

INVESTIGATION OF WOUND HEALING ACTIVITY OF LINSEED OIL (LINUM USITATISSIMUM) IN ANTISEPTIC CREAM

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ABSTRACT

Wounds are unavoidable in the day to life and it causes by physical, chemical and biological attack and its cause the cell death /cell damage. Wounds make the disorder the tissue integrity and loss of cellular level biological activity of living tissues. Antiseptics are used as a treatment to cleanse cuts, scratches, burns, grazes or other minor injuries where the skin is broken, or as a preventive measure before piercing the skin. Linseed oil and its derivative are containing the higher amount of essential fatty acid, *i.e.* alpha linolenic acid. The alpha linolenic acid is essential for the biological synthesis of omega-3 fatty acid and it help to heal the wound. It having the higher amount of secoisolariciresinol diglucoside and it help to behave as a broad

spectrum antimicrobial ingredients. Present study aims to develop the document of wound healing and microbial property of linseed oil containing antiseptic cream and the entire activity compared with the leading antiseptic benchmark samples. Results indicate that the antibacterial and antifungal activity of sample and benchmark sample found to be highly significant when compared to the control. Study results revealed that linseed oil accelerate the wound healing activity, wound healing activity of sample and standard found to be highly significant when compared to the control. In-house samples are having the slightly leading edge when compared to the benchmark samples. Incorporation of linseed oil in antiseptic cream is immense useful with respect to wound healing activity and microbial control.

KEYWORDS: Antiseptic cream; linseed oil, boric acid, antimicrobial; wound healing, alpha linolenic acid.

INTRODUCTION

Wounds are unavoidable in day to life and it cause by physical, chemical and biological attack and it causes by the cell death/cell damage. Wounds make the disorder the tissue integrity and loss of cellular level biological activity of living tissues.^[1,2] Various on research activity carried out to find out the accelerate the wound healing in biological science. Recent days many scientist exclusively studied the wound healing property with traditional approach i.e. using the plant extract, herbal powder, plant oil for the wound healing activity. Antiseptics are used as a treatment to cleanse cuts, scratches, burns, grazes or other minor injuries where the skin is broken, or as a preventive measure before piercing the skin (e.g. an injection). For this very reason, now a day's various forms of antiseptic products available in the market and deserves the various purpose of healing. Both antiseptic and disinfectant prevent the growth of disease-causing micro-organisms. The main difference between the two is that disinfectants are usually used an inanimate object (e.g. tables, floors, chairs) and antiseptics are more suitable for human skin. An antiseptic cream is useful to destroy or inhibit the growth and development of micro organisms in living tissues and also targets the broad spectrum.^[3,4] Many literatures available for aromatic oils cure the wound; however, the essential oils with respect to wound healing property are meager. Recent days many scientists worked that wound healing property of linseed oil. Linseed oil otherwise called as flaxseed oil and it's derived from the plant *Linum usitatissimum*. Oils are derived from the cold pressing techniques and it is a colourless to yellowish oil. Linseed oil also called as a drying oil, which is it can be polymerize and form a solid form with react with air. Linseed oil and its derivative are containing the higher amount of essential fatty acid, i.e. alpha linolenic acid.^[5-8] The alpha linolenic acid is essential for the biological synthesis of omega-3 fatty acid. Many type of research proved that omega fatty acids and linolenic acid help to healing the skin wounds, provides the skin nourishment and reduce the inflammation /itching sensation and soothing the wounded skin.^[5,9,10,11] Present articles deals about the wound healing property of Linseed oil in antiseptic cream base formula; articles deals the anti-microbial and anti-fungal activity of antiseptic cream. Antimicrobial and wound healing properties of developed formula compared with the market leading benchmark samples.

MATERIALS AND METHODS

An antiseptic cream prepared with below mentioned formula and complete ingredients are summarized in the below table 1.

Table 1: Formulation details of antiseptic cream.

| Ingredients | Functions |
|--------------------|-------------------|
| Emulsifier | Emulsion |
| Linseed oil | Active |
| Olive oil | Active |
| Alovera oil | Active |
| Propylene glycol | Solvent / carrier |
| Boric acid | Active |
| Zinc oxide | Active |
| Water | Carrier |
| Perfume | Odor |

Anti-microbial and anti-fungal screening

Antimicrobial and anti-fungal activity of study materials was tested in 'National College of Pharmacy', Shimoga, India. The cylinder plate assay of drug potency is based on the measurement of the diameter of zones of microbial growth inhibition surrounding the cylinder containing various dilutions of test compounds. The antimicrobial activity of standard drugs, topical cream formulations, and base used, were carried out by the cup plate method. The zone of inhibition was measured by anti biotic zone reader.^[12,13,14,15]

Wound healing activity

Animals are divided into two groups, one act as a control and remaining one act as a treatment. Hair at the back of the animal was clipped with scissors and the circular area of 300 – 500 sq.mm. The skin was excised to its full thickness with scalpel and scissor under light ether anesthesia. Next day the initial wound area was traced and later the respective formulations were applied. Wound contraction was measured by tracing the wound margin on tracing paper and the area assessed using a graph paper.^[15,16,17,18]

Statistical analysis

Treated group results were compared with the control group. The results were analyzed statistically using Student's t-test to identify the differences between the treated and control.

RESULTS AND DISCUSSION

Physicochemical parameters of antiseptic cream developed and benchmark sample analyzed and the analytical value summarized in table 2.

Table 2: Physicochemical properties of tested antiseptic creams.

| S. No. | Parameters | Benchmark | Sample |
|--------|--|---|---|
| 1 | Description | White to off white colored cream with characteristic odor | White to off white colored cream with characteristic odor |
| 2 | pH | 5.4 | 5.5 |
| 3 | Viscosity in (cps) | 82600 cps | 85100 cps |
| 4 | Density @ 25°C | 0.952 | 0.962 |
| 6 | Total Aerobic Bacterial count (CFU/gm) | <10 | <10 |
| 7 | Total yeast and mould (CFU/gm) | <10 | <10 |

Microbiological screening test

The anti-microbial activity of developed antiseptic cream compared with the leading benchmark cream by cup plate method.^[15] Results of anti-microbial activity of the antiseptic cream formulation observed equivalent results against the benchmark samples and the results are summarized in table 3. The study reveals that the antibacterial and antifungal activity of sample and benchmark sample found to be highly significant when compared to the standard. Yogesh *et al.*^[19] exclusively studied about the antimicrobial property of linseed oil with gemifloxacin and authors concluded that linseed oil was a good antimicrobial agent and also the boost the antimicrobial activity with the combination of gemifloxacin. However, the similar kind of results observed in our study we have noticed the synergistic combination of linseed oil and boric acid. Boric acid is a white powder and derived from the reaction of boron with water. It works against the fungal and bacterial infections and act as a broad spectrum. Incorporation of linseed oil alone having the antimicrobial property, the synergistic effect will help to heal the wound in rapid manner and prevent the biological attack from the external source. Many reports stated that the linseed oil having the good source of secoisolariciresinol diglucoside (SDG) and it having the higher anti-bacterial and antimicrobial properties.^[20]

Table 3: Anti-microbial study of antiseptic cream formulation.

| Microorganism | Concentration | Standard | Benchmark | Sample |
|-------------------------------|---------------|----------|-----------|-----------|
| Gram Positive Bacteria | | | | |
| Bacillus Subtilis | 200µg/disc | 30±0.92 | 23± 0.23 | 25± 0.36 |
| Bacillus cereus | 200µg/disc | 32±0.65 | 25± 0.56 | 25± 0.25 |
| Gram Negative bacteria | | | | |
| Shigella shiga | 200µg/disc | 29±0.23 | 26± 0.25 | 26± 0.41 |
| Escherichia coli (E.Coli) | 200µg/disc | 34±0.32 | 25± 0.36 | 22.± 0.65 |
| Fungi | | | | |
| Aspergillus fumigattus | 200µg/disc | 32±0.22 | 24± 0.36 | 23± 0.26 |
| Candida albicans | 200µg/disc | 32±0.62 | 22± 0.52 | 24± 0.45 |

Wound healing activity of antiseptic cream formulation

Effect of antiseptic cream on a percentage of wound area contraction/healing furnished in Table 4. Significant results ($p = 0.001$) noticed in 1st day itself when compared to the control, continuous wound healing noticed on 2nd day, 4th day, 8th day and 21st day intervals. Results clearly indicate that wound heal activity completely achieved on 8th day on benchmark and samples treated side, however, it will take around 21 – 24 days for control sites.

Table 4: Effect of antiseptic cream on percentage of wound area contraction/healing.

| Treatment details | 1 st day | 2 nd day | 4 th day | 8 th day | 21 st day |
|-------------------|---------------------|---------------------|---------------------|---------------------|----------------------|
| Control | 29.09 | 41.74 | 49.30 | 59.49 | 93.24 |
| Benchmark | 27.85* | 55.89* | 67.88* | 96.51* | -- |
| Samples | 30.25* | 60.88* | 70.18* | 96.55* | -- |

*-statistically significant at $p=0.001$ level.

Acceleration of wound healing activity is also monitored by the tensile strength of the incision wound. Wound healing property molecules increase the deposition of collagen content and it contribute the strengthen the damaged/wound skin.^[5] Granuloma Tensile strength on post wounding day measured and furnished in Figure 1.

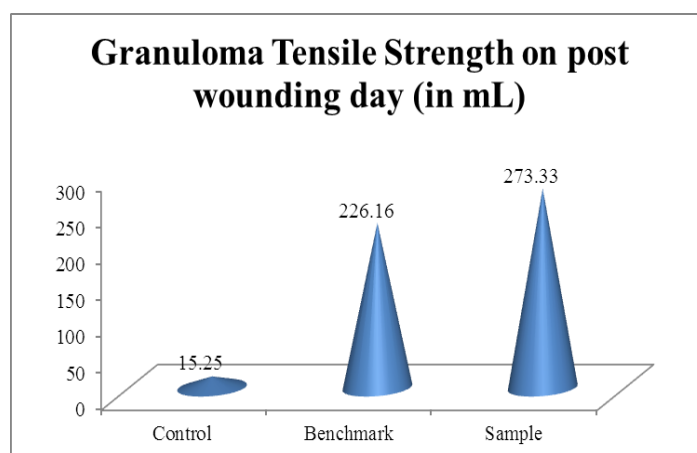


Figure 1: Effect of antiseptic cream on granuloma tensile strength on wound skin.

Study results indicated that the samples treated wounds were found to heal much faster as indicated by improved rates of epithelialization and wound contraction, also confirmed by histopathological examinations. Similar kind of trend was noticed in the tensile strength of drug-treated wounds. The study reveals that the wound healing activity of sample and benchmark samples to be highly significant ($p=0.001$) when compared to control. However, the in-house samples are having the slightly leading edge when compared to the benchmark samples. Study results revealed that linseed oil accelerate the wound healing activity, similar

kind of observations noticed by Farahpour *et al.*^[5] Cardoso *et al.*^[21] and Bardaa *et al.*^[22] Linseed oil has the higher amount of α -linolenic acid approximately containing around 25%. Many researchers identified that α -linolenic acid and linolenic acid are contributing the cell membrane for the structural integrity.^[5,6,8] As mentioned earlier linseed oil act as a dry oil, due to the polymerization behavior it forms a film layer, it will immense useful to cure the wound and prevent the wound from the external biological infections. Linseed oil having the higher amount of polyphenolic derivative and it will act as a good anti-oxidant. Sen *et al.*^[23] proved that anti oxidant potential help to recover the wound clearness and rapid recovery.

CONCLUSION

Linseed oil has the good antimicrobial activity and broad spectrum. The incorporation of linseed oil alone having the antimicrobial property, the synergistic effect along with boric acid immense useful to heal the wound and prevent the biological attack from the external. Results indicate that the antibacterial and antifungal activity of sample and benchmark sample found to be highly significant when compared to the standard. Study results revealed that linseed oil accelerate the wound healing activity, wound healing activity of sample and benchmark sample found to be highly significant when compared to the control. In-house samples are having the slightly leading edge when compared to the benchmark samples. Incorporation of linseed oil in antiseptic cream is immense useful with respect to the wound healing activity and microbial control and it helps the rapid recovery from the wound.

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