment alone (negative control group) and gentamycin ointment (positive control group), respectively. All of the formulations were applied to corresponding groups twice daily for 24 days, beginning from the day of wounding. The wound contraction as well as the wound closure time were taken as the criteria for evaluation of the wound healing property. Every three days, the wound area was measured by applying a transparent piece of paper over the wound and outlining it; then, the area of this measurement was calculated using a graph sheet (Werner et al., 1994). The wound contraction was described as the % of contraction. The time for wound closure was noted when total healing occurred. The protocol of the study for animal experiments was approved by the Institutional Animals Ethical Committee of the department.

2.2.3. Histopathological studies

The skin specimens were collected in 10% buffered formalin from rats of the six groups. Then, 5 μm thick sections were sliced and stained with haematoxylin and eosin. The light microscope was used to evaluate the sliced sections in terms of collagen formation, fibroblast proliferation, keratinisation, and epithelisation.

2.2.4. In vitro antimicrobial activity

The cup plate agar diffusion method (BSAC) was used to evaluate the antimicrobial activity of four hydrophilic ointments containing henna extract, myrrh extract, pomegranate extract and a blend of the three extracts.

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