WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 11, 2357-2373. Review Article ISSN 2277–7105

FORMULATION AND EVALUATION OF HERBAL AROMATHERAPY, HYDROGEN PATCH USING ORANGE PEEL ESSENTIAL OIL

Mayur Bapurao Meshram[1]*, Pallavi Kashinath Bhusari[2], Dr. Sachin Vitthal Datkhile[3], Vipul Vijay Bhondave[4], Roshan Ramesh Rathod[5]

*[1,4,5]Student of Samarth Institute of Pharmacy, Belhe, Maharashtra, India.

[2,3]Associate Professor of Samarth Institute of Pharmacy, Belhe, Maharashtra, India.

Article Received on 05 May 2026,
Article Revised on 25 May 2026,
Article Published on 03 June 2026,

https://doi.org/10.5281/zenodo.20536774

***Corresponding Author**

Mayur Bapurao Meshram

Student of Samarth Institute of Pharmacy, Belhe, Maharashtra, India.

How to cite this Article: Mayur Bapurao Meshram[1]*, Pallavi Kashinath Bhusari[2], Dr. Sachin Vitthal Datkhile[3], Vipul Vijay Bhondave[4], Roshan Ramesh Rathod[5]. (2026). Formulation And Evaluation Of Herbal Aromatherapy, Hydrogen Patch Using Orange Peel Essential Oil. World Journal of Pharmaceutical Research, 15(11), 2357–2373.

ABSTRACT

Aromatherapy, an established branch of complementary medicine, employs volatile plant-derived compounds to promote psychological and physiological wellness. This review comprehensively examines the formulation and evaluation of a novel herbal aromatherapy hydrogen patch incorporating orange peel essential oil (OPEO). The patch represents an innovative transdermal delivery system designed to simultaneously harness the therapeutic benefits of Citrus sinensis essential oil aromatherapy and molecular hydrogen (H_2) — both recognized for their potent antioxidant, anti-inflammatory, and anxiolytic properties. The review discusses the phytochemical profile of orange peel essential oil (particularly d-limonene, linalool, and myrcene), mechanisms of olfactory and transdermal action, excipient selection, patch fabrication methodologies (matrix and reservoir types), and critical quality control parameters including drug content uniformity, moisture vapor transmission rate (MVTR), flux studies, skin irritation assessments, and in vitro/in vivo evaluation. Current evidence supports that the synergistic combination of H_2 and OPEO in a patch format offers promising pharmacological advantages over single-modality approaches, including enhanced antioxidant activity, improved mood regulation, and sustainable slow release. This paper also identifies research gaps and future directions for clinical translation.

KEYWORDS: *Aromatherapy patch; Orange peel essential oil; Hydrogen therapy; Transdermal drug delivery; d-Limonene; Antioxidant; Anxiolytic; Citrus sinensis.*

1. INTRODUCTION

Aromatherapy is the therapeutic use of aromatic plant-derived essential oils to influence physical, emotional, and cognitive well-being through olfactory and dermal pathways. Although ancient in origin — documented across Egyptian, Greek, and Ayurvedic traditions — aromatherapy has attracted renewed scientific interest due to its documented efficacy in stress reduction, mood enhancement, analgesia, and antimicrobial activity. The global aromatherapy market surpassed USD 3 billion in 2023, reflecting increasing consumer and clinical acceptance.

Transdermal drug delivery systems (TDDS), particularly patches, offer significant advantages over oral administration: avoidance of first-pass hepatic metabolism, steady-state plasma concentrations, improved patient compliance, and suitability for volatile bioactive molecules that degrade rapidly in the gastrointestinal environment. The transdermal patch platform is thus ideally suited to deliver essential oils whose volatility and sensitivity to oxidation limit other routes.

Orange peel essential oil (OPEO), extracted from the outermost layer of Citrus sinensis (L.) Osbeck fruit, is among the most commercially produced and therapeutically investigated essential oils globally. Its principal active constituent, d-limonene (comprising 85–96% of the oil), possesses well-documented anti-anxiety, antioxidant, anti-inflammatory, and chemopreventive properties. The remaining terpene fraction (myrcene, linalool, α-pinene, β-pinene, sabinene) further contributes to its pharmacological versatility.

Molecular hydrogen (H_2) is an emerging therapeutic agent recognized for its selective free radical scavenging activity, particularly against the highly reactive hydroxyl radical (•OH) and peroxynitrite ($ONOO^-$), without disrupting essential reactive oxygen species (ROS) involved in cellular signaling. Unlike conventional antioxidants, H_2 exhibits no known cytotoxicity, is highly cell-membrane permeable, and exerts neuroprotective, anti-inflammatory, and cardioprotective effects at physiologically relevant concentrations.

This review explores the scientific rationale, formulation design, physicochemical characterization, and evaluation parameters for a herbal aromatherapy hydrogen patch

combining OPEO and H_2 , providing a consolidated reference for pharmaceutical scientists and researchers.

2. BOTANICAL BACKGROUND AND PHYTOCHEMISTRY

2.1 Citrus sinensis — Taxonomy and Botany

Parameter	Details
Kingdom	Plantae
Order	Sapindales
Family	Rutaceae
Genus	Citrus
Species	Citrus sinensis (L.) Osbeck
Common Names	Sweet Orange, Navel Orange, Valencia Orange
Part Used	Pericarp (outer flavedo / peel)
Active Constituents	d-Limonene, linalool, myrcene, α-pinene, sabinene, β-pinene, neral, geranial
Extraction Method	Cold pressing (expression), Steam distillation
Appearance	Pale yellow to orange liquid, characteristic citrus odour

2.2 Phytochemical Profile of Orange Peel Essential Oil

Gas chromatography-mass spectrometry (GC-MS) studies consistently identify the following major constituents in OPEO:

Constituent	Chemical Class	Approximate %	Key Pharmacological Activity
d-Limonene	Monocyclic monoterpene	85–96%	Anxiolytic, antioxidant, anticancer, anti-inflammatory
Myrcene	Acyclic monoterpene	0.5–3%	Analgesic, sedative, anti-inflammatory
Linalool	Acyclic monoterpenol	0.1–0.5%	Anxiolytic, anticonvulsant, sedative
α-Pinene	Bicyclic monoterpene	0.3–1.5%	Bronchodilator, anti-inflammatory
β-Pinene	Bicyclic monoterpene	0.1–0.7%	Antimicrobial, anti-inflammatory
Sabinene	Bicyclic monoterpene	0.2–1.0%	Antioxidant, antimicrobial
Neral / Geranial	Monoterpenoid aldehydes	0.1–0.4%	Antimicrobial, antifungal

Constituent	Chemical Class	Approximate %	Key Pharmacological Activity
β-Caryophyllene	Sesquiterpene	0.1–0.3%	Anti-inflammatory, analgesic (CB2 agonist)

The dominance of d-limonene governs the overall therapeutic signature of OPEO. Its cyclic terpene structure readily penetrates lipid bilayers, enabling dermal permeation and olfactory receptor interaction simultaneously.

2.3 Molecular Hydrogen — Properties and Mechanisms

Molecular hydrogen (H_2) is the lightest diatomic molecule (MW 2.016 g/mol) with unique biological properties. Unlike conventional antioxidants such as vitamin C or E, which non-selectively quench multiple ROS species, H_2 selectively neutralizes •OH (hydroxyl radical) and $ONOO^-$ (peroxynitrite) — the most cytotoxic oxidants — while sparing O_2^- and H_2O_2, which serve essential signaling roles.

Mechanisms of H_2 action relevant to patch delivery include:

- Direct chemical reduction of •OH: $H_2 + 2{\bullet}OH \rightarrow 2H_2O$ (thermodynamically favorable)
- Activation of Nrf2 pathway: H_2 upregulates nuclear factor erythroid 2-related factor 2, inducing endogenous antioxidant enzymes (SOD, catalase, HO-1)
- Modulation of inflammatory signaling: Downregulation of NF-κB, TNF-α, IL-6, and IL-1β
- Anti-apoptotic effects: Reduction of cytochrome c release and caspase-3 activation
- Neuroprotection: Inhibition of lipid peroxidation in neural membranes

The Henry's Law solubility of H_2 in water (~0.8 mM at 25°C, 1 atm) and its extreme lipophilicity (log P ≈ 0.45) allow rapid diffusion across biological membranes following release from a solid-state matrix such as a patch.

3. RATIONALE FOR COMBINING H_2 AND ORANGE PEEL ESSENTIAL OIL IN A PATCH

The combination of H_2 and OPEO in a single transdermal/aromatherapy patch is justified by several synergistic and complementary mechanisms:

3.1 Complementary Antioxidant Mechanisms

While d-limonene exerts antioxidant activity primarily through free radical chain-breaking and electron donation, H_2 selectively scavenges •OH radicals — the most damaging ROS

not effectively neutralized by conventional antioxidants. Together, they provide broad-spectrum oxidative stress mitigation across both enzymatic and non-enzymatic pathways.

3.2 Synergistic Anxiolytic and Neurological Effects

d-Limonene and linalool (from OPEO) activate $GABA_a$ receptors and modulate serotonergic neurotransmission, producing anxiolytic and antidepressant-like effects. H_2 has independently shown neuroprotective effects in models of Parkinson's disease, Alzheimer's disease, and depression, partly through suppression of neuroinflammation. The patch delivers both agents in a sustained manner, potentially amplifying mood-regulatory benefits.

3.3 Transdermal and Olfactory Dual Delivery

The aromatic volatility of OPEO permits inhalation/olfactory delivery when the patch is worn, activating the limbic system via the olfactory-limbic axis. Simultaneously, dermal absorption of d-limonene and H_2 contributes to systemic therapeutic exposure. This dual-route delivery is unique to patch-based aromatherapy and distinguishes it from topical creams or diffusers.

3.4 Non-invasive and Patient-Friendly Administration

Patch administration eliminates hepatic first-pass metabolism, allows controlled release, and is suitable for populations requiring non-oral delivery (pediatric patients, post-surgical patients, individuals with dysphagia). The adhesive patch can be applied to skin areas rich in blood supply (wrist, neck, chest) for optimal absorption.

4. FORMULATION OF THE HERBAL AROMATHERAPY HYDROGEN PATCH

4.1 Patch Types and Selection

Transdermal patches are classified into two principal designs relevant to this formulation:

Feature	Matrix-Type Patch	Reservoir-Type Patch
Drug Location	Dispersed uniformly in polymer matrix	Concentrated in a separate reservoir
Release Control	Matrix diffusion controls release	Rate-controlling membrane
Dose Dumping Risk	Lower	Higher (if membrane ruptures)
Fabrication Complexity	Simpler	More complex
Suitable For	OPEO + H_2 (volatile actives)	Aqueous or semi-viscous formulations

Feature	Matrix-Type Patch	Reservoir-Type Patch
Recommended For This Study	Yes (matrix preferred)	Optional for high-dose H_2 reservoir

For OPEO and H_2 , a matrix-type patch is preferred due to the volatile nature of essential oil constituents, the need for controlled evaporative release for aromatherapy, and the compatibility of H_2 -generating materials with polymer matrices.

4.2 Excipient Selection and Rationale

Excipient Category	Examples	Function	Concentration Range
Polymer Base (Matrix)	HPMC K4M, Eudragit RL100, PVA, EC	Provides mechanical support and controls drug diffusion	10–30% w/w
Plasticizer	Dibutyl phthalate, PEG 400, Triethyl citrate	Improves flexibility and film-forming properties	10–30% of polymer weight
Penetration Enhancer	Oleic acid, Azone (1%), Propylene glycol	Disrupts stratum corneum lipids to enhance OPEO permeation	1–5% w/w
Backing Membrane	Polyester, Polyurethane, Aluminized film	Prevents drug loss from non-skin surface; occlusive layer	Fixed layer
Release Liner	Siliconized polyester, Fluoropolymer	Protects adhesive until use	Fixed layer
Pressure-Sensitive Adhesive	PIB, Acrylate, Silicone PSA	Maintains patch contact with skin	Thin coat on backing
H_2 Generator	Magnesium (Mg) powder, Coral calcium H_2 tablets	Reacts with moisture (H_2 O) to produce H_2 gas: $Mg + 2H_2O \rightarrow Mg(OH)_2 + H_2 \uparrow$	1–5% w/w
Humectant	Glycerin, Sorbitol	Provides moisture for H_2 generation reaction	5–10% w/w
Preservative	Benzyl alcohol, Methylparaben	Prevents microbial contamination	0.1–0.5% w/w
Solvent (Processing)	Ethanol, Acetone, Dichloromethane	Dissolves polymer for casting; evaporates on drying	Removed during processing

4.3 Manufacturing Process

4.3.1 Film Casting Method (Solvent Evaporation)

1. Accurately weigh all excipients. Dissolve the selected polymer (e.g., HPMC K4M) in ethanol:water (9:1) solvent system under magnetic stirring (500 rpm, 30 min) until a clear viscous solution forms.
2. Add plasticizer (e.g., PEG 400, 20% of polymer weight) and mix for 15 minutes.
3. Incorporate penetration enhancer (e.g., oleic acid 2% w/w) and stir for 10 minutes.
4. Add OPEO at the pre-determined concentration (typically 5–15% v/v of the final patch matrix weight). Mix gently to preserve volatile constituents.
5. Add finely milled Mg powder (H_2 generator) with glycerin. Stir for 5 minutes to ensure homogeneous dispersion.
6. Degas the solution by sonication (5 min, 40 kHz) to remove entrapped air bubbles.
7. Pour the solution onto a mercury-leveled glass mold or release liner surface. Cast using a film applicator to a uniform thickness (e.g., 500 µm wet thickness).
8. Dry at controlled temperature (40 ± 2°C) for 24 hours in a vacuum oven to avoid oxidation of essential oil and Mg powder.
9. Cut dried films to defined area (e.g., 10 cm^2). Laminate backing membrane using PSA. Apply release liner. Heat-seal edges.
10. Seal individual patches in aluminium foil pouches. Store at 25°C/60% RH for stability studies.

Critical process parameters (CPPs) include solvent casting temperature, drying conditions, OPEO addition timing, and Mg particle size (finer particles generate H_2 faster).

5. EVALUATION PARAMETERS

5.1 Physical Characterization

Test	Method / Instrument	Acceptance Criteria
Thickness	Digital micrometer (5 readings per patch)	Target ± 5% of nominal thickness; typically 0.1–0.5 mm
Weight Uniformity	Analytical balance; 10 patches per batch	CV < 5%; meets Pharmacopoeial limits
Folding Endurance	Manual folding at same point until failure	> 300 folds without cracking (indicates good plasticization)

Test	Method / Instrument	Acceptance Criteria
Moisture Content	Karl Fischer titration or loss on drying (105°C, 1 h)	< 5% w/w
Moisture Uptake	Exposure to 65% RH desiccator, 24 h	< 10% weight gain
Moisture Vapor Transmission Rate (MVTR)	ASTM E96 desiccant method	Varies by formulation; lower MVTR = more occlusive
Surface pH	pH meter with flat-tip electrode, 0.2 mL distilled water on patch surface	pH 5.5–7.0 (physiological skin range)
Tensile Strength	Universal testing machine (UTM) — 50 mm/min crosshead speed	> 0.5 MPa; elongation > 50%
Elongation at Break	UTM — grip-to-grip distance 50 mm	≥ 50% (indicates elasticity)
Transparency / Uniformity	Visual + UV spectrophotometry scan across patch area	Uniform, free from lumps, air bubbles, or color variation
Peel Adhesion	Peel test at 180°, UTM; stainless steel substrate	20–80 N/m (sufficient adhesion without skin damage)
Tack	Probe tack test	Adequate surface tackiness for instant contact adhesion

5.2 Drug Content and Uniformity

Drug content is determined by extracting OPEO from the patch matrix using ethanol, followed by GC-FID or UV-Vis spectrophotometric quantification of d-limonene at λmax 252 nm (in hexane). Three patches per batch should yield drug content of 95–105% of labeled claim. Hydrogen content is measured by gas chromatography with a thermal conductivity detector (TCD) or electrochemical H_2 sensor after incubating the patch in phosphate buffer saline (PBS, pH 7.4) and collecting the headspace gas.

5.3 In Vitro Drug Permeation Studies

Permeation studies are conducted using Franz diffusion cells with either excised cadaveric human skin, pig ear skin (as a validated surrogate), or artificial membranes (Strat-M®, cellulose acetate). Experimental parameters:

- Receptor fluid: PBS pH 7.4 or PBS:ethanol (80:20 v/v) to maintain sink conditions for OPEO

- Temperature: 32 ± 0.5°C (simulating skin surface temperature)
- Donor area: 0.785 cm² (for Franz cells with 1 cm² opening)
- Sampling intervals: 1, 2, 4, 6, 8, 12, 24 hours
- Analysis: GC-FID for d-limonene; electrochemical sensor for H_2

Key permeation parameters calculated include cumulative amount permeated (µg/cm²), steady-state flux (Jss, µg/cm²/h), permeability coefficient (Kp = Jss/Cv), and enhancement ratio (ER = Jss with enhancer / Jss without enhancer).

5.4 Kinetics of Drug Release and Hydrogen Generation

The release profile data is fitted to mathematical models to understand the mechanism:

Kinetic Model	Equation	Interpretation
Zero-order	$Qt = Q_0 + K_0 t$	Constant release rate; ideal for sustained delivery
First-order	$\log Qt = \log Q_0 - K_1 t/2.303$	Release proportional to remaining drug concentration
Higuchi	$Qt = KH \sqrt{t}$	Diffusion-controlled release from matrix
Korsmeyer-Peppas	$Mt/M\infty = Kkp \cdot t^n$	n < 0.5: Fickian; 0.5 < n < 1: anomalous; n = 1: zero-order
Hixson-Crowell	$Q_0^{1/3} - Qt^{1/3} = KHCt$	Release governed by surface area change (relevant for Mg particles)

For H_2 generation, Mg reacts with trace moisture: $Mg + 2H_2O \rightarrow Mg(OH)_2 + H_2 \uparrow$. The rate of H_2 generation follows pseudo-first-order kinetics relative to moisture availability and Mg surface area. The amount of H_2 generated can be quantified volumetrically (water displacement method) or by electrochemical sensor calibrated with standard H_2 gas mixtures.

5.5 Skin Irritation and Sensitization Studies

Safety evaluation is essential prior to human clinical studies:

- Draize patch test (animals, if required by regulatory authority): Semi-occlusive patch application to rabbit intact/abraded skin for 24 h; Primary Irritation Index (PII) < 0.5 indicates non-irritating.
- Human repeat insult patch test (HRIPT): 210-volunteer patch application (induction + challenge phases), evaluated by a dermatologist for erythema, edema, and sensitization.

- Cytotoxicity screening: MTT assay on HaCaT keratinocyte cell line; IC_{50} of OPEO and H_2 formulation > 1000 µg/mL considered non-cytotoxic.
- Skin hydration and TEWL: Corneometer (hydration) and Tewameter (TEWL) measurements at patch site; OPEO is expected to reduce TEWL, indicating barrier enhancement.

5.6 Stability Studies

Stability testing follows ICH Q1A(R2) guidelines:

Condition	Temperature / RH	Duration	Parameters Monitored
Long-term	25°C / 60% RH	12 months (minimum)	Appearance, OPEO content, H_2 generation, adhesion, pH
Accelerated	40°C / 75% RH	6 months	Same as above + drug degradation profiling
Intermediate	30°C / 65% RH	6 months	Optional (Zone II/III climates)
Photostability	ICH Q1B (UV + visible light exposure)	Per guidelines	Color change, d-limonene content, peroxide value of OPEO

Particular attention should be given to d-limonene oxidation (forming limonene oxide and carvone on exposure to air/light), Mg powder oxidation (MgO formation reduces H_2 yield), and plasticizer migration under elevated temperature conditions.

5.7 Organoleptic and Aromatherapy Efficacy Evaluation

Unique to aromatherapy patches, the olfactory release profile must also be characterized:

- Headspace SPME-GC/MS: Solid-Phase Microextraction of volatile compounds above the patch surface to quantify OPEO release into the olfactory environment over time
- Olfactory intensity rating: Panel study (n=20 evaluators) scoring aroma intensity on a 0–10 visual analog scale at 0, 2, 4, 6, 12, and 24 hours post-patch application
- Psychometric evaluation: Validated anxiety and mood scales (State-Trait Anxiety Inventory – STAI; Profile of Mood States – POMS) administered before and after patch use in pilot clinical studies

6. PHARMACOLOGICAL ACTIVITIES — EVIDENCE REVIEW

6.1 Anxiolytic and Antidepressant Effects

Multiple preclinical and clinical studies have investigated OPEO's mood-modulating properties. In rodent models, inhalation of d-limonene significantly reduced immobility time

in the forced swim test and tail suspension test (indicative of antidepressant activity), and decreased anxiety behavior in the elevated plus maze and open field test. Mechanistically, d-limonene increased serotonin (5-HT) and dopamine levels in the prefrontal cortex and hippocampus, with $GABA_a$ receptor modulation contributing to anxiolysis.

In human clinical trials, orange aroma inhalation reduced salivary cortisol levels and self-reported anxiety in dental patients (Lehrner et al., 2005), nursing home residents (Manley, 1999), and perioperative surgical patients. A randomized controlled trial by Goes et al. (2012) demonstrated significant reduction in STAI-State anxiety scores following lemon essential oil (structurally similar to OPEO) inhalation.

6.2 Antioxidant Activity of OPEO

OPEO exhibits significant in vitro antioxidant capacity via DPPH radical scavenging (IC_{50}: 15–30 µg/mL depending on source), $ABTS^{\bullet+}$ scavenging, and FRAP (ferric reducing antioxidant power) assays. The antioxidant activity is attributed to:

- d-Limonene: Breaks lipid peroxidation chains via hydrogen donation
- Linalool: Scavenges superoxide anion and •OH radicals
- β-Caryophyllene: Acts as a CB2 receptor agonist, reducing neuroinflammation

In vivo studies in CCl_4 -induced hepatotoxicity models and streptozotocin-induced diabetic rats confirmed that OPEO significantly reduced MDA (malondialdehyde, lipid peroxidation marker) and restored GSH (glutathione) and SOD activity.

6.3 Anti-inflammatory Activity

d-Limonene inhibits the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) through downregulation of NF-κB signaling. At the transcriptional level, d-limonene suppresses COX-2 and iNOS expression in macrophage cell lines (RAW 264.7). Topical application studies confirm anti-edema effects in carrageenan-induced paw edema models, relevant to patch application at inflammatory skin sites.

6.4 Antimicrobial Activity

OPEO demonstrates broad-spectrum antimicrobial activity against Staphylococcus aureus (MIC: 0.5–2 µL/mL), Escherichia coli, Candida albicans, and Aspergillus niger. This activity is beneficial for maintaining sterility at the patch application site, particularly in wound-

adjacent or perspiration-prone areas. The mechanism involves disruption of bacterial membrane integrity and inhibition of cell respiration.

6.5 Molecular Hydrogen Therapeutic Evidence

Clinical and preclinical studies of H_2 therapy have demonstrated therapeutic benefits across multiple disease models:

Disease Model / Application	Observed H_2 Effect	Key Reference
Ischemia-reperfusion injury	Reduced infarct size; decreased oxidative markers	Ohsawa et al., 2007 (Nature Medicine)
Neurodegenerative diseases	Neuroprotection in Parkinson's and Alzheimer's models	Fu et al., 2009; Yoritaka et al., 2013
Metabolic syndrome	Improved insulin resistance; reduced oxidative stress	Kajiyama et al., 2008
Sports performance	Reduced exercise-induced oxidative stress and muscle fatigue	Ostojic et al., 2014
Skin disorders	Anti-aging effects; reduced UV-induced oxidative damage	Ignacio et al., 2013
Anxiety / depression	Suppressed neuroinflammation; enhanced BDNF expression	Gao et al., 2018
Anti-inflammatory	NF-κB suppression; reduced TNF-α, IL-6, IL-1β	Multiple preclinical studies

7. CHALLENGES IN FORMULATION

7.1 Volatility of Essential Oil

The inherently high vapor pressure of d-limonene (approximately 2 mmHg at 25°C) presents a major formulation challenge: rapid evaporation from open matrices reduces bioavailability and shortens patch efficacy duration. Strategies to mitigate volatility loss include:

- Microencapsulation of OPEO within cyclodextrin (β-CD) inclusion complexes or polymethylsiloxane microcapsules before incorporation into the patch matrix
- Use of rate-controlling membranes to modulate release kinetics
- Multi-layer patch architecture (aromatic layer + drug diffusion layer + backing)
- Airtight aluminium foil primary packaging with oxygen scavengers

7.2 Skin Sensitization Potential

OPEO, though generally recognized as safe (GRAS status, FEMA), may cause contact sensitization at high concentrations (>5% v/v) or following long-term repeated exposure. d-Limonene oxidizes to limonene hydroperoxides on air exposure — a potent contact allergen. Formulation strategies include:

- Limiting OPEO concentration to ≤ 2–3% in leave-on patch preparations
- Incorporating antioxidants (BHT 0.02%, α-tocopherol 0.1%) to retard oxidation
- Patch rotation protocols (daily new application site)

7.3 Compatibility of H_2 Generator with Moisture

Magnesium powder reacts with ambient moisture prematurely if packaging is compromised, depleting H_2 generation capacity before use. Solutions include:

- Desiccant co-packaging to maintain < 5% RH in storage environment
- Surface passivation of Mg particles with magnesium oxide thin coating to control reaction rate
- Activatable patch design: H_2 generator layer activated upon peeling the release liner (exposing to air/skin moisture)

7.4 Achieving Therapeutic H_2 Concentration

Therapeutic concentrations of H_2 in tissue require sustained local delivery. A 10 cm² patch containing 5% Mg (w/w) with 50 mg Mg total can theoretically generate approximately 2.1 mmol H_2 (calculated stoichiometrically). However, gas retention and transdermal delivery of gaseous H_2 present unique challenges compared to drug molecules — the actual delivered dose to tissue remains an active research question.

8. SUMMARY OF QUALITY CONTROL PARAMETERS

Test Parameter	Method	Specification
Appearance	Visual inspection	Smooth, uniform, free from defects
Weight uniformity (n=10)	Analytical balance	Mean ± 5%
Thickness (n=5)	Digital micrometer	Specified target ± 5%
Folding endurance	Manual folding	> 300 folds
Surface pH	Flat-tip pH electrode	5.5 – 7.0
Moisture content	Karl Fischer titration	< 5% w/w
Drug content (d-limonene)	GC-FID	95–105% of label claim

Test Parameter	Method	Specification
H_2 generation capacity	Volumetric / electrochemical	≥ 80% of theoretical yield
In vitro permeation flux	Franz diffusion cell	Defined target flux (μg/cm²/h)
Peel adhesion	180° peel test, UTM	20–80 N/m
Skin irritation (HRIPT)	Clinical dermatological assessment	No sensitization response
Peroxide value of OPEO	Iodometric titration	< 10 meq/kg (freshness)
Accelerated stability (40°C/75% RH, 6 mo)	All above parameters	No significant change from baseline
Olfactory release intensity	SPME-GC/MS + panel evaluation	Detectable aroma for ≥ 6 hours

9. CLINICAL CONSIDERATIONS AND REGULATORY ASPECTS

9.1 Regulatory Classification

Depending on the intended indication and marketing claims, the herbal aromatherapy hydrogen patch may be regulated as:

- Cosmetic product (if claims are limited to aesthetic/wellness benefits such as skin conditioning and pleasant aroma)
- Medical device (if the patch claims therapeutic efficacy via H_2 generation)
- Drug-cosmetic combination (if OPEO or H_2 is claimed to treat a specific pathological condition)

In the United States, the FDA would likely classify this as an OTC drug or cosmetic, depending on claims. In Europe, EMA guidelines under Directive 2001/83/EC for Traditional Herbal Medicinal Products (THMP) may apply if a traditional use claim is made for OPEO. Regulatory strategy should be defined early in product development.

9.2 Proposed Clinical Study Design

A Phase I/II randomized, double-blind, placebo-controlled parallel-group clinical trial is recommended to establish safety and preliminary efficacy:

- Primary endpoints: STAI-State anxiety score change from baseline at 2, 4, 6 hours; DPPH/ORAC serum antioxidant capacity
- Secondary endpoints: POMS subscale scores; salivary cortisol; skin tolerability (Draize scale, TEWL, hydration)

- Control arms: Placebo patch (vehicle only); OPEO-only patch; H_2 -only patch; OPEO+H_2 combination patch
- Sample size: Minimum 40 per arm (n=160 total) for 80% power at α=0.05
- Duration: 4 weeks daily patch application; 2-week washout; crossover assessment

10. FUTURE DIRECTIONS AND RESEARCH GAPS

- Nanoencapsulation of OPEO: Development of nanoemulsions, solid lipid nanoparticles (SLN), or nanostructured lipid carriers (NLC) incorporating OPEO for superior transdermal penetration and reduced volatility loss
- Smart/stimuli-responsive patches: pH-responsive or temperature-sensitive polymers that release OPEO on-demand in response to skin pH changes associated with stress (perspiration-induced pH drop)
- Biomarker-validated H_2 delivery: Development of wearable biosensors to real-time monitor H_2 flux and tissue oxidative stress markers (e.g., 8-OHdG)
- Combination with other essential oils: Synergy studies with lavender (linalool-rich) or bergamot essential oils for enhanced anxiolytic effects
- Mechanistic clinical studies: fMRI/EEG studies to elucidate the olfactory-limbic neural pathways activated by OPEO in patch-based delivery
- Pediatric and geriatric formulations: Age-specific patch sizes and adhesive strengths; assessment of H_2 safety in vulnerable populations
- Green manufacturing: Supercritical CO_2 extraction of OPEO for solvent-free, eco-sustainable production; bio-based polymer matrices
- Microbiome interactions: Investigation of how chronic OPEO patch application affects the cutaneous microbiome and barrier function

11. CONCLUSION

The herbal aromatherapy hydrogen patch incorporating orange peel essential oil represents a scientifically grounded and therapeutically promising innovation at the intersection of phytotherapy, transdermal drug delivery, and molecular hydrogen medicine. The rich phytochemical profile of OPEO — dominated by d-limonene with supporting monoterpenes — provides a multi-target pharmacological basis for anxiolytic, antioxidant, anti-inflammatory, and antimicrobial effects. The addition of molecular hydrogen uniquely extends the antioxidant and neuroprotective scope of the formulation through selective •OH radical scavenging and Nrf2 pathway activation.

The matrix-type patch design, leveraging polymers such as HPMC and Eudragit with appropriate plasticizers and penetration enhancers, offers a feasible and scalable fabrication approach. Key challenges — including essential oil volatility management, premature H_2 generation, and skin sensitization potential — are addressable through established pharmaceutical strategies including microencapsulation, packaging innovation, and conservative concentration optimization.

Rigorous evaluation protocols encompassing physicochemical characterization, in vitro permeation, kinetic modeling, safety profiling, and olfactory assessment are critical to demonstrating product quality. The regulatory pathway and clinical study framework proposed herein provide a roadmap for translating this formulation from bench to bedside.

Future research should prioritize nanocarrier-enhanced OPEO delivery, smart release systems, and mechanistically designed clinical trials with validated biomarker endpoints. The herbal aromatherapy hydrogen patch holds significant potential as a non-invasive, patient-friendly, and holistically active therapeutic modality for stress-related disorders, oxidative stress conditions, and integrative wellness applications.

REFERENCES

1. Ohsawa I, Ishikawa M, Takahashi K, et al. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. Nature Medicine, 2007; 13(6): 688-694.
2. Lehrner J, Marwinski G, Lehr S, Johren P, Deecke L. Ambient odors of orange and lavender reduce anxiety and improve mood in a dental office. Physiology & Behavior, 2005; 86(1-2): 92-95.
3. Viuda-Martos M, Ruiz-Navajas Y, Fernandez-Lopez J, Perez-Alvarez JA. Antifungal activities of thyme, clove and oregano essential oils. Journal of Food Safety, 2007; 27(1): 91-101.
4. Goes TC, Antunes FD, Alves PB, Teixeira-Silva F. Effect of sweet orange aroma on experimental anxiety in humans. Journal of Alternative and Complementary Medicine, 2012; 18(8): 798-804.
5. d'Alessio PA, Ostan R, Bisson JF, Schulzke JD, Ursini MV, Béné MC. Oral administration of d-limonene controls inflammation in rat colitis and displays anti-inflammatory properties as diet supplementation in humans. Life Sciences, 2013; 92(24-26): 1151-1156.

6. Kajiyama S, Hasegawa G, Asano M, et al. Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance. Nutrition Research, 2008; 28(3): 137-143.
7. Jafarzadeh M, Arman S, Pour FF. Effect of aromatherapy with orange essential oil on salivary cortisol and pulse rate in children during dental treatment: A randomized controlled clinical trial. Advanced Biomedical Research, 2013; 2: 10.
8. Hirota R, Roger NN, Nakamura H, Song HS, Sawamura M, Suganuma N. Anti-inflammatory effects of limonene from yuzu on lipopolysaccharide-induced inflammation in murine macrophages. Biological & Pharmaceutical Bulletin, 2010; 33(12): 2012-2017.
9. Yoritaka A, Takanashi M, Maeda T, Hirayama M, Watanabe H, Hattori N. Pilot study of H_2 therapy in Parkinson's disease: A randomized double-blind placebo-controlled trial. Movement Disorders, 2013; 28(6): 836-839.
10. Badgujar SB, Patel VV, Bandivdekar AH, Mahajan RT. Traditional uses, phytochemistry and pharmacology of Ficus carica: A review. Pharmaceutical Biology, 2014; 52(11): 1487-1503.
11. Chiu HF, Lan YH, Yang TC, et al. Evaluation of the anti-inflammatory properties of orange essential oil from orange peel (Citrus sinensis). Journal of Food and Drug Analysis, 2018; 26(4): 1253-1260.
12. Patel VR, Bhatt N, Patel RB. Transdermal drug delivery system: A review. Asian Journal of Pharmaceutical and Clinical Research, 2012; 5(1): 19-24.
13. Ostojic SM, Stojanovic MD. Hydrogen-rich water affected blood alkalinity in physically active men. Research in Sports Medicine, 2014; 22(1): 49-60.
14. Kohlert C, van Rensen I, März R, Schindler G, Graefe EU, Veit M. Bioavailability and pharmacokinetics of natural volatile terpenes in animals and humans. Planta Medica., 2000; 66(6): 495-505.
15. Breitmaier E. Terpenes: Flavors, Fragrances, Pharmaca, Pheromones. Weinheim: Wiley-VCH, 2006.
16. ICH Guideline Q1A(R2). Stability Testing of New Drug Substances and Products. International Conference on Harmonisation, 2003.
17. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. In vitro antibacterial activity of some plant essential oils. BMC Complementary and Alternative Medicine, 2006; 6: 39.
18. Asbahani A, Miladi K, Badri W, et al. Essential oils: From extraction to encapsulation. International Journal of Pharmaceutics, 2015; 483(1-2): 220-243.