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Formulation and Evaluation of Calendula Officinalis and Melaleuca Alternifolia- based Poly-Herbal Paper Soap for Enhanced Antimicrobial and Antioxidant Efficacy.

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KEYWORDS
Calendula officinalis, Tea tree oil, Herbal hygiene, Antimicrobial formulation, Natural hand wash

ABSTRACT:

Background: Herbal hygiene products have become incredibly popular in a time when people are gravitating toward natural and sustainable options. This study investigates the possibility for developing novel personal care products using two well-known medicinal plants: Melaleuca alternifolia (tea tree oil) and Calendula officinalis (pot marigold). While tea tree oil has potent antibacterial and antifungal properties, calendula officinalis is renowned for its wound-healing, anti-inflammatory, and antioxidant properties. The goal of the study was to create and evaluate product that combine both herbal agents for better skincare and hygiene: polyherbal paper soap.

Methodology: Three formulations (F1, F2, and F3) with different ratios of herbal oils and excipients were created in the form of paper soap. The liquid soap coating process was used to make the paper soap. Agar diffusion was used to assess its pH, moisture content, skin irritation, foam retention, antioxidant activity, and antibacterial properties against S. aureus and E. coli.

Results and Discussion: F3 was the most successful formulation out of all of them. It had the most antioxidant capacity and the strongest antibacterial action, especially against S. aureus. The product's effectiveness was greatly enhanced by the addition of calendula and tea tree oil, which worked in concert to provide antibacterial and skin-soothing effects.

Conclusion: Three forms of a safe, all-natural, and efficient herbal hygiene product were effectively developed by the study.

1. Introduction

Introduction of plants:
Calendula officinal

Figure 1: Calendula officinalis

Source The plant is the botanical origin of Calendula officinalis, and it belongs to the family Asteraceae. It is also known as pot marigold, ruddles or common marigold. It is native to the Mediterranean region but widely cultivated in Asia, North America, and Europe.

Chemical Properties:
Terpenoids, flavonoids (quercetin, isorhamnetin, rutin), coumarins, quinones, carotenoids (violaxanthin, neoxanthsdddDin, lycopene, lutein), essential oils, and amino acids (alanine, leucine, lysine, valine, histidine).

Activities:

1. **Anti-inflammatory effect:**
   Reduces inflammation via triterpenoids and flavonoids, , making it useful in treating arthritis, muscle pain, joint inflammation, psoriasis and sunburns.
2. **Antioxidant activity:**
   Exhibits antioxidant properties due to its high content of phenolic compounds and flavonoids, lowering the risk of chronic diseases such as cancer, diabetes, and cardiovascular diseases.
3. **Antibacterial:**
   Displays broad-spectrum antibacterial and antifungal effects.
4. **Antifungal:**
   Treats fungal skin infection (athlete's foot, ringworm, candidiasis), for improving gingivitis, mouth ulcers, and sore throats and to manage vaginal infections.
5. **Anticancer:**
   Cytotoxic to cancer cells, particularly in leukaemia,

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colon cancer, and melanoma.

6. **Hepato-protective**:
Hydro-alcoholic extracts of calendula are hepato-protective to liver cells against toxic impairment (CCl4-induced liver damage models).
Beneficial in fatty liver disease and hepatitis.
7. **Wound Healing:**
External treatments for wound, burn, laceration, and ulcer care due to its capacity to induce collagen formation and promote epithelial regeneration.
8. **Immuno-stimulant:**
Enhance immune cell activity, promoting phagocytosis and lymphocyte activation.
9. **Antiviral:**
Calendula extracts inhibit herpes simplex virus (HSV) and influenza virus replication.
It is mentioned in folk remedies as an effective cure for cold sores, influenza, and viral infections.

Melaleuca alternifolia:

Figure no.2 Melaleuca alternifolia

Source

Tea tree oil (TTO) is taken from the leaves of the Myrtaceae species Melaleuca alternifolia. Due to its antibacterial, anti-inflammatory, and antioxidant properties, this native Australian plant is widely used extensively.

Chemical Characteristics:

Tea tree oil (Melaleuca alternifolia essential oil) is a complex mixture of over 100 bioactive compounds, primarily composed of terpenes and terpene alcohols, which contribute to its antimicrobial, antifungal, anti-inflammatory, and antioxidant properties

Activities:

1. **Antibacterial Activity**
These include Gram-positive and Gram-negative bacteria, such as Staphylococcus aureus, E. coli and Pseudomonas aeruginosa can be effectively prevented by tea tree oil.
2. **Antifungal Activity**
It is Effective against Candida species, dermatophytes, and other pathogenic fungi.
3. **Anti-Inflammatory Properties**
Tea tree oil's terpinen-4-ol reduces inflammation by inhibiting pro-inflammatory cytokines
4. **Wound Healing & Skin Repair**
Promotes collagen synthesis and tissue regeneration, accelerating wound healing. It is used in burns, cuts, and surgical wounds to prevent infection.
5. **Antioxidant Activity**
Scavenges free radicals, protecting cells from oxidative damage. It reduces lipid peroxidation and enhances wound healing. [14, 15.16]

PAPER SOAP

Made of incredibly thin soap sheets that dissolve in water to create a cleaning lather, paper soap is a novel, portable hygiene solution The soap is usually made using either cold or hot process methods and is made using oils such as linseed, coconut, calendula, and palm. After being created, the soap is sliced, shaped into thin sheets, and then placed in travel containers. Parameters like pH, clarity, color, odor, foam height and retention, skin sensitivity, irritation, and paper stability are used to assess the finished product's quality.

ADVANTAGES OF PAPER SOAP: [18]

1. Portable and lightweight
2. Ready to Use
3. Skin-friendly
4. Ease of Carrying
5. Environmentally Friendly
6. Economical

DISADVANTAGES OF PAPER SOAP:

1. Reduced Efficacy in Hard Water
2. Moisture Susceptibility

MATERIAL AND METHODS

The formulation of paper soap involves the Liquid Soap method to achieve optimal dissolution, lathering, and portability.

This technique involves applying liquid soap directly onto the paper. Brush the liquid solution onto paper using a soft-bristled paint brush. This ensures a thin and even layer of soap on the paper. Hang the treated sheets in a dry area for at least 24 hours or until all moisture evaporates. Once dried, cut the sheets into desired shapes. Because of its ease of use and efficiency, the liquid soap method is employed in this project while making paper soap. This

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method involves immediately putting liquid soap on paper then letting it dry. Since the Liquid Soap Method requires no need for specialist instruments, it may be used for small-scale manufacturing. This technique makes it simple to add other fragrances, essential oils, or other improvements.

Paper Soap Formulation

Ingredients	F1	F2	F3
Distilled water (70%) + Glycerine (30%)	14 ml	16 ml	18 ml
Castor oil	2.5 ml	2.5 ml	1ml
Calendula oil	10 ml	11 ml	12 ml
Curcumin powder	1-2 drops	1-2 drops	1-2 drops
Tea tree oil	1-2 drops	1-2 drops	1-2 drops
Petroleum jelly	1 g	1 g	0.5 g
SLS	8 g	4 g	6 g

Table No: 1 Formulation Table

Procedure for preparation of paper soap

1. **Water phase preparation**
   Combine distilled water and glycerine in a 70:30 ratio in a heat-safe beaker. Heat to around 40°C while stirring at a moderate pace.

2. **Oil Phase preparation**
   a) Combining Oils**:** Put petroleum jelly, calendula oil, 1-2 drops of tea tree oil and 1-2 drops of curcumin powder in a different beaker.
   b) Heating and Dissolving**:** Set this beaker in a water bath swirl gently with a stirrer until everything is fully blended and the texture looks consistent. Heat the petroleum jelly in the calendula oil until it dissolves fully, around 40°C.
   c) Emulsification
   It involves Phase Combination. Ensure both the water and oil phases are at a similar temperature (around 40°C). With the water phase continuously stirred on the magnetic stirrer, slowly pour the oil phase into it.

3. **Mixing:**
   Continue stirring at a moderate speed for 5–10 minutes until a uniform emulsion is formed with no visible separation.

4. **Cooling:**
   Reduce the temperature of the soap to around 35°C while still stirring.

5. **Application of herbal soap to the paper:**
   a) Dipping technique
   The dipping technique involves immersing the paper sheet into a liquid hand wash solution to allow uniform absorption of the cleansing agent. To achieve adequate absorption, allow the paper to soak for 5–10 seconds. Then, lift the paper carefully and let excess liquid drip off.
   b) Brush technique
   In this method, a soft bristled brush is used to coat the butter paper with the liquid soap. This ensures a thin and even layer of soap on the paper.

6. **Drying:**
   Let the treated sheets dry in a cool and dry area, away from sunlight for at least 48 hours or until all moisture evaporates.

Notes and Precautions

- Warm the mixture to around 40°C to ensure better blending with the oil phase.
- Stir gently to ensure even distribution without losing the essential oil's properties.
- Stir gently to avoid excess foam formation.
- Dry the soap sheets in a clean, dust-free environment to prevent contamination.
- Avoid drying at excessively high temperatures, as this may degrade the active ingredients.

RESULTS AND DISCUSSION

To assess the effectiveness and quality of the formulated polyherbal paper soap (F1, F2, F3), a series of evaluation tests were conducted, focusing on physicochemical properties, antimicrobial potential, stability, and user safety.

1. **pH:** The pH of the paper soap was determined in order to be sure that the formulation does not shows any irritancy to the skin. The pH was found to be 6.3-7.3 ± 0.5, it indicates that the ingredient present in

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formulation does not alter the pH of formulation.

Figure 3: -pH meter

Formulation	pH
F1	6.3
F2	6.9
F3	7.3

Table 2 :-pH readings of paper soap

2. **Skin Irritation and Patch Test**: No irritation & adverse reaction was observed after applying the paper on a hand of healthy volunteer after 24 hrs.

Figure 4:- Skin Irritation and Patch Test of Paper Soap

3. **Washability:** The paper soap foam was easily washed off just by water.

Figure 5 :- Washability of paper soap

4. **Stability:**

CONDITIONS	DAYS	RESULT
Refrigerator (2-8 Degree Celsius)	6 months	Purging of oil from paper
Room temperature (25-27 Degree Celsius)	6 months	No oil purging, no microbial growth observed
Hot air Oven (40 Degree Celsius)	6 months	Purging of oil from paper

Table:3 Stability of paper soap

Thus, the paper soap is stable at room temperature (25-27 Degree Celsius).

5. **Moisture Content:**

The maximum permissible moisture content of paper soap is 1-14%. Moisture content was calculated using the

Formula:-

Moisture content (%)= (loss in weight / weight of sample) x 100

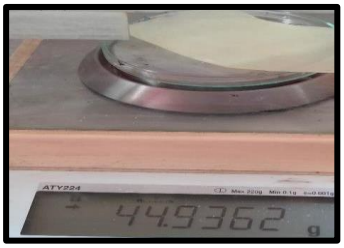

Figure 6:- Moisture Content of paper soap

Sample	Empty Plate(g)	Initial Weight (g)	Final Weight (g)	Moisture Content (%)
F1	40.05	42.03	41.85	10.11
F2	44.34	47.18	46.87	12.17
F3	43.27	47.57	47.13	11.23

Table 4: Moisture Content of paper soap

6. **Foam Height and Foam Stability Test**

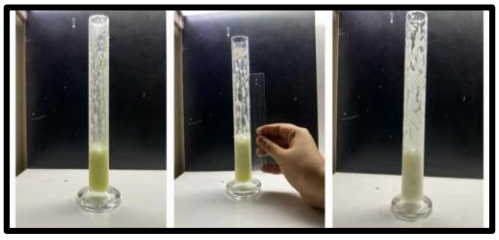

Figure 7:- Foam Height and Foam Stability of paper soap

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Time (sec)	F1 (cm)	F2 (cm)	F3 (cm)
Immediately	3	4.3	5.5
10	2.9	4.3	5.3
20	2.9	4.2	5.3
30	2.7	4.2	5.3
40	2.7	4.2	5.3
50	2.7	4.1	5.2
60	2.4	4.1	5.1
70	2.4	3.9	4.9
80	2.3	3.8	4.7
90	2.2	3.8	4.6

Table 5 :- Foam Height and Foam Stability

F3 remains relatively stable, indicating it is more resistant to disintegration. If a formulation retains its size longer, it suggests better stability and a slower dissolution rate. F3 appears to have the highest stability, followed by F2 and then F1.

7. Antioxidant Test

Sample Conc (µg/mL)	Absorbance (Control)	Absorbance (Standard)	% Scavenging Activity
0.5	1.5082	1.4180	1.86
0.6	1.5082	1.3953	7.48
0.7	1.5082	1.3867	8.05

Table No: - 6 Anti-oxidant Activity of Ascorbic acid

Sample Conc (µg/mL)	Absorbance (Control)	Absorbance (Sample)	% Scavenging Activity
F1	1.5082	1.4987	0.62
F2	1.5082	1.4804	1.84
F3	1.5082	1.3969	7.37

Table No: - 7 Anti-oxidant Activity of Paper Soap

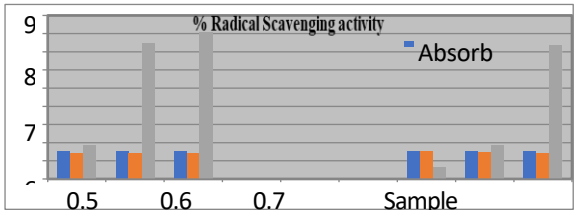

Figure 8: Anti-oxidant Activity of Paper Soap

Inference:

1. Concentration-Dependent Activity: A dose-dependent effect is indicated by the antioxidant activity in the standard group increasing with sample concentration.
2. Effective Formulation: In terms of antioxidant potential, F3 is the most potential formulation, indicating a stronger synergy between the herbal substances utilised.
3. Potential for Further Development: The results indicate that the polyherbal paper soap has potential as a natural antioxidant source, particularly with optimised formulation and concentration, even though the action is rather moderate in comparison to strong antioxidants.

8. Antimicrobial Activity of Paper soap

Bacteria	Sample	Zone of Inhibition(mm)
E.coli	F1	20
	F2	24
	F3	25
S.aureus	F1	23
	F2	22
	F3	25

Table 8:-Inhibition zone by bacteria

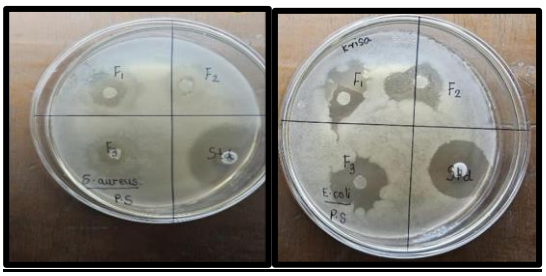

Figure 9:-Inhibition zone by bacteria of paper soap

Inference-

The most effective antibacterial formulation is F3.

Although the formulations still have substantial antibacterial activity against both S. aureus and E. coli, they are more effective against the former.

CONCLUSION

A poly herbal paper soap and hand wash exhibiting combined antibacterial activity was developed using *Calendula officinalis* extract and *Melaleuca alternifolia* (tea tree) oil, along with suitable excipients, surfactants, and stabilizers. *Calendula officinalis* (commonly known as pot marigold), belonging to the Asteraceae family, is well-documented for its anti-inflammatory, antimicrobial, wound-healing, and antioxidant

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properties. These therapeutic actions are largely attributed to the presence of triterpenoids, flavonoids, saponins, carotenoids, and essential oils. Traditionally, it has been used in herbal remedies for treating skin infections, minor burns, and wounds due to its ability to stimulate tissue regeneration and inhibit microbial growth.

Melaleuca alternifolia, commonly known as tea tree and native to Australia, is renowned for

it's potent broad-spectrum antimicrobial action, derived from its essential oil rich in compounds like terpinen-4-ol, γ-terpinene, and α-terpinene. Tea tree oil has been shown to exert antibacterial, antifungal, antiviral, and anti-inflammatory effects, making it a key ingredient in many dermatological and hygiene formulations. The formulation process began with the collection of materials. *Calendula officinalis* and

Melaleuca alternifolia essential oil was procured from an authenticated herbal source. Selected surfactants, humectants etc were incorporated to stabilize the formulation. The liquid soap method was used to formulate the paper soap. Antimicrobial activity was assessed using the disc diffusion method (Kirby-Bauer technique) against Staphylococcus aureus and Escherichia coli, with test samples prepared. The combination of *Calendula officinalis* and tea tree oil exhibited a synergistic effect, showing larger zones of inhibition than either component alone. Thus, a synergistic poly herbal formulation combining *Calendula officinalis* and *Melaleuca alternifolia* has been successfully developed into a dual-format paper soap and liquid hand wash with promising antiseptic, antioxidant, and antifungal properties. While laboratory-scale tests have confirmed its efficacy and safety, further studies, including clinical evaluation, are recommended to explore its potential for commercial and therapeutic applications.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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