

Wound Healing Activity of Topical Application of *A. marmelos* and Cow Ghee

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Abstract

The formulation of *Aegle marmelos* (AM) leaves extract and Cow Ghee (CG) claimed to promote wound healing in traditional practices. However no systematic studies are reported in modern scientific literature with regard to the verification of the traditional medicinal claims. The study incorporates the wound healing potential of AM leaves extract and CG by topical application. The effect produced by topical application of AM leaves extract and CG with reference to wound contraction, wound closure, decrease in surface area of wound and tissue regeneration at the wound site has been studied. The present study thus provides scientific rationale for the traditional use of *A. marmelos* and Cow ghee for management of wounds. *A. marmelos* has been evaluated for many pharmacological activities such as anti-inflammatory, antipyretic, analgesic, antidiarrheal, antidiabetic, antifungal, antihyperlipidemic, antimicrobial, antibacterial, antiparasitic, anticancer, antimalaria, hepatoprotective, anticollitis, and cardioprotective activities [2,7]. Research has found the essential oil of the Bael tree to be effective against 21 types of bacteria [16]

Key words: *Aegle marmelos*, cow ghee, wound healing

Introduction

Aegle marmelos (AM;family: Rutaceae), commonly known as bael, Bengal quince, golden apple, stone apple, wood apple, bili, is a species of tree native to India. The bael tree is well-known for its medicinal properties and is used in Ayurveda, the traditional Indian medicine. *Atharvaveda* (around 1200 BC), *Charak Samhita* and *Sushrut Samhita* (1000–500 BC) are main classics that give detailed descriptions of bael tree [15]. The Shiv Puran tells the story of the effect of worshipping Lord Shiva with the bael leaf. The bael leaf is known as *bilva patra* in Sanskrit or the leaf of wood apple. Many meanings are derived from the trifoliate (three-pronged) shape of the bael leaf. The dark trifoliate leaves symbolize the three eyes of Lord Shiva, and contain a small percentage of Shiva's alchemical substance – mercury.

It is said that offerings of water sprinkled on these leaves at any shrine will always remain fresh. The consumption of Bilva leaves alleviates diseases caused by excess vata and kapha (mucus). They are also useful in diabetes mellitus. For this a few leaves should be chewed daily and their fresh juice drunk. They are diaphoretic (producing more perspiration), thus reducing temperature and lowering fevers, and an aphrodisiac. A decoction of leaves is a favorite remedy for ailments that often occur during seasonal changes, such as fever, flu and fatigue. The essence of its leaves possess an evaporating oil which is very good for human system. The leaves successfully inhibited *B. subtilis*, *S. typhi*, *S. aureus* and *E. coli*. *B. subtilis* was found to be more sensitive to the AM leaves. Many of these organisms are natural flora of the skin and also known etiologic agents of several skin and mucous membranes infections of man [6,20].

A.M. leaves are widely used to treat diarrhea, dysentery, skin and eye disease [11,12]. They contain terpenoids which act as an antifungal agent [18]. Fruits, leaves and stem bark of wood apple have been studied for anti-tumor [19] and antimicrobial activity [17]. In addition to this, wood apples also have hypoglycemic activity, larvicidal and hepatoprotective activity [22]. Imbalance in free radical generations and antioxidants caused delayed wound healing. Therefore, elimination of ROS could be an important factor in wound healing [13]. Unfortunately we are not able to utilize these qualities of sacred tree. Instead tones of leaves get wasted daily on the name of worship. Thus the number of trees is declining day by day. As we consider, the use of leaves of *Aegle marmelos* in worship was mentioned in ancient literature just because of its medical qualities but we had taken it in wrong way and instead of using its qualities, the leaves are disposed off daily in large quantity.

Similarly, Cow Ghee (CG) is also rich in medical properties. CG is obtained by clarification of milk fat at high temperature. Ghee is almost anhydrous milk fat and there is no similar product in other countries. It is by far the most ubiquitous indigenous milk product and is prominent in the hierarchy of Indian dietary [6]. According to ancient Ayurveda cow ghee is full of essential nutrients, fatty acids, antibacterial, anti fungal, anti-oxidants and antiviral properties. It is cool, sweet and full of saturated fat. It is used to heal wounds, chapped lips and mouth ulcers. It detoxifies body. It is

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best for cancer patients, having power to stop growing cancer cells. Bleeding nose, brain stroke, sinus, headaches and migraine problem get cured completely. It is also useful when applied to burns. It is excellent for glowing skin. Chemically, ghee is a complex lipid of glycerides (usually mixed), free fatty acids, phospholipids, sterols, sterol esters, fat soluble vitamins, carbonyls, hydrocarbons, carotenoids (only in ghee derived from cow milk), small amounts of charred casein and traces of calcium, phosphorus, iron, etc. It contains less than 3% moisture. [6].

The best part of Ayurvedic medicine is that they are without any side effect. The time has come to utilize its potential and present it in new form to serve the mankind. Ayurveda describes hundreds of medicines to treat skin ailments. We have researched upon wound healing effect of CG and AM. The medicine is known to our family for long time and large numbers of patients have been benefitted with its qualities. It treats even the deep wounds in less time and with better effects in comparison to other available medicines. However it is essential to verify its claims using modern day technology and currently accepted scientific norms. The study provides a valuable insight into the claimed pharmacological activity of the formulation.

Material and Methods

Fresh leaves of *Aegle marmelos* were collected and washed properly. These leaves were ground to paste with water. Known quantity of *A. marmelos* leaves paste was subjected to cold extraction and the aqueous extract was

collected. The extract was stored in a sterile container.

A definite quantity of AM leaves extract and ghee was heated at 125°C until it loses water. The formulation was ready for use. It was absorbed in cotton and applied topically on wound.

Results and Discussion

The results revealed that the composition of AM leaves extract and Cow Ghee significantly promotes wound healing. The wound healing effects of *Aegle marmelos* seemed to be due to decreased free radical and myeloperoxidase generated tissue damage, promoting effects on antioxidant status, faster collagen deposition as evidenced by increase in collagen determinants and decrease in inflammation confirmed histopathologically [8].

In fact, the alternative medicine is less toxic and with negligible side effects compared with conventional medicine and hence it is important to introduce a scientific validation for the given formulation. The wounds gap were measured in millimeters using a ruler at magnification 60X or 32X (32X for extremely large wounds) and wound sizes were compared. Changes in wound area were measured regularly and the rate of wound contraction calculated as given in the formula below. Figure 1 is showing wound contraction with use of formulation and the comparison of contraction rate with other ointment is shown in chart 1.

$$\% \text{ Wound contraction} = \frac{\text{Healed area}}{\text{Total wound area}} \times 100$$



Fig. 1- Progress in wound healing is shown. In eight days wound is completely healed

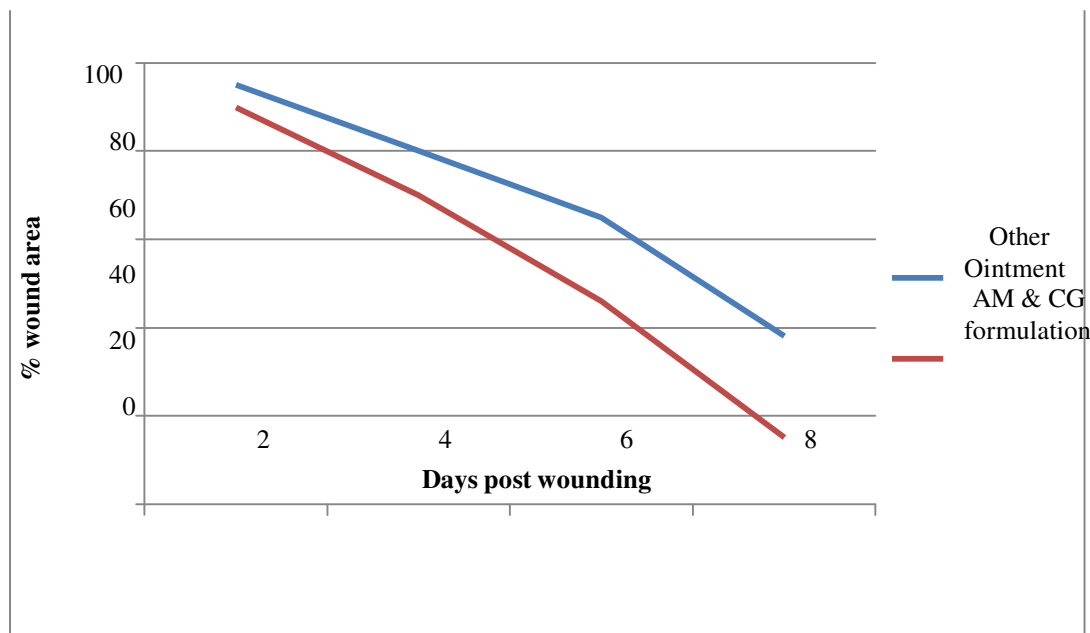


Chart 1: Showing comparison of wound contraction

Significance in wound healing of the test groups are derived by comparing healed wound area on respective days with healed wound area of control group. The period of epithelization that is, day of fall of eschar and scar area were also noted down, where, healed area = original wound area – present wound area [1].

The formulation is effective in almost all type of wounds including laceration, abrasion, puncture and avulsion. The treatment was equally effective in diabetic patients and wounds with pus discharge. The composition is itself bactericidal, antimicrobial; antifungal thus there is no requirement of antibiotics during treatment thus avoiding side effects of antibiotics. Wood apple has anti-diabetic and antioxidant potential by reducing the level of blood glucose and malondialdehyde [14]. The formulation also worked successfully in treating nasal sinusitis, which could not be cured completely even by operation.

Wounds are still a major problem in developing countries, often having severe complications and involving high costs for therapy. Healing is a complex process that involves a series of biochemical and cellular reactions initiated in response to an injury that restores the function and integrity of damaged tissues [8]. A

major goal of wound healing biology is to figure out how skin can be induced to reconstruct the damaged parts more perfectly. Proper and timely wound healing is a vexing problem faced by all clinicians. In majority of patients normal healing establishes tissue integrity quickly and effectively. However at times this healing is delayed and the ability to accelerate the wound healing becomes a highly desirable objective[3]. Wound healing involves different phases such as contraction, epithelization, granulation, collagenation [10].

Most wounds to the skin will cause leakage of blood from damaged blood vessels. The formation of a clot then serves as a temporary shield protecting the denuded wound tissues and provides a provisional matrix over and through which cells can migrate during the repair process. The clot consists of platelets embedded in a mesh of cross linked fibrin fibers derived by thrombin cleavage of fibrinogen, together with smaller amounts of plasma fibronectin, vitronectin, and thrombospondin [4]. Platelets, the cells present in the highest numbers shortly after a wound occurs, release a number of things into the blood, including growth factors [9]. The ¹H NMR spectrum of leaves extract of AM revealed the presence of secondary methyl. The presence

of the aromatic ring is easily established and identified by the IR characteristics signal V_{max} 1623 cm^{-1} and 1H and ^{13}C spectra. The 1H spectrum gives the aromatic proton at δ H

6.66482. The IR spectrum showed peaks at V_{max} 3420 cm^{-1} (OH), 2926 cm^{-1} (CH), 2853 cm^{-1} (aliphatic C-H stretching) and 1059 cm^{-1} (C-O) stretching [20]. AM leaves extract is reported to contain important bioactive compounds such as carotenoids, phenolics, alkaloids, pectins, tannins, coumarins, flavonoids, and terpenoids. Flavonoids are most commonly known for their antioxidant activity. Tannins have been reported to possess antioxidant, wound healing, and antimicrobial activities. Phenolic compounds are commonly known for their antioxidant, anti-inflammatory, and antimicrobial activities, while triterpenoids have been reported to show immune modulatory property. Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity [8]. The healing effects of AME in wound healing could be due to the presence of above active principles.

Plants contain a spectrum of secondary metabolites such as triterpenoids, phenols, flavanoids, quinines, tannins and their glycosides, alkaloids and their essential oils. The importance of these substances as antimicrobial agents against pathogens has been emphasized by several workers [21].

Traditionally prepared cow ghee has two types of properties *Samshodhana* (cleansing or detoxifying) and *Samshana* (palliative). Such ghee is considered to be extremely useful for treating wounds, inflammatory swellings and blisters for promotion of quick healing. The short- and medium-chain fatty acids in butter have strong anti-tumor effects. Conjugated linoleic acid (CLA) in butter from grass-fed cows also gives excellent protection against cancer.

These properties of AM & CG can be better utilized to treat sinusitis and further research can provide good results in cancer too.

The trifoliolate leaves of bael patra are used in worshipping Lord Shiva. Observation was done to identify the wastage of leaves in Mahakal temple, Ujjain, Madhya Pradesh, India. It was found that quintals of leaves were disposed off in pond on the back side of temple. These leaves along with flowers get rotten and in turn pollute water. The district administration was also informed about the same.

Conclusion

It is a low cost alternative medicine, without any side effect and easy to prepare even at home. The constituents are easily available. The leaves which are used in worshipping can be used to prepare medicine instead of

throwing them. This would not only prevent pollution but also preserve the sentiments of people.

It is also required to carry on more research to study its effect on nasal sinusitis which is very common problem now a days and its satisfactory and economic treatment is yet not available.

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