FORMULATION AND EVALUATION OF THE TOPICAL HYDROGEL INTEGRATING ORANGE PEEL OIL LOADED NANOSPONGE TO ENHANCE ITS ANTIFUNGAL ACTIVITY

A Thesis is submitted to

ASSAM SCIENCE AND TECHNOLOGY UNIVERSITY,
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In the Partial Fulfillment of the Requirement for the
Award of the Degree of

MASTER OF PHARMACY (M.PHARM)
in
PHARMACEUTICS

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I wish him all success in life.

Prof (Dr.) Gouranga Das
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I wish him all success in life.

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DECLARATION BY THE CANDIDATE

I hereby declare that the matter embodied in the dissertation entitled **"*Formulation and Evaluation of a topical hydrogel integrating orange peel oil loaded nanosponge to enhance its antifungal activity***" a bonafide and genuine research work carried out by me under the supervision of **Dr. Bhupen Kalita**, Assistant Professor, Department of Pharmaceutics, ***Girijananda Chowdhury Institute of Pharmaceutical Science, Hatkhowapara, Azara, Guwahati-17.*** The work embodied in this thesis is original and has not been submitted for the award of degree, diploma, associateship or fellowship of any other university or institution.

Date: **Robina Jidung**

Place: **GIPS Guwahati**

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संख्या /No.: BSI/ERC/ Tech/2021/ 118 दिनांक /Dated:29.07.2021

सेवा में/To,

Rubina Jidung
M. Pharm. Student
Girijananda Chowdhury Institute of Pharmaceutical Sciences
Azara, Guwahati-17, Assam.

विषय/Sub.: Identification and authentication of plant specimen.

Dear Miss Jidung

With reference to your letter No.Nil dated 09-07-2021, regarding the subject cited above, I am to inform you that your plant specimen has been identified and confirmed as below:-

1. *Citrus sinensis* Pers. (Rutaceae)

Thanking You/ धन्यवाद

भवदीय /Yours sincerely

29/7/2021

(Dr.N. Odyuo)
वैज्ञानिक-'ई' एवं कार्यालय प्रमुख/ Scientist-E,HoO

Affectionately Dedicated To
My Beloved Parents

ABSTRACT

Orange peel oil is a volatile oil extracted from the peel of Citrus sinensis. Because of its various pharmacologic and therapeutic effects, has become one of the most important natural oils in the pharmaceutical industry. However, orange peel oil has a limited water solubility, which could reduce its effectiveness. Furthermore, the instability of citral, the product's main active ingredient, could result in volatilization, reactivity with other formulation ingredients, and, as a result, skin irritation. To address these issues, this study intends to develop orange oil loaded ethyl cellulose nanosponges with a topical hydrogel that has a stronger antifungal impact while causing less irritation. Steam distillation method is used for extraction of oil after which the percentage yield was calculated. Characterisation of the oil was done to check whether the oil is pure or not. The emulsion solvent evaporation technique was used for the preparation of the nanosponge. Nine formulation were prepared employing the ethyl cellulose: polyvinyl alcohol ratio and stirring rate as independent variable. The size of the prepared formulation was evaluated. Result revealed that the size of the nanosponge depends upon the stirring rate and the ratio of ethyl cellulose: polyvinyl alcohol.

Key words: Volatile oil, Ethycellulose, Nanosponge, Volatilization

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Abbreviation and symbols	Full forms
°	Celsius
Cm	Centimetre
Nm	Nanometre
Et. Al	Et alia/ and others
g	Gram
s	Second
PCR	Polymerase Chain Reaction
GRAS	Generally Recognised as Safe
FDA	Food and Drug Administration
DNA	Deoxyribonucleic acid
BCS	Biopharmaceutical classification system
TLC	Thin Layer Chromatography
FT-IR	Fourier Transform Infrared
PVA	Polyvinyl alcohol
LD	Lethal Dose

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INTRODUCTION

1.1. Fungal diseases

The human species coexists peacefully with the microorganisms that surround them for the most part, and only when the defence system is compromised or pathogen concentrations reach an unusually high density can an infection occur. Most infections go unnoticed, but infecting agents occasionally elicit a response from the body, resulting in clinically evident signs and symptoms, a condition known as infectious illness. Bacteria, viruses, parasites, fungus, prions, worms, and helminthes have all been implicated in infectious diseases, the most common of which are caused by common viruses, and the most dreaded of which were caused by bacteria until a few decades ago. Fungi have become the most dangerous pathogens as efforts to control bacterial infections in humans have improved.

Data from the US showed that invasive fungal disease was ranked as the 10th most fatal infection in 1980 rising to seventh in 1997 [1]. Invasive fungal infections have become more common in patients who are not at the terminal stage of their underlying disease since the 1980s. A fungus is a vegetative organism that isn't a plant because it lacks the ability to generate chlorophyll. It's a non-motile life form with a fundamental structural unit that's either a chain of cylindrical cells (hyphae) or a single cell, or both. The most prevalent species, such as Aspergillus and Candida, can be found all over the world. Candida spp. and Cryptococcus spp. are the most usually isolated yeasts. The most common moulds isolated are Aspergillus spp., but Fusarium spp., Penicillium spp., and zygomycetes are also becoming more common. Molds are multicellular organisms with branching filamentous hypae that reproduce sexually or asexually by spore production, whereas yeast are unicellular organisms that reproduce through budding or binary fission of bacteria.

Over 69.000 fungus species have been described over time, with the total number of fungal species believed to be in the 1.5 million range. Fungi species number in the millions [1, 2]. A fungus causes a fungal disease or infection, often known as mycosis, which is a skin disease caused by a fungus. Mycosis and skin illnesses can be spread through the air, direct touch, or food water. Both human and fungal factors influence the risk of fungal illnesses. Fungi can enter the body when exterior barriers are breached, causing skin irritation, itching, swelling, redness, and scaly skin.

1.1.1. Structure

The main body of most fungi is made of fine branching, usually colourless threads called hyphae. An individual fungus filament is called hypha. Several of these hyphae, all most fungi has cell wall that contains chitin, unlike the cell wall of plants which contains cellulose.

1.1.2. Morpholigical Classification

- Moulds: They are hyphae in form e.g. Ringworm or Dermatophytes.
- Yeast: Single cell that use bud to reproduce e.g. Cryptococcus Neoformans.
- Yeast like: Form pseudo hyphae e.g. Candida albicans
- Dimorphic fungi: Fungi having yeast form in tissue and mould in culture e.g. Blastomyces dermatitis.

1.1.3. Factors promoting invasive fungal infection

- Poor hygiene
- Genetic predisposition
- Use of antibacterial, manipulation of a patient microbiological flora
- Use of H_2 receptor antagonist

- Mucosal barrier injury
- Depressed cellular immunity

1.1.4. Organism and Diseases cause

Candida spp: Candida spp. are yeasts that colonise human skin, the gastrointestinal, and genitourinary systems without infecting humans. Although Candida albicans is the most common fungus found in clinical specimens, other species are becoming more prevalent (eg, Candida glabrata). This is significant because antifungal drugs used in the past to treat these additional species may be ineffective. Candida spp. infections are particularly common on intensive care units, where patients frequently have invasive devices inserted. Furthermore, broad-spectrum antibiotics might alter a patient's normal skin flora, making Candida spp colonisation more common, which can lead to vascular invasion via such invasive devices. Endorgan disease, such as endophthalmitis (inflammation of the intraocular cavities) or endocarditis, can occur once the organism has gained access into the bloodstream (candidaemia). If left untreated, Candida spp. endocarditis has a terrible prognosis and almost always requires heart valve replacement.

Cryptococcus spp : The most prevalent Cryptococcus species is Cryptococcus neoformans, which is pervasive in the environment and frequently discovered in bird droppings, particularly from pigeons. Individuals who are immunocompetent or immunocompromised can develop a pulmonary syndrome characterised by fever, cough, and lung infiltrates. Although cryptococcosis can affect immunocompetent hosts, it is much more common in HIV patients. Meningitis is the most common clinical symptom for these immunocompromised hosts. Untreated cryptococcal meningitis has a near-100 percent fatality rate, making it a huge healthcare burden in underdeveloped nations.

Aspergillus spp: Aspergillus spp. are moulds, with Aspergillus fumigatus being the most usually isolated species. Invasive aspergillosis is a particular issue for patients having allogeneic bone marrow transplantation who have haematological malignancies; up to 13% of individuals have been observed to be afflicted.

Similarly, people who have had a solid-organ transplant are at risk of invasive aspergillosis, while the risk is higher for lung transplant recipients. Aspergillus spp. enter the body through the lungs. Invasion can start in the lungs or sinuses and spread throughout the body via the bloodstream. The most prevalent form of aspergillosis is pulmonary aspergillosis, which should be suspected in haematology patients who have lung symptoms and indications that are consistent with infection and are not responding to broad-spectrum antibiotics.

Zygomycete: Zygomycetes is an umbrella word for a group of fungi that produce zygomycosis, a disease. Zygomycetes are distinguished by the formation of sporangiospores (spores formed in a fungal structure called the sporangium), which spread rapidly in the air and can then be breathed; this is why rhinocerebral and pulmonary disease are the most prevalent clinical manifestations of zygomycosis.

Zygomycosis is the most severe and widespread fungal illness. The pathogen can spread locally into the orbit once it has settled in the sinuses, causing pain and visual disruption. Neutrophil dysfunction and neutropenia, particularly chronic neutropenia, are known to increase the risk of zygomycosis. Invading zygomycetes spread through the bloodstream, producing embolization and tissue necrosis. Because central nervous system invasion is associated with a high mortality rate despite vigorous treatment, prompt diagnosis is critical.

Dimorphic fungi: Although dimorphic fungi can cause invasive infections, they are uncommon in the United Kingdom. As a result, each patient suspected of having a fungal disease should have their travel history documented.

1.1.5. Treatment:

Invasive fungal illness can be difficult to diagnose. It is treated differently depending on the type of sickness. Antifungal medicines can be used to treat it. The most effective diagnostic approach is determined by the fungus that is causing the sickness. Fungi are widespread, which means that when a fungus is isolated in a microbiology lab. The gold standard for diagnosis is to isolate fungus from the illness site. Direct microscopy will reveal the presence of fungus but will not identify the species. Fungus can be cultured on particular media, which can help with identification. Fungal disease is diagnosed by culture of a fungus from generally sterile tissue or fluid, such as cerebrospinal fluid, bone, or blood, however laboratory contamination is possible. Candidaemia is diagnosed using blood cultures. Serology is a technique for identifying fungal antigens in body fluids, and it's very beneficial for detecting cryptococcosis. Serology data can be difficult to use because of the low specificity and sensitivity of the tests, especially in immunocompromised patients. The polymerase chain reaction (PCR) has been used to diagnose invasive aspergillosis, however the test results have not been standardised, and the outcomes of PCR studies have varied widely.

1.2. Essential Oils

Essential oils are a class of volatile and liquid fragrance molecules derived from natural sources, most commonly plants. Depending on the species, it is produced from plant material contained within a specific part of the plant or a specific component of the plant cell. The odoriferous chemicals (essential oils) are produced in the chloroplast of the leaf, the vesinogenou layer of the cell wall, or through the hydrolysis of certain glycosides. They can be found in a variety of places on the plant. Some can be found in leaves, seeds, flowers, peels, rhizomes, roots, bark, wood, resin, and petals. Essential oils extracted from several areas of the same plant can have a wide range of fragrances and qualities. Geranium, for example, produces oil from both the flowers and the leaves, and the components, fragrances, and other attributes of each differ. Many connected elements, including as meteorological, seasonal, and geographical circumstances, harvest timing, and extraction procedures, influence the amount of essential oil recovered from the plant. The stages of plant growth can also affect the output of oils from the plants. Essential oils contain oxygenated molecules that act as chemical messengers in the cells, bringing life to the plants, eliminating pests, promoting growth, and boosting healing. Previous studies have found that essential oils have therapeutic qualities.

1.2.1. Healthy Benefits of essential oil:

Despite their widespread use, little is known about essential oils' ability to treat certain illnesses. Here are some essential oil as the evidence which is use as an aromatherapy to address some of the most common health issues.

- Stress and anxiety

It has been estimated that 43% of people who have stress and anxiety use some form of alternative therapy to help relieve their symptoms. Initial research on aromatherapy has proven

quite encouraging. Many studies have demonstrated that the scent of various essential oils can help cure anxiety and stress in addition to regular therapy.

- Headaches

It has been studied that many essential oil use to treat headache. According to the study applying a mixture of chamomile and sesame oil to the temples may treat headaches and migraines. This is a traditional Persian headache remedy

- Sleep and insomnia

Lavender, one of the most popular essential oil, is used commonly for relaxation and sleep. When used before bed, studies have shown that lavender oil can not only help you fall asleep but also improve the overall quality of rest.

- Reducing inflammation

Essential oils are a great support tool for relieving the discomfort from inflammation that can occur on the surface of our skin. Essential oil like thyme, clove, rose, eucalyptus helps in reducing inflammation.

- Antibacterial and antimicrobial

Various studies have proved that essential oils do have antibacterial and antimicrobial properties. The property of essential oil are generally determined by the major compounds present in them.

1.2.2. Side effects:

Aromatherapy which is a natural healing process in which essential oil are used for treating different problems like sleeplessness, headache. Although, essential oils are obtained from

plant extract and are safe for use in-spite of having so many benefits it still have some common side effects.

- Skin irritation:

Despite the fact that essential oils are natural and harmless, only a small percentage of people are allergic to them. It can be caused by inhaling essential oils or massaging them into various regions of the body. Mild irritation and redness may occur as a result of an allergic reaction. Allergies develop when your immune system reacts to an allergen, causing redness and itching.

- Nasal irritation

Inhaling essential oils might irritate the nasal passages. Some of the most frequent symptoms of essential oil allergies include sneezing, running nose, and congestion. If you have asthma, you should avoid using essential oils.

- Hormonal Issue

The effects of some essential oils are similar to those of hormones. These oils mimic oestrogen and prevent the effects of androgens. As a result, before using any essential oil, it is important to seek advice from a professional.

1.2.3. Extraction of essential oils:

Essential oils are valuable plant products, generally of complex composition comprising the volatile principles contained in the plant and the more or less modified during the process of preparation. The oil droplets being stored in the oil glands or sacs can be removed by either

accelerate diffusion through the cell wall or crush the cell wall. The adopted techniques depend on the part of the plants where the oil is to be extracted, the stability of the oil to heat and susceptibility of the oil constituents to chemical reactions. Common techniques used for the extraction of essential oils are Hydrodistillation, Hydrodiffusion, Effleurage, Cold pressing, Steam distillation, Solvent extraction.

Essentials oils are broadly used in pharmaceutical components, in nutritional suppliments, for cosmetic industry and for aromatherapy.

1.2.4. Essential having antifungal property

Fungal infections are caused by eukaryotic organisms, and it is therefore more difficult to ascertain their presence and apply the appropriate therapeutic treatment compared to bacterial infections. The cell wall of fungi may be considered as the prime target for selectively toxic antifungal agents because of its chitin structure, which is absent in human cells. Chemical treatments are largely effective, but resistant strains and intrinsically resistant species can be developed. The onset and severity of the fungal infection depends on the inoculum charge, the host's immunological state and resistance. Essential oil can represent one of the most promising natural products for fungal inhibition.

In fact, many kinds of Essential oil obtained from different plants or herbs exhibited intense antifungal properties. Essential oils are classified as "Generally Recognised as Safe" (GRAS) by the Food and Drugs Administration (FDA), thus they are not harmful and, due to their natural origin, are more widely accepted by consumers than "synthetic" agents. The antifungal activity of essential oil might be caused by the properties of terpenes/terpenoids that— due to their highly lipophilic nature and low molecular weight— are capable of disrupting the cell membrane, causing cell death or inhibiting the sporulation and germination of food spoilage

fungi. Therefore, several in vitro tests indicate that terpenes/terpenoids show ineffective antimicrobial activity when used as singular compounds compared to the whole Essential oil.

Table no.1. List of Essential oil having antifungal property

Sl no.	Essntial Oils	Scientific name of the plant	Plant part from which oil is extracted
1	Tea tree oil	*Melaleuca alternifolia*	Leaves
2	Lemon grass oil	*Cymbopogon citratus*	Leaves, Stalks
3	Clove oil	*Syzygium aromaticum*	Dried flowe bud
4	Orange oil	*Citrus sinensis*	Peel
5	Thyme oil	*Tymus vulgaris*	Leaves, Flower
6	Peppermint oil	*Mentha piperita*	Leaves

1.3. Nanosponges

The therapeutic efficiency of a medicine is determined by its bioavailability, which is determined by its solubility. Nearly 70% of medications examined in the pharmaceutical industry are poorly water soluble and fall into the BCS class II group. For medications with issues including limited bioavailability, poor water solubility, or a small absorption window, oral administration is preferred. Various medication delivery techniques are utilised to circumvent these constraints. Nanotechnology is one of the most major revolutions in this sector. Nanotechnology is described as the fabrication and conversion of materials at the nanoscale level to produce end products with unique properties. Nanoparticles, nanocapsules, nanospheres, nanosponges, nanosuspensions, nanocrystals, and other formulations are

included. Nanoparticles are used in biocompatible materials, textile functionalization and antimicrobial coatings, medication delivery, DNA delivery, and other applications. Polymeric nanoparticles, solid lipid nanoparticles, nanoemulsions, nanosponges, carbon nanotubes, dendrimers, and other dosage forms are available. Nanosponges are microscopic mesh-like highly crosslinked polymer-based colloidal structures that encapsulate a wide range of medicinal molecules in their core. They have a proven spherical colloidal nature, and their inclusion and non-inclusion behaviour indicate that they have a very high solubilization capacity for BCS class II (poorly soluble pharmaceuticals). They've only recently been produced and proposed as a medicine delivery system. It has the ability to solubilize poorly water soluble medicines, allowing for longer release and increased bioavailability. Because of their interior hydrophobic chambers and exterior hydrophilic branching, nanosponges can carry both hydrophilic and hydrophobic medicinal molecules, providing versatility. They resemble a three-dimensional network or scaffold (3D). A lengthy length of polyester is combined in solution with small molecules known as crosslinkers, which serve as tiny grappling hooks to connect different regions of the polymer.

In comparison to conventional nanoparticles, it has a significant benefit. It can be easily regenerated using a variety of methods, including washing with environmentally friendly solvents, stripping with relatively inert hot gases, light heating, or adjusting pH or ionic strength. As a result, nanosponges have already been used in a variety of disciplines, including cosmetics and pharmaceuticals. They are water soluble, but they do not chemically break down in water. They also use water as a transport fluid when mixed with it. They're employed to cover up undesirable odours and tastes, as well as to turn liquids into solids. The chemical linkers allow the nanosponges to attach to the target location preferentially. They are solid in nature and have been found to be safe for oral and invasive routes of administration and so that they could serve as a potential carrier for drug delivery system. The small shape of nanosponges

enables the pulmonary and parenteral delivery successfully. For oral administration, the complexes may be dispersed in a matrix of excipients, diluents, lubricants and anti-caking agents suitable for the preparation of capsules or tablets, for parenteral administration the complex may be simply carried in sterile water, saline or other aqueous solutions and for topical administration they can be effectively incorporated into hydrogel.

1.3.1. Structure of nanosponge

A polymeric nanoparticle area was covered by red blood cell membranes in nanosponges. Polymers, copolymers, and cross-linkers are among the materials utilised to make nanosponges. The nanomaterial (polyester) produces a biodegradable three-dimensional network, allowing it to decay gradually in the body and release the medicine gradually.

Fig no 1: Structure of nanosponge that shows a cavity for drug loading

Toxin absorption is possible, and the core can be loaded with a variety of medications, including enzymes, proteins, vaccines, and antibodies. The capacity to carry both hydrophilic and lipophilic pharmaceuticals through the body is a substantial benefit, with nanosponges enhancing the solubility, stability, and bioavailability of treatments

1.3.2. Nanosponge advantage and disadvantage

Advantages

- They provide targeted site specific drug delivery.
- Allows incorporation of immiscible liquids which improves material processing. Liquid can be converted to powders.
- Improved formulation flexibility, increased elegance, and improved stability.
- These are self-sterilizing due to their 0.25 small size, which bacteria cannot penetrate.
- They improve the solubility of the medicine that isn't very soluble.
- The drug delivery methods developed by Nanosponge are non-irritating, non-mutagenic, and non-toxic.
- Nanosponges complexes 3 are stable at a temperature of 130 °C and over a large pH range (i.e. 1- 11).
- There are less negative side effects (since smaller quantities of the drug have contact with healthy tissue).
- By adjusting the quantity of cross-linker to polymer, particles can be made smaller or larger.
- Biodegradable and predictable release.

Disadvantages

- Nanosponges can only encapsulate tiny molecules and are therefore unsuitable for larger molecules.
- Dose dumping is a possibility
- Only rely on the medication molecules' loading capabilities.

LITERATURE REVIEW

- (Aldawsari H.M et al., 2015) concluded that the hydrogels integrating lemongrass-loaded nanosponges was prepared by emulsion solvent evaporation method. The best formulation was chosen for further study on the basis of its particle size and controlled release profile. And then it was subjected to surface investigation, using SEM and TEM, which revealed the spongy structure with minute pores and the sustained integrity of the nanosponge structure when incorporated in the hydrogel. The results was found to be promising with respect to the practical application of the incorporation of LGO in pharmaceutical formulations with the benefit of decreasing the hazards of its use in folk medicine in the crude form.

- (Simionata I et al., 2019) have discussed how essential oils is encapsulated in cyclodextrin nanosponge and the assessment of their antimicrobial activity against foodborne pathogens. They described that the essential oil is successfully encapsulated in cyclodextrin nanosponge to shows its activity.

- (Silva F et al., 2019) have discussed how a coriander oil is incorporated into a cyclodextrin nanosponge to create a stable controlled release system. They also described that the incorporation of the whole essential oil nanosponge and their ability to provide a controlled oil release to inhibit the bacterial growth creating a new strategy to overcome the poor efficacy of current antimicrobial food packaging.

- (Wadhwa G et al., 2019) has carried out a study that babchi essential oil having a variety of biological activity due to its properties the oil posess and immense potential for the treatment of dermatological disorder. The results of photostability and stability analysis indicated improved stability of BEO loaded microsponges. Hence, encapsulation of BEO in microsponges resulted in efficacious carrier system in terms of stability as well as safety of this essential oil alongwith handling benefits.

- (Oikeh et al., 2020) has carried out a study investigated the phenolic content and antimicrobial activities of fresh and dry Citrus sinensis peel extracts. The results from the study conclude that the fresh Citrus sinensis peel extract contains more phenolics and possesses better antimicrobial activities against the studied microbial strains compared to the dry peel extract. The findings in this study suggest that drying plant parts before extraction for phytonutrients may lead to loss of active components.

- (Fekadu et al., 2019) has discussed how extraxtion of oil is done from the peel of orange through distillation method by using different solvent. In this study physical, chemical characterisation of the oil is determined and the maximum yeild of the essential oil is obtained.

- (Velázquez-Nuñez et al., 2013) has carried out a study that the antifungal efficacy of orange peel essential oil at selected concentration applied by vapour exposure or direct addition was faster, orange peel essential oil vapors were more effective, since lower concentrations were required to achieve the same antifungal effect.

- (Rezende et al., 2020) has concluded that essential oils from citros have been mainly acknowledged as compounds that carry secondary metabolites whose biological functions act either synergically together or alone. Essential oil from both varieties of Citrus exhibited high contents of limonene, a monoterpene that may be related to the promising in vitro antifungal activity against Rhizopus stolonifer.

- (Abbas et al., 2019) characterised that Nanosponge-based hydrogel formulations of FZ demonstrate a sustained drug release pattern. *Invitro* drug release showed Higuchi model as the best fit model. The developed topical nanocarrier system shows the potential benefits such as reduction in frequency of application, total dose and systemic side-effects. The better retention ability of nanosponge based hydrogel makes it potentially suitable for the treatment of topical fungal infections which may improve patient compliance.

- (Hay et al., 2014) concluded that fungal disease causes a huge burden in the global context of health. Skin conditions were the 4th leading cause of nonfatal burden expressed as years lost due todisability in 2010; taking into account health loss due to premature death expressed as disability-adjusted life years skin remains the 18th leading cause of health burdenworldwide.

- (Jain et al., 2010) conclude that the incidence of local and systemic fungal infections is increasing at an alarming rate. This is primarily due to advances in medical practice, which have resulted in the proliferation of severely ill, immunocompromised, hospitalized patients. Superficial fungal infections are mild but may spread to other areas of the body or, occasionally, to other individuals. More seriously, but less

frequently, they may develop into invasive forms.. It is therefore desirable to treat local fungal infections to prevent the risk of spread. Although the incidence of fungal infections can be reduced by minimizing risk factors in hospitals, such as poor hygiene practice, our inability to reliably prevent such infections highlights the need for effective treatments.

AIM AND OBJECTIVES

3.1. Aim: The primary aim of the study is to formulate and evaluate a topical hydrogel integrating orange peel oil loaded nanosponge to enhance its antifugal activity.

3.2. Objective

- To indentify the plant
- To extract oil from the peel of the orange.
- To identify the Physico-chemical property of the oil
- To prepare a nanosponge
- To prepare a hydrogel by loading a nanosponge
- To check the antifungal activity of the prepared formulation (invitro).

PLANT PROFILE

4.1. ESSENTIAL OIL REVIEW

4.1.1. Orange peel oil:

- Obtained from the dried or fresh outer part of *Citrus sinensis* belonging to the family Rutaceae. The peel contains essential oil.

Fig no 2: Sweet orange (*Citrus sinensis)*

- Botanical name: Citrus sinensis
- Common name: Sweet orange peel oil, citrus sinensis oil
- Plant part use: Peel
- Extraction method: Steam distillation

➢ Physico chemical properties:

- Molecular weight: 136.234
- Boiling point: 349˚C
- Density: 0.845g/mL at 25˚C
- Color: clear liquid

- Odor: Fresh orange aroma
- Solubility in water: Insoluble

Taxonomical Classification

Taxon name: Citrus sinensis

Common name: Orange peel oil

The taxonomical classification of the drug is shown in Table no 2

Table no 2: Scientific Classification of the plant

Kingdom	Plantae
Order	Sapindales
Family	Rutaceae
Genus	*Citrus*
Species	*Sinensis*

- Pharmacology

Description

It is distilled from the peel of the fruit. It has a lively, fruity, sweet aroma.

Constituents:

Orange oil has various chemical compounds that include pinene, sabinene, myrcene, limonene, linalool, citronellal, neral, geranial, decylic aldehyde, Terpineol and carotin.

Properties :

Analgesic, antifungal, anti-emetic, antioxidant, antirheumatic, antiseptic, antispasmodic, antiparasitic, aphrodisiaac, cardiac, larvicidal, laxative, digestive, stimulant(energetic) and tonic.

Toxicity profile:

Acute toxicity of sweet orange peel oil in the animals is low, moderately irriating to rabbits, LD_{50} in rabbits >5 g/kg, LD_{50} in rats >5 g/kg.

Safety:

Citrus essential oil are non-toxic, non-mutagenic, and non-carcinogenic. They are not hazardous. However, there is a possible sensitization issue if cold or oxidised oil is used. Applying essential oil to the skin in high dose than the maximum dermal use level it is recommended to avoid sunlight.

Precautions:

- Don’t apply undiluted essential oil to your skin.
- Keep the oil away from your eyes.
- Store the oil out of reach of children and pets.
- If you use the oil for aromatherapy, make sure that the space you’re in is ventilated well.

- If you're pregnant, breastfeeding, or taking prescription medications, speak to your doctor before using orange essential oil.

Benefits & Uses:

- Orange oil is a tonic for anxiety and depression. It also stimulates the digestive system and is effective for constipation.
- Orange oil can be used effectively on the immune system as well as for colds and flu and to eliminate toxins from the body. It is a good diuretic and is most useful in balancing water retention and obesity.
- It is widely used as a flavoring of food and drinks.
- It is also used in perfumery, soap making, skin care products and other cosmetics products.

Orange peel oil is a volatile oil which is extracted from the peels of *Citrus sinensis*. It is used largely for its antimicrobial and antifungal properties. Orange peel oil is incorporated in many formulations used to treat skin infection as an active ingredient. It is mainly found in Southern China, Northeast India and Myanmar. It has an excellent source of vitamin C. It has also been traditionally used.

Fig no 3: Orange peel

4.1.2. Oil Extraction

Orange peel oil is extracted by steam distillation of *Citrus sinensis* peels. After condensation the oil part is separated with tha aqueous part. Alternative method of extraction can also be done by using hydro distillation method.

4.2. EXCEPIENTS REVIEW

Ethyl Cellulose

- Molecular formula: $C_{20}H_{38}O_{11}$
- Synonyms: Cellulose ethylate, Ethocel 150
- Molecular weight: 454.5
- IUPAC name: 2-[4,5-diethoxy-2-(ethoxymethyl)-6-methoxyoxan-3-yl]oxy-6-(hydroxymethyl)-5-methoxyoxane-3,4-diol
- Chemical structure:

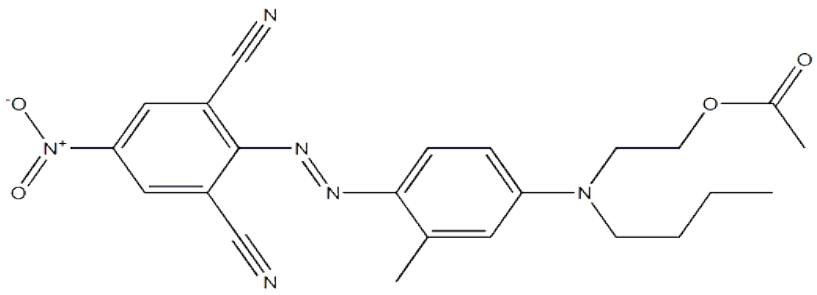


Fig no 4: Structure of ethyl cellulose

- Melting point: 240-255˚C

- Description: Slightly hygroscopic white to off white, odourless and tasteless powder
- Solubility: Partially insoluble in water
- Category: Coating agent, flavouring fixative, Binder, Filler
- Use: Use in oral and topical pharmaceutical formulations for various purpose.
- Storage: It should be stored in a well tight container and the temperature should not exceed 39˚C.

Polyvinyl alcohol (PVA)

- Molecular formula: C_2H_4O
- Synonyms: Ethenol, Vinyl alcohol
- Molecular weight: 44.05
- IUPAC name: Ethenol
- Chemical sturture:

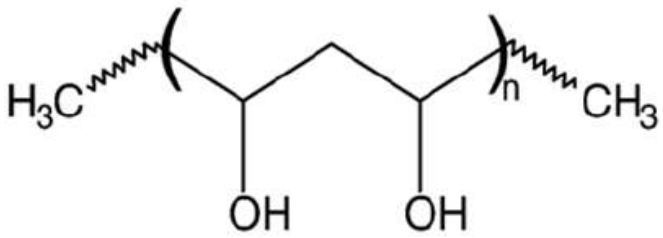


Fig no 5: Structure of PVA

- Melting point: 442˚F
- Description: Odourless white to cream colored granules or powder
- Solubility: Soluble in water, sparingly soluble in ethanol
- Category: Thickner agent, Emulsion stabiliser
- Use: It is used as a lubricant to increase viscosity.

- Storage: Stored in a cool, dry, ventilated area.

Dicholoromethane

- Molecular formula: CH_2Cl_2
- Synonyms: Methylene chloride, Methylene dichloride
- Molecular weight: 84.93
- IUPAC name: Dichloromethane
- Chemical sturture:

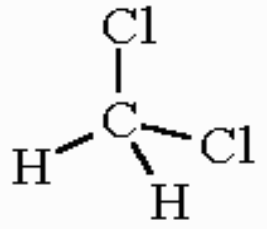


Fig no 6: Structure of Dichloromethane

- Melting point: -142.1˚F
- Description: Dichloromethane appears as a colourless liquid with a sweet, penetrating, ether-like odor.
- Solubility: Miscible with ethanol, alcohol.
- Category: Reducing agent
- Use: It is used as a solvent in the manufacturing of pharmaceutical products.
- Storage: It is highly volatile so it should be stored in a cool, dry area in tightly closed container.

Carbopol 940

- Molecular formula: CH_2-$CHCO_2H)n$
- Synonyms: Carbomer 940, Polyacrylic acid
- Molecular weight: 713.1
- IUPAC name: Poly(acrylic acid), poly(1- carboxyethylene)
- Chemical structure:

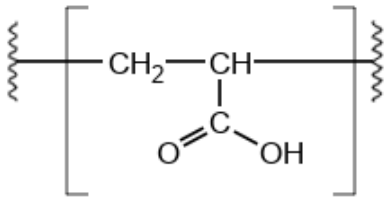


Fig no 7: Structure of Carbopol 940

- Melting point: 116˚C
- Description: It is a white powder, crosslinked polyacrylic acid polymer. It is extremely efficient rheology modifier capable of providing high viscosity and forms sparkling clear gels or hydro-alcoholic gels and creams
- Solubility: Soluble in water after neutralization, they are soluble in 95% ethanol and glycerine.
- Category: Mucoadhesive
- Use: Carbopol is used as a thickener in lotions, creams and gels. It is also used to stabilize, suspend and control the release of pharmaceutical products.
- Storage: Tightly closed container and it should be stored out of contact with water. Temperature should be below 104°C.

Triethanolamine

- Molecular formula: $C_6H_{15}NO_3$
- Synonyms: TEA, Triethalolamine
- Molecular weight: 149.19 g/mol
- IUPAC name: 2,2',2''- Nitrilotriethanol
- Chemical sturture:

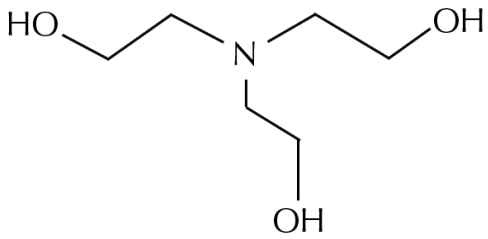


Fig no 8: Structure of triethanolamine

- Melting point: 20-21˚C
- Description: It is a clear, colourless to pale yellow-colored viscous liquid having a slight ammoniacal odor.
- Solubility:
- Category: Emulsifying agent; alkalizing agent.
- Use: It is use as a stabilizer which helps to balance a pH of the product.
- Storage: To be stored in an airtight container protected from light, in a cool and dry place.

MATERIALS AND METHOD

Table no 3: List of materials

Sl no	Materials	Supplier
1	Ethyl Cellulose	HiMedia Laboratories Pvt. Ltd, Mumbai
2	Dichloromethane	Merck Specialities Pvt. Ltd, Mumbai
3	Polyvinyl alcohol	HiMedia Laboratories Pvt. Ltd, Mumbai
4	Carbopol 940	Merck Specialities Pvt. Ltd, Mumbai
5	Triethanolamine	HiMedia Laboratories Pvt. Ltd, Mumbai
6	Toulene	Merck Specialities Pvt. Ltd, Mumbai
7	Ethyl acetate	Merck Specialities Pvt. Ltd, Mumbai
8	Vanillin	Merck Specialities Pvt. Ltd, Mumbai
9	Sulphuric acid	HiMedia Laboratories Pvt. Ltd, Mumbai

Table no 4: List of Equipments / Instruments

Sl no	Equipments / Instrument	Model/ Company
1	Digital Weighing Balance	Citizen, Denver Instrument
2	FT-IR	Bruker Alpha E
3	UV Chamber	U- Tech
4	Digital pH meter	Systronic
5	Magnetic Stirrer	Rolex India
6	Heating mantle	Indo-sati 1000ml
7	Zeta Sizer	Malvern, (Nano-s90)

METHOD

5.1. Authentication of species

We have collected the leaves, branch and bud of the species and prepared a herbarium sheet. After that the it was send to the Botanical Survey of India, Eastern Regional Centre, Shillong, Meghalaya for authentication of the species.

5.2. Extraction of oil

Steam distillation method is use for extraction which is the oldest and the easiest method. And the apparatus is known as Clevenger apparatus which consist of distillation flask, heating mantle, condenser, round bottom flask. A portion of peel was weighed in a digital balance and was transferred in a round bottom flask with a large amount of water. The flask was connected to a still column which was connected to a condenser. . Heat is supplied to the distillation unit by temperature controlled heating mentle. The distillate is having two part one is the oil part and one is the solvent part. The oil part remains upward whereas the Solvent part remain at the bottom. As it is a volatile oil it is collected in an efendrof tube. The percentage yield is calculated.

% yield= $\frac{weight\ of\ oil\ extract}{weight\ of\ sample\ used}$×100

5.3. Identification test of essential oil

The different identification test decribed in the BP involve physical, chemical and chromatographic test for essential oil. The physical test involves determination of . The chromatographic test is based on TLC to check the materials purity and composition and to check the presence of impurities. All the test are used to validate the essential oil purity, quality and identity.

Physical c.aracteristic of extracted essential oil

- Physical appearance

The oil was subjected to sensory investigation in order to identify its physical qualities. This required the use of the senses of sight, smell, and touch

- Determination of solubility of the essential oil in water.

In a test tube with a small amount of water, a few drops of the oil were introduced. A stirring rod was used to properly stir the test tube. There were two distinct phases noted. The oil's insolubility in water was deduced from the experiment.

- Determination of density of the oil

The density of the oil extracted was evaluated by measuring and noting the weight of an empty beaker. The essential oil was then put into the beaker, and the weight was recorded. The density of the oil was calculated using equation.

Density = $\frac{weight\ of\ oil\ sample}{volume\ of\ oil\ sample} \times 100$

- Determination of specific gravity

All of the experiments were done in triplicate, and the average values were taken. A weighing balance was used to weigh a clean and dry bottle. The bottle was filled with distilled water and weighed. The same amount of oil was poured into the same bottle and weighed in the same way. The specific gravity is calculated as the ratio of oil to that of water as per the equation

Oil specific gravity= $\frac{weight\ of\ particular\ volume\ of\ oil\ extract}{weight\ of\ equal\ volume\ of\ water} \times 100$

- Moisture content determination

The moisture content determination was calculated by expressing the weight loss upon drying a fraction of the initial weightr of the sample used.

Dry matter%= $\frac{weight\ after\ drying}{weight\ before\ drying}$×100

Chromatographic study:

TLC: Chromatographic conditions for the identification of components of orange peel essential oils are given in Table no. 5 Mobile phase was prepared accordingly in a beaker then was covered with a watch glass. A moistened filter paper in the mobile phase was placed on the inner wall of the chamber to maintain equal humidity (and also thereby avoids edge effect this way) and was left for 10-15 min so that the mobile phase gets saturated. Then over a pre-coated TLC plate a line was drawn 2 cm above its bottom line by pencil to apply the sample spot. Then the sample solution was applied on the spots. The plate was then placed in the TLC chamber so that the side of the plate with the sample line is facing the mobile phase. Then the chamber is closed with a lid. The plate is then immersed, such that the sample spots are well above the level of mobile phase (but not immersed in the solvent) for development. Sufficient time for the development of spots was allowed. Then the plates were removed and allowed to dry. The sample spots were seen in UV light chamber and the length of the spot and movement of the solvent was measured.

Table no 5: Chromatographic conditions

Essential oil	Stationary phase	Mobile phase	Detection
Orange peel oil	Silica gel	Toulene : Ethyl acectate (93: 7)	Spray vanillin - sulphuric acid heat it and examine in a day light

5.4. Drug excipient compatibility study

The physical and chemical interaction between the mixture of oil and excipients was studied using the Fourier transform infrared (FTIR) technology (Model ALPHA-E, Bruker, Germany). By placing the samples on the sampling plate of an FTIR spectrophotometer, the FTIR spectra of the oil, ethylcellulose, PVA, and mixture of oils were obtained.

5.5. Formulation

A topical hydrogel integrating orange peel oil loaded nanosponge was prepared according to their formulas given in the Table no 6. The ratio of ethyl cellulose and polyvinyl alcohol were altered accordingly in the formulation. The stirring rate is also altered respectively. Out of them the best one was selected on basis of their results.

Table no 6: Formulation of topical hydrogel integrating orange peel oil loaded nanosponge

Ingredients	Oil	Ethyl cellulose: polyvinyl aclcohol	Dichloromethane	Distilled water	Stirring rate
F1	200µL	1:1	10ml	10ml	5000
F2	200µL	1:2	10ml	10ml	5000
F3	200µL	1:3	10ml	10ml	5000
F4	200µL	1:1	10ml	10ml	8000
F5	200µL	1:2	10ml	10ml	8000
F6	200µL	1:3	10ml	10ml	8000
F7	200µL	1:1	10ml	10ml	10000
F8	200µL	1:2	10ml	10ml	10000
F9	200µL	1:3	10ml	10ml	10000

5.5.1. Preparation of the nanosponge

Orange peel oil loaded nanosponge were prepared using the emulsion solvent evaporation method. The organic and aqueous phases of this technique are separated. The oil and ethyl cellulose are dissolved in 10ml of dichloromethane in the organic phase, which is the dispersed phase. Required amount of water was then progressively added to a specific amount of poly vinyl alcohol which is the aqueous phase. Then the organic phase was added slowly in the aqueous phase. The mixture was then stirred according to the specific time. The generated nanosponge was filtered and dried for 24 hours at 40°C in an oven. To guarantee that all

remaining solvent was removed from the dried nanosponge, it was placed in a vacuum chamber.

5.6. Particle Size

Particle size of the nanosponge dispersion was determined by light scaterring technique, using Zeta Sizer , Malvern, (Nano-s90) following an earlier described method [5]. At 25°C, aqueous NS dispersion was adequately diluted for scattering intensity. Samples were retained in a disposable cuvette, and measurements were taken for 20 seconds at 372.0 kcps (count rate).

Table no 7: Factors and level used in the applied 3^2 full factorial experimental design

Factor	-1 level	0 level	1 level
Ethycellulose: polyvinyl alcohol	1:1	1:2	1:3
Stirring rate	6000	8000	10000

RESULT AND DISCUSSION

6.1. Authentication of species

Authentication of the species was done by Botanical Survey of India, Eastern Regional Centre, Shillong, Meghalaya. The scientist Dr. N Odyuo confirmed that the plant specimen is *Citrus sinensis.*Pers. (Rutaceae) The certificate is enclosed.

6.2. Extraction of the oil

During the drying process of orange peel certain difficulties occurs like it was prone to microbial growth. So proper drying is needed for the extraction process. Extraction of the oil was done by steam distillation method. Also the clean and dry apparatus should be used during extraction so that the oil doesn't mixed with the other impurities. After the extraction the Percentage yield of essential oil is found to be 4.2%. Which is under acceptable limit as it was reported in literature review (Fekadu et al., 2019).

6.3 Physical appearance of the oil: Visually the oil was observed to be clear liquid and the odor of the oil was fresh orange aroma.

6.4. Solubility: As oil is hydrophobic in nature the orange peel is insoluble in water.

6.5. Density: Because the extracted essential oil has a specific density of less than one, it is lighter than water and thus insoluble in water. The oil's density was calculated to be 0.84 g/cm^3. This matches the usual value of essential oil extracted from orange peel waste.

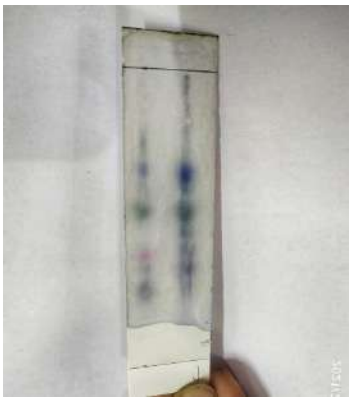
6.6. Specific gravity: The weight of an essential oil is determined by its specific gravity. It's also crucial for identifying essential oil quality and purity. The specific gravity of most essential oils ranges from 0.696 to 1.88 as per the literature. Except for a few oils containing oxygenated aromatic chemicals, most oils have specific gravity values less than one. The specific gravity of the oil was calculated to be 1.04166 and as per the study it is between the ranges.

6.7. Moisture content: As per literature the most serious problem with orange peels is their high moisture content (75–90%), which makes them highly perishable and has a short storage life (de la Torre et al., 2019). Because of the high moisture content the microbial growth may occur easily which leads to degradation of the product that is the orange peel. The moisture content of the peel was found to be 60%, it can be concluded that the moisture content of the peel which is used in the experiment is less than the range given above so, the degradation or microbial growth is less.

Table no 8: Physical properties of essential oil from orange peel

Parameters	Values
Solubility	Insoluble in water
Density	0.84 g/cm^3
Specific gravity	1.04166
Moisture content	60%

6.8. TLC: The TLC was done by using Toluene : Ethyl acetate(93: 7) as a solvent system the Rf value of the orange peel was found to be 0.75.



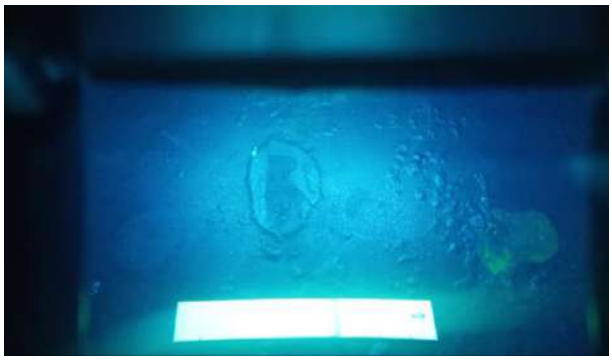


Fig no 9 : TLC plate of orange peel oil

6.9. Data of FTIR

Graph of FTIR is shown in the following figures and table of all the sample given below.

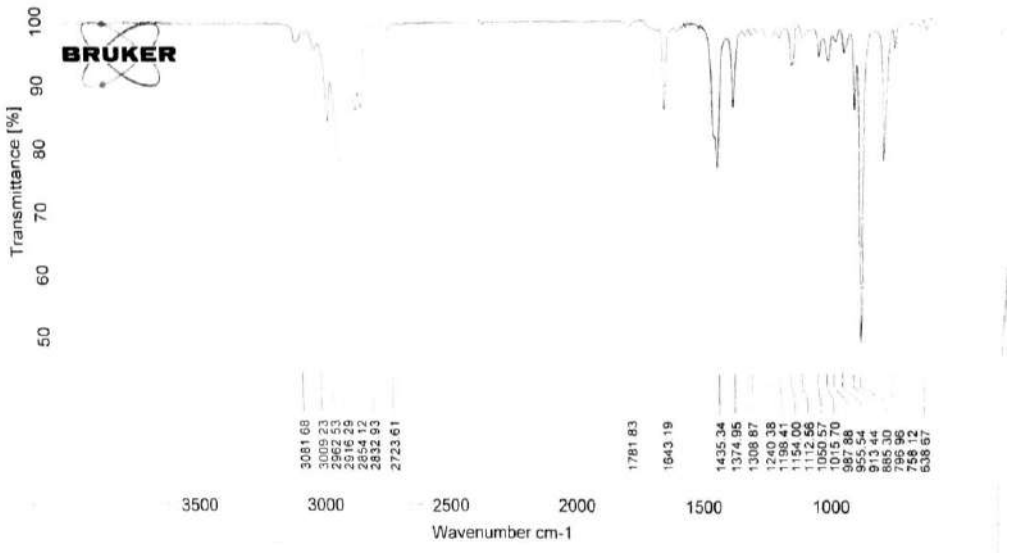


Fig no 10: FTIR of the extract

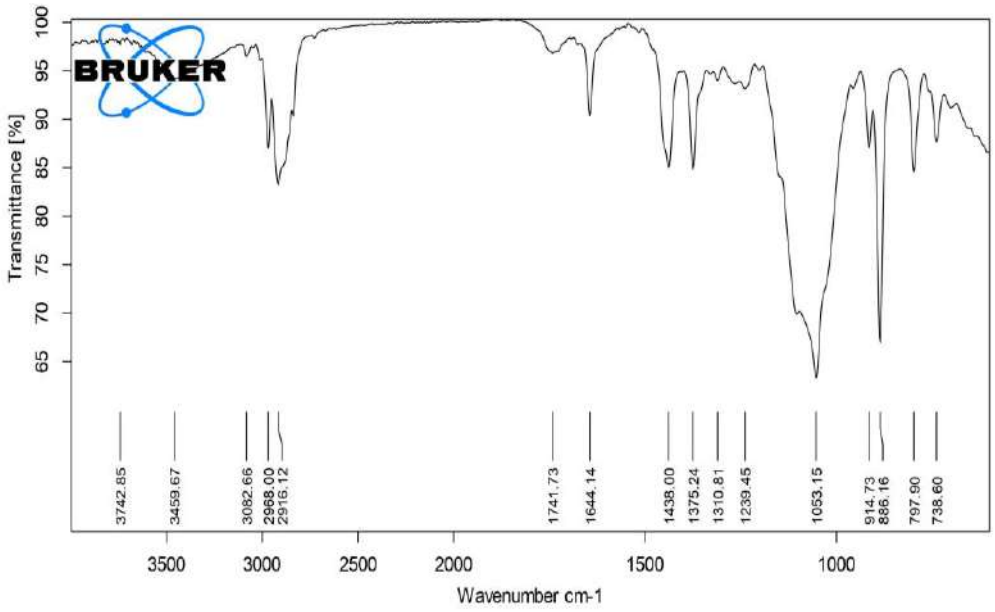


Fig no 11: FTIR of the mixtures

Table no 9: Characteristic peaks of the extract and the mixture of oil with the ingredients

Sl no	Functional Group	Range	Wavenumber (cm^{-1})	
			Extract	Mixture
1	CH bending aromatic	700-850	885.30	738
2	C=C stretching aromatic	1450-1600	1435.34	1438
3	C=C stretching (alkene)	1620-1680	1643.19	1644.14
4	CH stretching alkane	2960-2850	2962.53	2968
5	CH stretching aromatic	3000-3100	3081.68	3082.66

The FT-IR interpretation was done as shown in Table no 9. As the FT-IR study of the orange peel oil and the mixture of ingredient along with the oil we have found that the oil doesn't change its properties when it is interacted with the other excipient. So, the oil is compatible with the ingredients.

6.10. Partical size

Formulation	Particle size nm	PDI Value
F1	987.6	0.381
F2	876.9	1.00
F3	772.0	0.267
F4	539.2	1.00
F5	672.8	0.02
F6	625.7	0.02
F7	455.6	1.0
F8	367.6	0.02
F9	438.2	0.02

Table no 10: Particle size determination of the nanosponge

The particle size of the prepared nanopsonge is presented in Table no 10. The prepared nanosponge analysed by zeta sizer showed particle size varying from 367.6 – 987.6 nm. The average particle size was considerably affected by the drug to polymer ratio. The relatively smaller size is due to low concentration of polymer and also the stirring rate. A nanosponge product with the best sizre should be used for further evaluation.

CONCLUSION

The oil was extracted successfully after which the yield was determined. Physicochemical characterisation of the oil was done. Then the oil was successfully incorporated in the ethyl cellulose nanosponge using solvent evaporation method. 9 formulations were prepared by incorporating the oil in the nanosponge. Then the particle size of the nanosponge were determined by using zeta sizer. From the result we know that the main factor from which the particle size of the nanosponge differ is the ratio between the ethyl cellulose: polyvinyl alcohol and also the stirring rate. We have also seen that after incorporating the oil. the size of the nanosponge differs as compared with the placebo. As orange peel oil is having an antifungal property this could be promising with respect to the practical application of incorporating of the orange peel oil in pharmaceutical formulations with benefit of decreasing the hazards of its use in folk medicine in the crude from. The better retention ability of the nanosponge based hydrogel makes it potentially suitable for the treatment of topical fungal infections which may improve patient compliance.

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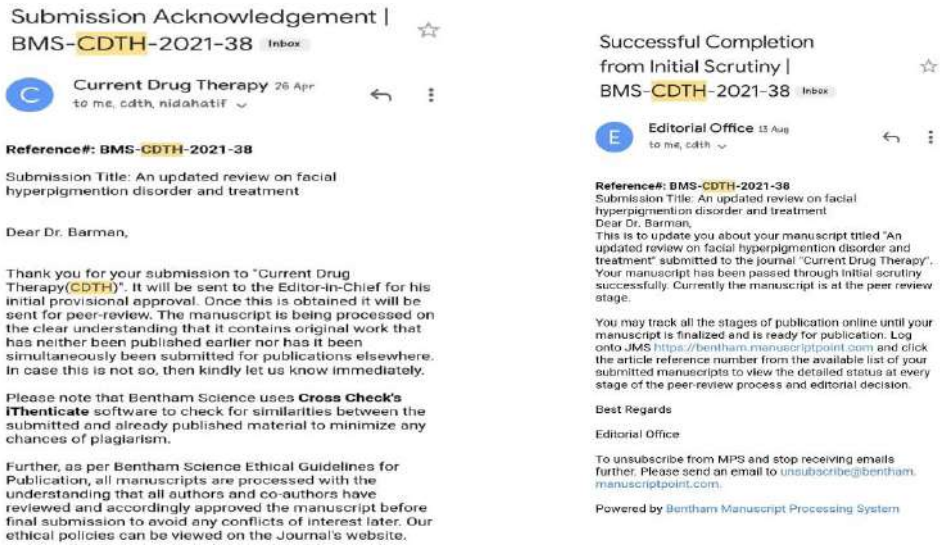
LIST OF PAPER

1. **Review article under process:**

    a. The article **An updated review on facial hyperpigmentation disorder and treatment** is under review process in **Current Drug Therapy** with corresponding author **Kamallochan Barman** and co-author: Bhupen Kalita, Mayuri Phukan, Robina Jidung and Payal dasgupta.



Submission Acknowledgement | BMS-CDTH-2021-38 Inbox

Current Drug Therapy 26 Apr
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Reference#: BMS-CDTH-2021-38

Submission Title: An updated review on facial hyperpigmention disorder and treatment

Dear Dr. Barman,

Thank you for your submission to "Current Drug Therapy(CDTH)". It will be sent to the Editor-in-Chief for his initial provisional approval. Once this is obtained it will be sent for peer-review. The manuscript is being processed on the clear understanding that it contains original work that has neither been published earlier nor has it been simultaneously been submitted for publications elsewhere. In case this is not so, then kindly let us know immediately.

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World Journal of Pharmaceutical Research ISSN 2277-7105
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