ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF PHARMACY

DEPARTMENT OF PHARMACEUTICS AND SOCIAL PHARMACY

PREPARATION, CHARACTERIZATION AND EVALUATION OF EMULGEL FORMULATION OF LOCAL *THYME* ESSENTIAL OIL *(THYME SERRULATUS HOCHST. EX BENTH*) FOR THE TREATMENT OF CANDIDA ALBICAN SKIN INFECTION

BY

ATSEDE SOLOMON (BVSC. PHARMA)

JANUARY, 2023

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By

Atsede Solomon (BVSc. Pharma)

A Thesis Submitted to the Department of Pharmaceutics and Social Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University, in Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmaceutics.

Under the Supervision of Dr. Nisha Mary Joseph, and Mr. Fantahun Molla, Department of Pharmaceutics and Social Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University.

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DEPARTMENT OF PHARMACEUTICS AND SOCIAL PHARMACY

This is to certify that the thesis studied by Atsede Solomon Mebratu, entitled: "Preparation, Characterization and Evaluation of Emulgel Formulation of Local *Thyme* Essential oil (*Thymus Serrulatus Hochst. Ex Benth*) for the treatment of Candida Albican skin infection". submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmaceutics complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Approved and signed by the examining committee:

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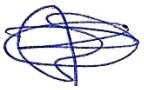
Dr. Dereje Kebede …  …….

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ABSTRACT

The genus *thyme* is an aromatic evergreen shrub with many branches and a woody base found all over the world, but it is thought to have originated in the Mediterranean region of temperate zones. The genus *Thyme* is represented in Ethiopia by two endemic species, *T. serrulatus* and *T. schimperi*. *T. serrulatus* is found in Gondar, Bale, Tigray, Wollo, and Semen Showa. Traditionally, this plant has been used primarily to treat fungal infections. However, no research has been conducted to evaluate the plant's emulgel formulation for the treatment of *candida albican* skin infection. Therefore, the aim of this study was to prepare, characterize and evaluate emulgel formulations of local *thyme* essential oil (*T. serrulatus*) for the treatment of *candida albican* skin infection. The essential oil of T. serrulatus was extracted using a hydro-distillation method with a Clevenger type apparatus. Gas chromatography-mass spectrometry (GC/MS) was used to examine the chemical composition of *T.serrulatus* oil. Seven emulgel formulations with 2% *T.serrulatus* oil, various types and concentrations of emulsifying agents (Span 80, Tween 80), gelling agents (Na CMC and HPMC at 1%, 2%, 3%, and 4% concentrations), and 7.5% virgin olive oil and liquid paraffin as oil phases were prepared. The physical appearance (consistency, homogeneity, phase separation, and texture), pH, spreadability, extrudability, viscosity, swelling index, and drug content uniformity of the prepared formulations were characterized and evaluated. Following the selection of the best formula, it was tested for *in vitro* drug release, *in vitro* antifungal activity against Candida albicans ATCC using the disc diffusion method, skin irritation on rat skin, and three-month stability study. According to the GC/MS analysis, the extracted *thyme serrulatus* essential oil was light yellowish in color and contained 22 compounds. The essential oil's main constituents are thymol (36.92%), O-cymene (26.05%), carvacrol (19.15%), and -terpinine (8.24%). Seven of the prepared emulgel formulations (F3, F6, F7, F10, F11, F14, and F15) demonstrated acceptable physicochemical properties with creamy white to pale white color appearance and excellent homogeneity, consistency, texture, and pleasant odor. Furthermore, no phase separation or aggregates were observed. The pH of the formulations was within an acceptable range for skin. The viscosity, spreadability and extrudability were optimum for the better patient compliance. In both the blank and drug-loaded emulgel formulations, the swelling index was rapidly increased and maintained for 90 minutes.

The drug content uniformity in five emulgel formulations was within the official pharmacopeia limit (F6, F10, F11, F14, and F15) 95%, 90.8%, 98.2%, 101.3%, and 97.5%, respectively. The *thyme serrulatus* oil emulgel drug release was good after 6 hour release time. *Thyme serrulatus* oil emulgel exhibited a strong inhibition zone (36 mm) of antifungal activity against the tested pathogen, a wider zone of inhibition than the marketed miconazol cream (28mm). The result of irritation test showed no edema and erythema. *Thyme serrulatus* oil emulgels maintained their Physico-chemical stability at 30 ±5℃ /65±5 RH and at 5 ±3 ℃ storage conditions. From this, the result of this study demonstrated the potential application of emulgel formulation of *thyme serrulatus* oil with acceptable physico chemical properties for the treatment of candida albicans skin infection.

Key words: *Thyme serrulatus*, topical, emulgel, *in vitro* antifungal activity, skin irritation test, *candida albican*.

ABBREVIATIONS

ATCC	American type culture collection
ATP	Adenosine Triphosphate
CLSI	Clinical and Laboratory Standards Institutes
EPHARM	Ethiopian Pharmaceuticals Manufacturing
EPHI	Ethiopian Public Health Institutes
FTIR	Fourier-Transform Infrared
GC/MS	Gas Chromatography-Mass Spectroscopy
HPMC	HydroxyPropyl Methylcellulose
HLB	Hydrophilic Lipophilic Balance
MRSA	Methicillin-Resistant Staphylococcus Aureus
MIC	Minimum Inhibitory Concentration
NaCMC	Sodium Carboxy Methylcellulose
NIST	National Institute of Standards and Technology
PDII	Primary Dermal Irritation Index
RH	Relative Humidity
RPM	Revolution Per Minutes
SDA	Sabouraud Dextrose Agar
TEA	Triethanol amine
TEO	*Thyme* Essential Oil
USP	United State Pharmacopeia

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1. INTRODUCTION

Skin infections caused by fungi in both humans and animals are known as mycosis. Fungal infections are characterized as opportunistic, pathogenic, and allergic fungi are known to cause a variety of disease, ranging from simple surface mycosis to complicated mycosis (Brown *et al*., 2012). Across the world, over one billion people suffer from fungi infection annually (Vos *et al*., 2010) and it is estimated that fungal infection is responsible for over 1.6 million deaths and predisposing to fatal outcome of many more diseases (Bongomin *et al*., 2017).

Fungi are eukaryotic microorganisms that cause a range of clinical infections including skin, hair, nail, mucosal, subcutaneous, and invasive infections. The infection could be superficial or subcutaneous. The superficial infections are worldwide in their distribution and affect hair, nails and the outer layers of the skin. Dermatophytoses, *ptyriasis* versicolor and cutaneous candidiasis are good examples of superficial infections. Among the most prevalent fungi infection, candidiasis is one of the most endemic infections in all age group. Particularly, it affects immune-compromised individuals and called opportunistic fungal infection (Watts *et al*., 2009).

1.1. Candidiasis and Its Treatment

Candidiasis is a fungal infection caused by yeast that belongs to the genus called *candida*. *Candida* normally lives on the skin and mucosal surfaces of the body, in places such as the mouth, throat, gut, and vagina, without causing any problems. However, some species of *candida* can cause infection in people; the most common is *candida albicans* which is responsible for a wide range of infections from non-life threatening muco-cutaneous illnesses to invasive conditions which may involve severe infection to destruction of vital organs (Khan *et al*., 2010).

The treatment of candidiasis by anti-fungal drugs usually depend on risk factors for patients with infection, immune status of the patient, the anatomic location of the infection, and upon the susceptibility of the *Candida* species (Güng, 2013).

There are various antifungal drugs found to be efficient and available for the treatment of candidiasis, like ketoconazole, miconazol, itraconazole and clotrimazole are used as topical application over the skin by spreading, but cause adverse effects such as itching and allergic reaction (Silva *et al*., 2014). Amphotericin B has served for five decades as a standard treatment

but its usage is limited due to toxic effects, fluconazole is as effective as amphotericin B with superior safety but certain non-albicans candida species are less susceptible to fluconazole. Among the echinocandins, caspofungin attacks the fungal cell wall and maintains its efficacy by retain the activity against isolates with resistance to azoles or polyenes. Furthermore, conventional formulation needs high dose or repeated administration, drug-drug interactions, and an increased risk of both local and systemic toxicity (Gauglitz *et al*., 2012).

In order to address the problem of decreasing availability of medications needed to treat candidiasis, and the development of resistant candida species has led to revival in medicinal plant utilization. In contrast to synthetic pharmaceuticals, they are renewable in nature, have fewer side effects, relatively inexpensive; and more accepted (Motsei *et al*., 2003).

1.2. Medicinal plants

In the previous few decades, the development of herbal medicine delivery systems has received a lot of attention. Nowadays, the use of herbal medicine has increased remarkably in the world and used to managing dermatological conditions and variety of infectious diseases with better therapeutic effects and less toxic/adverse effects. Natural products, either as pure compounds or as standardized plant extracts, provide good opportunities for the discovery of new drugs (Sasidharan *et al*., 2011). As reported by Cristiane *et al*., (2008) the genus *cymbopogon* is used as antifungal agents for skin diseases of cutaneous candidiasis and dermatomycosis. The genus eucalyptus also possesses a wide range of biological activity including antibacterial, fungicidal, insecticidal and herbicidal properties (Batish *et al*., 2008).

Eighty percent of the Ethiopian population use medicinal plants for more than century to fulfill their essential drug needs in different ways of dosage formulation such as topical and oral formulations and also they are inherent to the people's cultural ideas and attitudes (Beyene *et al*., 2016). Moreover, medicinal plants remain the most crucial or sometimes the only source of therapeutics by playing a key role in the increase and progress of modern studies by being a base for the development of novelties in drugs.

Most of the herbal medicines are believed to be safer, easily available, cheaper, time tested and effective than most of modern synthetic drugs, because of their long history of use in the treatment of different human diseases. Based on the WHO report, 80% of the world population depends on medicinal plants for their basic healthcare (Popović *et al*., 2016).

1.3. Essential oils and their Applications

Aromatic plants, particularly their essential oils and combinations of natural compounds, are among the herbs that have been used traditionally to cure bacterial and fungal infections. Essential oils are composed of lipophilic and highly volatile secondary plant metabolites. They are often created in a specific cell or set of cells that can be found in numerous leaves, flowers, stems, and roots. It is either concentrated or stored in a specific area of the plant or in several organs within the same plant (Hammer *et al*., 1999). At present, approximately 3000 essential oils are known. About 300 of them are used financially in the advertising of pharmaceuticals, spices and perfumes (Dahanukar *et al*., 2000).

Medicinal values (Antimicrobial properties) have been reported more frequently in a wide range of plant extracts such as essential oils in an attempt to discover new chemical classes that could resolve strains expressing resistance (Doughari, 2012). These properties are the result of their chemical composition as different essential oils are enriched in different phytochemicals such as the presence of small terpenoid and phenolic compounds (phenolic acids, polyphenols and flavonoids).

Essential oils may exhibit nearly similar modes of action against for both bacterial and fungal strains most of them considered as the damage of the cytoplasmic membrane, loss of energy substrate, inhibition of production of amylase and protease, direct lysis of bacteria and leakage of ions (Kotzekidou *et al*., 2008). For instance, the hydrophobicity of essential oils and their constituents is a crucial quality that allows them to partition into the lipids of the bacterial cell membrane, disrupting the structure of the cell, making it more permeable, and ultimately causing cell death (Bouyahya *et al*., 2019).

Essential oils are usually extracted as secondary metabolites. They are often collected in secretory organs, secretory ducts and/or lumens (Preedy, 2015). The essential oils contained in the plant cells are released from different parts of the plant material by heat and pressure; Leaves, flowers, fruits, grass, roots, wood, bark, and blossoms. The techniques used depend on the part of the plants from which the oil is to be extracted, the stability of the oil to heat and the sensitivity of the components of the oil to chemical reactions (Surburg and Panten, 2016).

Extraction of essential oils from plant material can be performed by different methods these are delegated traditional and imaginative which centered on the improvement of selectivity in extraction effectiveness by decreasing extraction time, decreasing volatility, utilization of vitality, dissolvable, and CO2 emissions. Of these, hydro-distillation- Clevenger apparatus system (Figure 1), steam and steam/water distillation are the most common methods of extraction (El Asbahani *et al*., 2015). Steam /water distillation is one of the more sophisticated processes for obtaining essential oils from plant sources formed by small, unstable, and really hydrophobic particles (Bakkali *et al*., 2008).

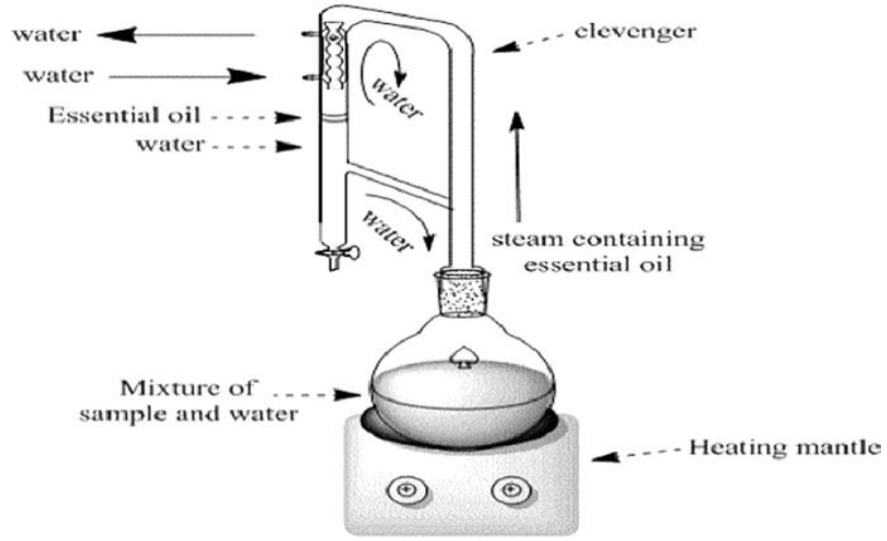


Figure 1: Hydro-distillation-Clevenger apparatus (Samadi *et al*., 2017)

The application of essential oils depends on a great degree on plant source, extraction method and quality of oil. Essential oils have modern uses in beautifying agents, fabricating of fragrances, soaps, shampoos, cleaning gels, toilet products, pharmaceuticals, soft drinks and distilled alcoholic beverages. The other interesting part of these oils is their potential as medicines in aroma based therapies and as a vehicle for drug delivery (Baser and Buchbauer, 2009). Essential oils and their individual aroma components have a suppressive activity when tested on a number of human cancer cells lines including Glioma, breast cancer, leukemia and others. Glioma is one of the most malignant human tumors (Fitsiou and Pappa, 2019). Intravenous administration of essential oil of basil (Ocimumgratissium) induced an immediate and significant hypotension and bradycardia (Lahlou *et al*., 2004).

Essential oils are used in part of pharmaceutics; for their potential application as therapeutic agents (Lawless, 2013). This is particularly the essential oils from peppermint, sage, thymus, eucalyptus, clove, and tea tree. These oils are utilized as antibacterial agents (peppermint, sage, thymus, clove and tea tree oil), as a decongestant of the respiratory tract (peppermint essential oil), as an expectorant for treating bronchitis and cough (eucalyptus essential oil) and as a carminative effect (anise essential oil). Furthermore, clove oil is used in dentistry for its antimicrobial and analgesic properties, while tea tree oil is used in the field of dermatology (as an acne treatment) because it has antimicrobial characteristics against Gram-positive microbes (Baser and Buchbauer, 2009).

Aromatherapy and improving the sensory properties of pharmaceutical medications are the two main applications of essential oils in pharmaceutics. There are various methods for administering essential oils derived from various plant sources (Koroch *et al*., 2007). The most popular method is to apply these oils locally along with some carrier oils after diluted them to a certain concentration. After adding a few drops, they can also be inhaled in the steaming water using an air humidifier (Boehm *et al*., 2012). They can also be used as infusions of extracts in the form of tea, which is considered to be more precise in dosage while avoiding adverse effects. However, it is conceivable that the harmfulness of essential oils could be higher with this method (Karlsen, 2010).

The antibacterial attributes of *thyme* essential oils have used to be for the most part due to carvacrol and thymol, which are the phenolic fractions of the oil. Carvacrol and thymol are structurally alike with a minor difference, which is the attachment of hydroxyl group to phenolic ring in thymol. The two substances seem to alter the cell membrane integrity (Lambert *et al*., 2001). Carvacrol and thymol can break down the gram negative and gram-positive microbe's outer envelope, leading to diffusion of lipopolysaccharides and alleviating the porousness of cytoplasmic layer to Adenosine Triphosphate (ATP). This mechanism was shown to be unaltered by the presence of magnesium chloride ions (Helander *et al*., 1998).

1.4. The genus thymus

The lamiaceae is the most well-known medicinal plant family that contains about 83 genera and 860 known species (Braidy *et al*., 2017). This family is much diversity in terms of ethno-medicine. It has diverse chemical composition like flavonoids, phenolic acids, terpene, saponins, polyphenols, tannins, iridoids, and Quinone (Özgen *et al*., 2011).

The genus *thymus* is one of the most taxonomically complexes genera in the lamiaceae family for its different numerous species and varieties. It comprises about 350 species worldwide and is primarily native to the Mediterranean region of the temperate regions. *Thyme*, a name taken from a Greek word, refers to courage and sacrifice. *Thyme* species are known as medicinal plants due to their biological and pharmacological properties which are also abundant in essential oils (Özgen *et al*., 2011).

In global scenes, *thyme* is distributed in almost everywhere, as an aromatic for culinary uses which is native to southern Europe such as, south France, Spain, and North America, while *thyme* is uncommon in tropical part of Africa (Stahl-Biskup, 2002). The common *thyme* found in European, *T. vulgaris* is widely grown around the globe for culinary, cosmetic, and medicinal uses. It has antibacterial properties, which are mostly attributable to the high concentration of phenolic components including thymol and carvacrol. In Ethiopia, the genus *Thyme* is recognized by two endemic species, *T .serrulatus* and *T. schimperi* (Damtie and Mekonnen, 2015) both of which are locally named 'Tosign' (Amharic), 'Tesni/Thasne' (Tigrigna) and Ethiopian *thyme* (English).

The volatile oils and flavonoid constituent have been attributed to the essential oil from the leaves of *T. schimperi* and *T. Serrulatus*, which has antibacterial, antifungal, and anthelmintic properties. They are also used to treat gonorrhea, liver disease, a cough, a stomachache, an earache, and a headache. Additionally, they can be used as flavors in tea, coffee, and various stews (Asfaw *et al*., 2000).

Thyme Serrulatus is a densely branched perennial subshrub that grows 5 to 40 cm tall, with pink corollas in its dense inflorescence, and has oblong to elliptic leaves with whole sides (Figure 2). *T. Serrulatus* is endemic to Ethiopia and widely distributed in Gondar, Bale, Tigray, Wollo and in the highlands of Semen Shoa from 2000 to 4000 meters above sea level (Dagne *et al*., 1998).

Photograph by Atsede Solomon. **Figure 2:** *Thyme* leaves

The volatile oil from *thyme* contains *p*-cymene, thymol, γ-terpine, carvacrol, rosmarinic acid, and eugenol. The essential oil of *thyme* is dominated primarily by thymol and carvacrol (Figure 3). These phytoconstituents are present in various proportions and characterized by a large amount of monoterpenes which accounts for 90% of the oil. Thymol and carvacrol occur more commonly and accompanied by the couple c-terpinine/p-cymene, the four monoterpenes being biogenetically closely correlated. *T. serrulatus* is belonging to the thymol-carvacrol chemo types (Debelo *et al*., 2015). Both the chemical composition and the isolation yield of *thyme* oils depend on a number of factors, such as the environment or region in which they are grown, the practice in cultivation, the development stage of the plant, harvesting time and habitat (Casiglia *et al*., 2019). The essential oils from *thyme vulgaris* were discovered as having high amount of oxygenated monoterpenes (56.53%) and less contents of mono-terpene hydrocarbons (28.69%), sesquiterpene hydrocarbons (5.04%) and oxygenated sesquiterpenes (1.84%). The most predominant compound among the essential oil components was thymol (51.34%) while the amount of all other constituents of the oil was less than 19%.

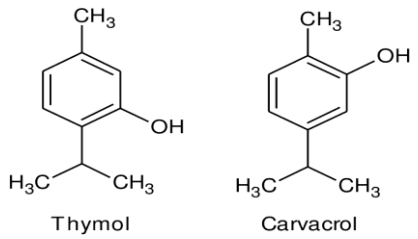


Figure 3: Chemical structure of thymol and xarvacrol (Safwan *et al*., 2012)

Sub-acute toxicity study of *T. serrulatus* on mice did not cause any major organ damage (liver and kidney) and rise in serum enzyme level as compared to the control group. In addition, the ointment prepared from essential oil of *T. serrulatus* did not show any abnormal skin irritation up on follow up for 14 days (Dires *et al*., 2018). Other report by Damtie and Mekonnen, (2015) on intra gastric uptake of extracts of n-butanol fraction of the aqueous crude extract and crude methanol leaf extract of *T. Serrulatus* with the oral limit at doses 300, 2,000, 5,000, and 10,000 mg/kg did not produce any sign of morbidity and mortality in female mice during the acute toxicity study time period and showed that the LD50 of the aqueous leaf extracts of *T. Serrulatus* were above 10,000 mg/kg.

Thyme serrulatus crude extract has diuretic activity. For instance, the lowest and highest urine excretions are measured at 125 mg/kg and 500 mg/kg with diuretic indices of 1.13 and 1.60, respectively (Abreham *et al*., 2015). In the other studies an increased in the urine volume at the dose of 250 mg/kg and 1,000 mg/kg *T. serrulatus* which is the lowest and highest urine yield, respectively, has been documented (Melka *et al*., 2016). In both studies the result was comparable to the reference medicine, hydrochlorothiazide.

The *in vitro* vasodilator effect of *T. serrulatus* aqueous leaf extract on guinea pig thoracic aorta, with successive administrations of relatively low to high concentrations of *T. serrulatus* from 0.5 to 5 mg/mL to the organ bath fluid was attenuated the force of contractions on guinea pig thoracic aorta that were provoked by potassium chloride in concentration dependent manner both in intact and denuded endothelium. And also the percent of aorta relaxation of *T. serrulatus* aqueous leaf extract exhibited a significant difference (*P*-value) between intact and denuded endothelium of the guinea pigs' thoracic aorta (Geleta *et al*., 2015).

Ethno -botanical study and knowledge of medicinal plants would contribute to improved human health on a local and/or a global level (Haile *et al*., 2008). The oil of *T. serrulatus* are responsible for antifungal activity against different species of fungus and antibacterial activity due to richer in medicinally important constituents such as carvacrol (66.2%) and thymol (50%) (Hailemariam and Emire, 2013) which was similar with pharmacologic activity of oils of other *thyme* species for example *T. hirtus, T. kotschyanusboiss, T.vulgaris, T. mastichina, T. serpyllum* and *T. algeriensis* species (Ayesh *et al*., 2014). Activity against microorganisms has been widely published against staphylococcus aureus, pseudomonas aeruginosa, salmonella typhimurium, escherichia coli, klebsiella pneumoniae, enterococcus faecalis, and candida albicans (Hossain *et al*., 2013). Its dried leaves are used to produce tea and are suggested as a treatment for liver disease, high blood pressure, general pain syndrome, gonorrhea, thrombosis, urinary retention, and respiratory issues (Dawit and Ahadu, 1993). *Thyme* is made as an infusion for laryngitis, spasmodic cough, mouthwash, and external use on wounds. (Damtie *et al*., 2019). It is also used as a decongestant, as a cholagoge, to reduce flatulence and to fight parasites. Antitussive, expectorant and antispasmodic actions are considered to be the major pharmacological properties of *thyme* and have been associated with the volatile oils and flavonoid constituents (Borugă *et al*., 2014). The screened phytochemical constituents such as, the presence of anthraquinones, phenolic compounds, cardiac glycoside and steroids could be attributed to antimicrobial activities of *T. serrulatus*. Topical antimicrobial therapy is the most important elements of wound care infections. Essential oils are also among the most popular natural products for the application of dermatological infections (Bajpai *et al*., 2009).

1.5. Emulgel

The drug delivery system is more advantageous in delivering the herbal drug at predetermined rate. A various drug delivery systems such as Emulgel, Niosomes, Liposomes, and Microspheres etc. have been formulated for the delivery of herbal drugs. The advantages of developing a novel drug delivery technology to plant actives can help in minimizing presystemic metabolism, degradation of drug in the gastrointestinal tract, accumulation of drug in the non-targeted tissues and/or organs, and decreased side effects. Hence, will have better the therapeutic efficacy and predominantly minimizes the toxic effects with increase in bioavailability of drugs (Saraf, 2010). Over all this avoid, the problem of delivery of drug at the below therapeutic concentration in the blood resulting in less or no therapeutic effect.

Varieties of medicinal items are used on the skin or mucous membrane that either improves their health, restores a fundamental function of the skin, or pharmacologically changes an action in the underlined tissues. These products are referred as topical or dermatological products (Bensouilah and Buck, 2006). Many widely used topical agents like ointments, creams and lotions have many disadvantages such as sticky in nature, feeling of discomfort in the patient when used and they have lesser spreading coefficient so applied by rubbing and they also exhibit stability problem (Rathod and Mehta, 2015). These and other factors within the main category of semisolid preparations have led to an increase in the usage of translucent gels in both medicinal and cosmetic preparations. Gels have several advantages, but one significant downside is the delivery of hydrophobic medications. This restriction is being solved by adopting an emulsion-based strategy, which enables even a hydrophobic drug component to be incorporated and successfully delivered using gels. Emulgels are the name given to the dosage forms created by combining gels with emulsions (Tanaji, 2018). Emulgels are basically emulsions, which are water- in oil or oil-in- water type that are gelled in which gelling agent is used for mixing as shown in (Figure 4).

For hydrophobic or poorly water soluble medications emulsified gels is better formulation because they are more stable. They have the benefits of gels and emulsions. Emulgel improved stratum cornum drug absorption because of this; they are utilized to deliver various medications into the skin. They exhibit greater hydrophobic medication release than conventional gel formulations (Rehman and Zulfakar, 2014). Different gelling agents are available on market with

such emulsion stabilizing characteristics, such as carboxymethyl cellulose sodium, guar gum, hydroxyl propyl cellulose, Hydroxylpropylmethyl cellulose, and sodium alginate (Khullar *et al*., 2011).

The structure of gels which are tied together by van der Waals forces to form amorphous and crystalline regions with in the system, form interlacing three dimensional networks that restricting the movement of dispersion medium. This makes an emulgel dosage form as an attractive part for topical drug administration where systemic absorption of drugs is not desired (Yadav *et al*., 2017).

Emulgels are used to reduce the side effects of medicinal ingredients and make the formulation more bearable. Different vegetable oils with emollient qualities have been employed as the oil phase to reduce the significant skin dryness and irritation brought on by some anti-acne and other medications, which can occasionally force the patient to stop the treatment (Thakur *et al*., 2012). These emulgel are having major advantages on novel vesicular systems as well as on conventional systems in various aspects. Emulgel for dermatological use have several favorable properties such as being easily spreadable, thixotropic, greaseless, easily removable, emollient, non-staining, long shelf life, bio-friendly, transparent & pleasing appearance (Kute and Saudagar, 2013).

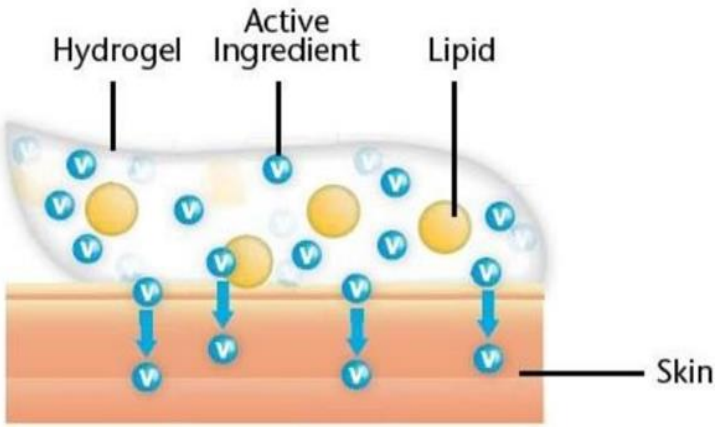


Figure 4: The structure of emulgel (Nikitha *et al*., 2021**)**

1.6. Formulation of emulgels

Emulgel formulation consists of quick, easy stages that boost manufacturing viability. The creation of emulgels does not require any specialist equipment. Furthermore, materials used are easy to be found and reasonably-priced. The initial step in the emulgel preparation process is make O/W or W/O type emulsions. In the prepared emulsion the drug is incorporated. In the second step, the gel base is formulated. In the third step, the prepared emulsion is incorporated into the gel matrix with a continuous stirring to make an emulgel (Figure 5) (Charyulu *et al*., 2021).

Emulsion are unstable biphasic liquid dosage forms consisting of two immiscible liquids in which one of the liquid is dispersed in the other one with the addition of a third agent, known as an emulsifying agent. Emulsifying agents are help to stabilize the globules in one another. Emulsions could be of either O/W or W/O. These emulsions are used as vehicles for the medicines (Sharma *et al*., 2014).

A physical state condition of gel exhibits features that are broadly in between those of solids and liquids properties. Gels are constituted of a polymer that swells when exposed to liquid. Gels are fragile and wet and look like a solid material and show a broad bending from solid to liquid state (Jain *et al*., 2011). Determining systems that are non-irritating, non-toxic, non-comedogenic, and non-sensitizing are difficult when making topical emulgels. Additionally, the formulation of the emulgel must be cosmetically appealing elegant and physiologically compatible (Vikas *et al*., 2011).

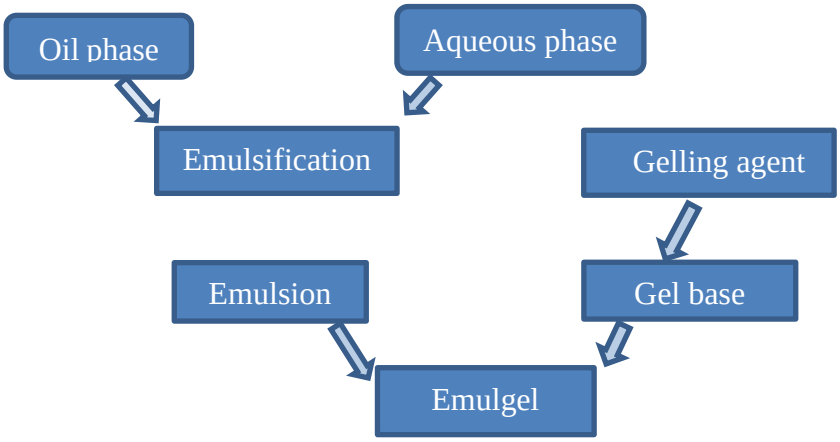


Figure 5: Flow chart for processes of emulgel formulation

1.6.1. Factors contribute in formulation of emulgel

Gels are made using gelling agents. These can be used as a thickening agent and to improve the consistency of any dose form. The characteristics of the external phase are typically taken into account when choosing a polymer to create gel. Additionally, the capacity of the polymer to achieve its intended release and to generate an efficient and stable dosage form depends on its ability to fulfill the acceptable physicochemical requirements (Baboota *et al*., 2011).

Drug release through the gel matrix is influenced by the type and concentration of the gelling agent in an inverse relationship with the drug release. In O/W emulsions, the addition of a highly hydrophilic polymeric increases the viscosity of the external aqueous phase, delaying the likelihood of collisions and the subsequent merging of the dispersed internal phase globules. (Scomoroscenco *et al*., 2021).

Polymers are important in a variety of topical gel characterization parameters, such as *in vitro* drug release study and viscosity and physicochemical properties. The use of a suitable gelling ingredient, usually a polymer, is required for the formulation of an efficient gel. The preferred characteristics of such polymer include inertness, safety, and biocompatibility with other ingredients, permission of drug permeation while not being absorbed into the body, and irritation-free (Gupta *et al*., 2002).

Emulsifier is used to promote emulsification throughout the manufacturing process as well as to maintain the drug's stability over time. An emulsifier or combination of emulsifiers is needed to stabilize droplets of the internal phase (Pinnamaneni *et al*., 2003). An emulsifying agent works with lowering of the interfacial tension of the two phases and decreasing the coalescence of the dispersed droplets. The emulsifying agent type and concentration and amount of external phase have the most significant impact on the drug release from the emulgel, followed by the oil phase concentration, which has a retarding effect. For example, polyethylene glycol 40 stearate, sorbitan mono-oleate (Span 80), polyoxy ethylene sorbitanmonooleate (Tween 80), stearic acid, and sodium stearate are some of the emulsifiers used in emulgel preparations (Chime *et al*., 2014).

Selection of emulsifier is done by proper hydrophilic and lipophilic balance (HLB). For example, a surfactant with HLB value greater than 8 is used in oil in water emulsion, whereas surfactant

with HLB value less than 8 are used in making of water in oil emulsion. Tweens are used for emulsification in the water phase, while spans are used in the oil phase (Hong *et al*., 2018).

Emulsion preparation involves the use of oils. These substances create the emulsion's oily phases. Most oil phases are mineral oils including paraffin (soft or hard) and petrolatum. Fixed oils such as almond oil, olive oil, coconut, sunflower oil and clove oil, which are widely used as the vehicle for the drug, for moisturizing skin, treat dandruff, relieving constipation and sensory characteristics. It must also be realized that the type and concentration of oil used may have an effect on the stability of the final product (Sonam *et al*., 2014). Many emulsions for topical use contain oils that are present as carriers for the active ingredient. Therefore the oily phase preferably has to be a good solvent for the active ingredient which also ensures a large drug loading capacity of the formulation (Talegaonkar *et al*., 2008).

An essential factor in choosing oils is the drug's solubility in the oil phase. This is crucial because the medication's solubility in the oil phase has a significant impact on an emulsion's capacity to sustain the drug in solubilized form in aqueous conditions (Lu and Gao, 2010). There may be a risk of precipitation if other formulation ingredients play a significant role in the solubilization of the medicine since the solvent capacity of these mediators will be reduced as the emulsion is diluted in aqueous media. Therefore, in order to build stable and suitable low-volume emulsion systems for drug delivery applications, it is crucial to understand the parameters that affect drug loading capacity (Shafiq *et al*., 2007).

1.7. The present study

Skin diseases are the most prevalent form of disorders in all age groups of people. Among the most common diseases of skin, superficial and subcutaneous mycoses affect millions of people around the world. The fungi tend to grow in moist parts of the body where the skin comes together such as between fingers, toes, corners of the mouth, skin folds, anus, nail, and scalp and in the genital area (Muyima and Nkata, 2005). Candidiasis is one of a fungal infection caused by yeasts that belong to the genus *candida* which are frequent colonizers of the skin and mucosal surfaces of our body, including the genitourinary tract, oral cavity, and gastrointestinal tract. *Candida albicans* is the most prevalent and cause of a variety of illnesses, ranging from more superficial and minor clinical signs (Havlickova *et al*., 2008).

The liquid, solid and semi-solids pharmaceutical dosage forms are preferred for topical treatment for external or systemic impact. Creams, paste, ointments and other preparations show patient incompliance due to several limitations such as difficulty to remove from the skin after application, greasy characteristics, staining behavior on the skin and state of stability. Topical gels are becoming more and more popular due to their simplicity of use and improved percutaneous absorption. They have an external solvent phase which is hydrophobic or hydrophilic character. However, it is very challenging to incorporate hydrophobic drug due to the difficulty to insert into gel because of high polarity difference between drug and gel base. To overcome these limitations of conventional dosage form, a novel formulation of Emulgel has been suggested which combine the properties of both gel and emulsion. Obviously, the presence of a gelling ingredient in the aqueous phase converts a conventional emulsion into an emulgel. Emulsion is one of the techniques to increase the solubility of hydrophobic drug (Gupta *et al*., 2002).

The antimicrobial properties of many plant extracts such as essential oils have been studied. For instance, studies on *T. schimperi* have shown antibacterial, antifungal and anthelmintic activity (Mekonnen *et al*., 2016). In Ethiopia, there are documentations of *T. schimperi* and *T. serrulatus* traditionally used as food spices, cures for various illnesses, and food preservatives. In various regions of the nation, dried or fresh *thyme* leaves are also used to flavor tea, add flavor to chili powder or “berbere” and make “shirro” (Meresa *et al*., 2017).

The composition and concentration of the local *thyme* species is different from foreign species in major chemical components such as thymol, caracrol, y-terpinine, and p-cymene and the local *thyme* can be easily accessed in different parts of Ethiopia. Therefore, extraction of essential oil of *thyme* will be economically feasible as well as the nature of *thyme* is safe.

With regard to cost, the *thyme* essential oil is cheaper compared to the commercial modern synthetic topical formulations. As a result, it will save foreign currency for importing of topical formulations in the country. In addition, the oily nature of the *thyme* is suitable to apply on the skin and it can be easily absorbed through the skin due to its penetration enhancer property. Therefore, it could be a promising alternative herbal treatment for *candida albicans*. Furthermore, the *T. serrulatus* essential oil emulgel dosage formulation will be another optional approach for drug delivery.

Drugs containing imidazole, such as clotrimazole, econazole, ketoconazole, miconazol, and oxiconazole, are available over the counter. Their mode of action reduces ergosterol synthesis by inhibiting fungus cytochrome P450 enzymes. They exhibit a lower level of selectivity, nonetheless, due to their greater affinity for fungus than for human cytochrome P450 enzymes. This disadvantage explains why they have a higher risk of drug interactions and side effects, which can include mild digestive discomfort, abnormal liver enzymes, and very infrequently clinical hepatitis. However, there is still the need for alternate dosage forms and drug which can be effective with lower side effects. So far, no work has been reported on the formulation of *thyme* oil emulgel for skin candidiasis (British pharmacopia, 2018). Numerous studies have revealed the antifungal, antibacterial, antioxidant, and preservation properties of the indigenous *thymus* plant. Its efficacy on *candida albican* in base formulations (paste and ointment) and direct use of oil extracts, which has a zone of inhibition of 28 mm as compared to Methicillin-resistant Staphylococcus aureus, which showed a zone of inhibition of 22 mm (Mekonnen *et al*., 2016; Pina *et al*., 2004; El-Shahran *et al*., 2017).

However, the development of topical emulgel formulations and its evaluation from essential oils of local *T. serrulatus* has not been investigated in Ethiopia. Thus, the aim of the present study was to fill this gap. Therefore, the preparation, characterization and evaluation of emulgel formulations of endemic *Thyme serrulatus* essential oil 2% v/v for the treatment of candida albicans skin infection were studied.

2. OBJECTIVES

2.1. General Objective

- To prepare, characterize and evaluate of emulgel formulations of local *Thyme* essential oil *(T. serrulatus Hochst. Ex Benth*) for the treatment of *Candida Albican* skin infection.

2.2. Specific Objectives

- To extract essential oil from local *thyme* species.
- To formulate emulgels containing indigenous *T. serrulatus* essential oil.
- To evaluate and characterize the physico-chemical properties of emulgel formulations.
- To compare the physical characteristics of the *T. serrulatus* essential oil emulgel formulations with marketed antifungal topical cream.
- To determine the in-*vitro* drug release of the best formulations.
- To evaluate the antifungal activity of the *thyme* essential oil formulation.
- To evaluate skin irritation effect of the best formulation.
- To evaluate the stability of the best formulated emulgels.

3. MATERIAL AND METHODS

3.1. Materials

Fresh leaves of local *Thyme* (T. serrulatus) (Gondar, Debark at limalimo Village); Sodium carboxy methyl Cellulose (NaCMC) (Hengshui Taocheng Chemicals Auxiliary Co., Ltd. China), Hydroxylpropylmethyl Cellulose (Shandong Head Co., Ltd. China), Polysorbate (Tween 80 and Span 80) (Alpha chemical Plc., India); Liquid paraffin (Dallul Pharmaceuticals PLC), Virgin Olive oil (Angel Camacho Alimentacion, Spain); Sodium sulphate (Uni-Chem®chemical reagents, India), Formaldehyde (8%) and Cellulose Acetate Membrane (Sartorius, Gottingen, Germany) were purchased from local market and used as received. Miconazol Topical cream (Galentic Pharma Pvt. Ltd, India) were purchased from local pharmacy and used as received. Albino Wister rats were received as a gift from the Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, Addis Ababa University. Aluminum Collapsible Tube, Propylene glycol, Methyl Paraben and Propyl Paraben was received as a generous gift from Ethiopian Pharmaceuticals Manufacturing Sh. Co. (EPHARM). Sabouraud Dextrose Agar (SDA) was received as a gift from University of Gondar, Microbiology Department; fungal strain (Candida Albicans ATCC) was obtained as a gift from Ethiopian Public Health Institutes (EPHI). All other chemicals used were of analytical grade, such as Monobasic Potassium Phosphate (Fisher Scientific LTD., UK), Sodium Hydroxide (Carlo Erba Reagents, Italy), ethanol (Carlo Erba Reagents, Italy).

3.2. METHODS

3.2.1. Plant collection and identification

The fresh leaves of local *Thyme species* were collected from North Gondar (Debark town, limalimo Village) by the sample code (*thyme* L1) (Figure 6) and the identity of the plant material was confirmed by a botanist in the Department of Biology, College of Natural Sciences, University of Gondar, Ethiopia.

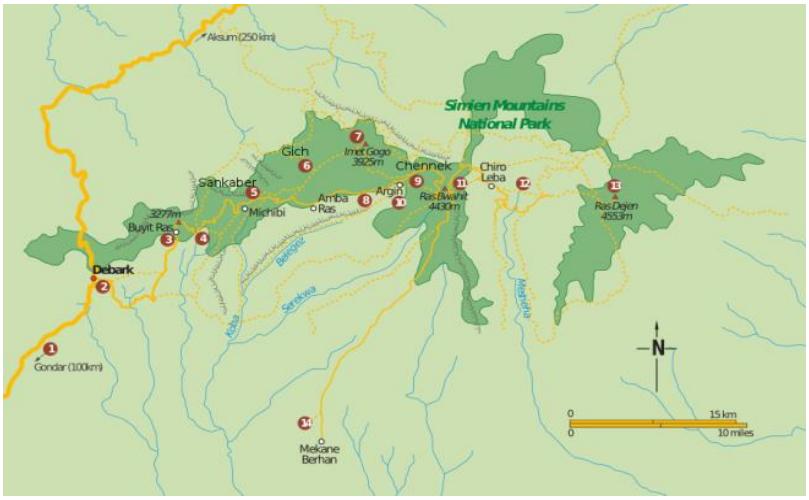


Figure 6: Geographical location for thyme plant in Gondar, debark

3.2.2. Extraction of essential oil

Freshly collected local *thyme* plant was washed with tap water to remove debris and cut into smaller pieces using the knife. From the cut *thyme* pieces, 5 kg of was weighed (ADAMS=160L, AAA analytical digital weighing balance) and transferred into a round-bottomed flask containing 3000 ml of distilled water. Then, it was subjected to hydro-distillation using a Clevenger-type apparatus and heated using a heating mantle to boil the water at 100℃ for 3 hours (European Pharmacopoeia, 1996). After boiling, the essential oil, along with the water vapor, was passed through a condenser and the oil was accumulated in a graduated side arm of the Clevenger

apparatus. The essential oil was clearly separated from water and then the water was removed manually and the oil left in the Clevenger- apparatus was drained out carefully. Then, the volume of each of the essential oil was measured in milliliters (mL) using a measuring cylinder. The percentage yield of *thyme* essential oil was calculated based on the weight of fresh leaves and finally, the essential oil was collected and kept at 4℃ in the refrigerator in a clean, dark brown amber bottle before analysis and formulation (Abreham *et al*., 2015).

3.2.3. Essential oil analysis

After three days of storage, The extracted oil of T. serrulatus was analyzed by the Perkin Elmer gas chromatograph (Clarus 600) coupled with a Perkin Elmer (Clarus 600 T) mass selective detector. Subsequently, 1 μL of the aliquots of the extracts were injected into Elite 5-MS capillary column (30m × 250 μm I. D, 0.25 μm film thickness; Perkin Elmer, Shelton, CT, United States) in the split less mode of 1:20. The column temperature was kept at 40°C during sample injection and was raised to 150°C for 2 min having rate of 10°C/min and further increased to 300°C held for 2 min. The injector temperature was at 280°C, inlet line temperature at 220°C, and source temperature was 220°C. The total run time was 32 min. Helium was used as the carrier gas at a flow rate of 1.0 ml per min. Detection was carried out by using MS detection in electron-ionization mode using a single quadruple detector and full-scan monitoring mode (m/z 40–600).The result of the analysis was recorded based on the essential oil mass spectra with multiple mass spectral libraries included in National Institute of Standards and Technology (NIST, 2005), retention time included in (Wiley, 2006) and component was confirmed by comparing their retention indices by using (Chemstation data system) with those from the literatures (Tirillini *et al*., 2008 ; Adams, 2007).

3.2.4. Determination of λ Max of *Thyme* oil

To determine the maximum absorption (λ max) of indigenous *T. serrulatus* essential oil, UV/Visible Spectrophotometer (SOLAR CM2203, Minsk, Belarus) was used, phosphate buffer solution (pH 5.5) was used as a solvent over wavelengths ranging from 200-400nm and further used to plotting of calibration curve and drug release of the *T. Serrulatus* essential oil from the formulation (Sundari *et al*., 2014).

3.2.5. Construction of calibration curve for *Thyme* essential oil

Initially stock solution of 0.5mg/ml was prepared in phosphate buffer of pH 5.5. From the stock solution, five different concentrations (25µg/ml, 50µg/ml, 75µg/ml, 100µg/ml, and 125µg/ml) were prepared and diluted. Then the absorbance of each of these concentrations was measured using UV/Visible spectrophotometry at λ_{Max} **(**275 nm) in the concentration range 25–125 µg /ml. Phosphate buffer solutions was used as a blank. The standard graph of *T. serrulatus* essential oil shows linearity with the correlation co-efficient ($R^{2)}$ value of 0.9992, and the linear regression equation was Y=0.0052x+0.1349 (Figure 7) (Lin *et al*., 2018).

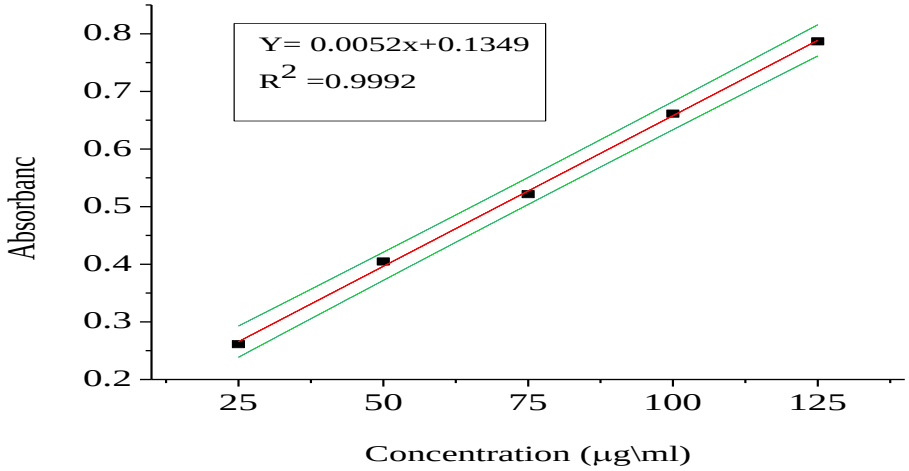


Figure 7: Calibration curve of thyme serrulatus in phosphate buffer pH 5.5 at 275nm with 95% confidence interval

3.2.6. Preliminary studies

Factors that could have significant effects on the response variables such as pH, viscosity, spreadability, extrudability, and drug release, according to literatures, were considered in the preliminary studies. These factors include gelling agents type (NaCMC and HPMC) and concentration, oil phases type (liquid paraffin and virgin olive oil) and concentration, and emulsifiers type (Tween 80 and Span 80) and concentration and amount of external phase (water) (Verma and Mishra, 2016).

Commonly used gelling agents in emulgels preparation with high-viscosity grade were chosen to prepare *thyme* oil emulgel. (Na CMC) that provides a viscosity of more than 6000cp at 2% in

water, used for preparation of emulgels F1- F4, and F9 - F12 and (HPMC) that provides a viscosity of more than 15,000cp at 2% in water, used for emulgel F5 - F8 and F13- F16 preparations. The gelling properties of both gelling agents were evaluated at a concentration of 1%, 2%, 3% and 4% w/w (Gabriel *et al*., 2013). The gel bases were formed by dispersing the gelling agents in sufficient quantity (q.s) of warm distilled water to form 50g final emulgels formulation, with constant stirring using magnetic stirrer and hot plate (Jain *et al*., 2011). The formulations of gel are presented in Table 1.

Table 1: Formulation of gel

Formulation code	Gelling agent type and Concentration	
	NaCMC (%)	HPMC (%)
F1	1%	-
F2	2%	-
F3	3%	-
F4	4%	-
F5	-	1%
F6	-	2%
F7	-	3%
F8	-	4%
F9	1%	-
F10	2%	-
F11	3%	-
F12	4%	-
F13	-	1%
F14	-	2%
F15	-	3%
F16	-	4%

3.2.6.2. Selection and composition of oil phase and emulsifiers

In the preliminary studies, all the emulgel batches were prepared using emulsions consist of either virgin olive oil (Formulations F1to F8) or liquid paraffin oil (Formulations F9-F16) as the oil phase in the concentration of 7.5%. High HLB Tween 80 and low HLB Span 80 were used as emulsifying agents in the level of 5% concentration to prepare oil in water emulsion (Sonam *et al*., 2014).

To formulate physically stable oil in water emulsion the amount of Tween 80 (HLB = 15) and Span 80 (HLB = 4.3) using liquid paraffin (HLB =10.5) and olive oil (HLB = 7) as oil phase was calculated as per the equation 1:-

$A = 50 (x\text{-}HLB_B) / (HLB_A\text{-}HLB_B)$……Eq 1: HLB value of oil phases

Where; 50= the total percentage of the formulation

X = the required HLB of the surfactant (oil) mixture (liquid paraffin or olive oil),

A = Tween 80, B = Span 80.

The fraction of Tween 80 (HLB of 15) and Span 80 (HLB of 4.3) that used to produce physically stable liquid paraffin (HLB of 10.5) as oil phase for oil in water emulsion was calculated as follows:-

A = 50 (10.5-4.3) / (15-4.3)

A = 28.97%

B = 50 – A

B = 50 - 28.97 = 21.03 %

A = 28.97 × 5/50

A = **2.9 g**

B = 5 – 2.9 = **2.1 g**

For the oil in water type emulsion, 5.0 g emulsifier was required. For this reason, for a 50 g emulgel, 2.897g Tween 80 should be taken and the remainder 2.103 g should be completed with Span 80.

The fraction of Tween 80 (HLB of 15) and Span 80 (HLB of 4.3) that used to produce physically stable olive oil (HLB of 7) oil in water emulsion was calculate as follows:-

A = 50 (7-4.3) / (15-4.3)

A = 12.6%

B = 50 – A

B = 50 – 12.6% = 37.4 %

A = 12.6 × 5/50

A = **1.3 g**

B = 5 – 1.3 = **3.7 g**

For the oil in water type emulsion, 5.0 g emulsifier was required. For this reason, for a 50 g emulgel, 1.3 g Tween 80 should be taken and the remainder 3.7 g should be completed with Span 80.

3.2.7. Drug-excipient compatibility study

FTIR spectroscopy was performed to ensure the compatibility between the polymers and *thyme* oil. The FTIR spectra of *thyme oil*, sodium CMC, HPMC, and the physical mixtures of drug and polymers were carried out using FTIR (FT-IR- 8400-S SHIMADZU®, Japan). The pure drug, the polymer and the physical mixture of drug polymer were size reduced in a mortar and 3 mg of each sample was mixed in mortar and pestle. The sample mixture was then placed onto the face of a potassium bromide (KBr) disk, and the second window was placed on top of the first salt plates to form a thin film of the mull by compression between two plates. The sandwiched plates were placed in the infrared spectrometer and the spectra were obtained by scanning the sample in the resolution range of 4000-400 cm^{-1} once at a time to be interpreted in the PerkinElmer spectrum 10 software. The wave numbers of characteristic peaks of physical mixtures were compared with the pure samples and interpreted (Haneefa *et al*., 2014).

3.2.8. Preparation of *Thyme Serrulatus* oil emulgel

Emulgels were prepared in two steps method and the composition of emulgel formulations is presented in Table 2. In the primary step, gels were prepared using either HPMC or NaCMC as a gelling agent at different concentrations of 1%, 2 %, 3% and 4%. The weighed amount of either HPMC or NaCMC were added into 25ml glass beaker containing distilled water and stirred continuously using a magnetic stirrer at 12 rpm until a homogeneous gel was formed. Based on the preparation procedure of NaCMC gelling agent on its package, the prepared gel was left overnight to confirm hydration of the gel. And also the pH of HPMC gel was adjusted to

pH range of 5.5- 6 by adding 1-2 drops of Triethanolamine (TEA) due to the more acidic pH of the gelling agent (Abdul and Rajab, 2014).

Then the aqueous and oil phases of the oil in water (O\W) emulsion were prepared separately in the following procedures: - the aqueous phase was prepared by making a solution of Tween 80 (2.9% and 1.3% in liquid paraffin and olive oil, respectively) in distilled water (Solution 1). In the second step (Solution 2), methyl paraben (0.03%) together with propyl paraben (0.02%) and Sodium sulphate (0.3%) was dissolved in propylene glycol (5%) separately. Both solutions (1 + 2) were thoroughly mixed with continuous stirring until a homogenous mixture formed.

The oil phase was prepared by mixing Span 80 (2.1% and 3.7% for olive oil and liquid paraffin, respectively) with *thyme serrulatus* oil (2%) and virgin olive oil (7.5%) for formulations F1 to F8 and light liquid paraffin (7.5%) for formulations F9 to F16 respectively. The quantity of emulsifiers was used according to the HLB theory of the oil phase as mentioned in the part of selection and composition of emulsifier (Ambala and Vemula, 2015).

Both the aqueous and oily phases were separately heated at 70℃ on the water bath (Polytron®, PT-1008, Germany) with a mechanical stirrer. Then the aqueous phase was added slowly, drop by drop, into the oily phase with a constant stirring using a hot plate with a magnetic stirrer at rotational speed of 12 rpm for 20 minutes, then the emulsion was left to cool down to room temperature. Finally, the prepared emulsions and gels were mixed in a 1:1 ratio and stirred constantly at room temperature until a uniform semi-solid emulgel was formed (Mounika *et al*., 2018).

Table 2: Percentage composition of batches of emulgel formulations

Formulation Code	HPMC (%)*	Na-CMC (%)*	Liquid paraffin (%)	Olive Oil (%)	Tween 80(%)	Span 80(%)	Methyl paraben (%)	Propyl paraben (%	Propylene glycol (%)	Na-Msulphate	DW up to ml (qs.)
F1	-	1	-	7.5	1.3	3.7	0.03	0.02	5	0.3	50
F2	-	2	-	7.5	1.3	3.7	0.03	0.02	5	0.3	50
F3	-	3	-	7.5	1.3	3.7	0.03	0.02	5	0.3	50
F4	-	4	-	7.5	1.3	3.7	0.03	0.02	5	0.3	50
F5	1	-	-	7.5	1.3	3.7	0.03	0.02	5	0.3	50
F6	2	-	-	7.5	1.3	3.7	0.03	0.02	5	0.3	50
F7	3	-	-	7.5	1.3	3.7	0.03	0.02	5	0.3	50
F8	4	-	-	7.5	1.3	3.7	0.03	0.02	5	0.3	50
F9	-	1	7.5	-	2.9	2.1	0.03	0.02	5	0.3	50
F10	-	2	7.5	-	2.9	2.1	0.03	0.02	5	0.3	50
F11	-	3	7.5	-	2.9	2.1	0.03	0.02	5	0.3	50
F12	-	4	7.5	-	2.9	2.1	0.03	0.02	5	0.3	50
F13	1	-	7.5	-	2.9	2.1	0.03	0.02	5	0.3	50
F14	2	-	7.5	-	2.9	2.1	0.03	0.02	5	0.3	50
F15	3	-	7.5	-	2.9	2.1	0.03	0.02	5	0.3	50
F16	4	-	7.5	-	2.9	2.1	0.03	0.02	5	0.3	50

*F= Formulation; HPMC= Hydroxylpropylmethyl Cellulose; NaCMC= Sodium CarboxyMethyl Cellulose and DW= Distilled Water.

3.2.9. Evaluation of emulgel formulations

3.2.9. Physical parameters evaluation

The prepared all blank and drug-loaded emulgel formulations were evaluated for their physicochemical characteristics such as physical appearance, colour, homogeneity, consistency, and texture and phase separation after they have been filled into glass jars.

These physical characteristics of the formulations were examined by visually observed. Their colour was viewed after preparation. To evaluate the homogeneity the emulgels were applied on the skin and the feel was recorded psychorheologically (Vikrant and Sonali, 2014). The immediate skin feel (texture) including greasiness, stickiness, grittiness and stiffness of the formulations were also evaluated by mildly rubbing the emulgel between thumb and fingers (Effionora and Ramadon, 2014). Evaluations of the consistency of emulgels were on the basis of the polymer level of concentrations.

Phase separation Accurately weighed 6 grams of emulgel from all formulations was centrifuged at rotational speed of 6000rpm for 10 minutes at room temperature to evaluate the formulations for phase separation. The result was observed visually for formation of phase separation (Usha *et al*., 2020).

3.2.10. pH measurement

The pH of each emulgel formulations were measured at room temperature (24±1°C) using a digital pH meter which was pre-calibrated with standard buffer solutions of pH 4, 7, and 10. Emulgel of 50 g was put in a glass jar and then the tip of the pH electrode was submersed into the formulations directly and waited until the reading stabilized. The electrode was thoroughly rinsed with distilled water between each measurements of pH to remove all traces of the sample to avoid the error. After 3 min the result was recorded with the averages of three readings of the pH values (Marwaha, 2013).

3.2.11. Viscosity study

The viscosity of the emulgel formulations prepared with various concentrations of sodium CMC and HPMC (1%, 2%, 3%, and 4%) was determined at room temperature using a rotational viscometer (Kinematica, AG, Type Viscostar Plus L, Switzerland). Different spindle numbers of L1, L2, L3, and L4 at different shear rates 5, 10, 20, 30, 50, 60, 100 and 200 rpm were used for the viscosity determination (Singla *et al*., 2012).

3.2.12. Spreadability test

Different radiuses of concentric circles in 3cm, 5cm, 7cm, 9cm, 12cm, 15cm, and 19cm were drawn on the white paper and a $20cm^2$ glass plates was fixed onto it. First one gram emulgel was weighed and put on the center of the lower plate and spread over 2.5cm in diameter. Likewise, another twenty-centimeter square glass plate of 380 g was kept gently on the emulgel. Then, 200g standardized weight load was applied on the upper glass plate for 5 min. The increase in the diameter due to *thyme* oil emulgel spreading was measured by ruler and recorded as spreadability (Gabriel *et al*., 2013).

3.2.13. Extrudability test

Twenty five gram (25g) of emulgel formulation was filled in a clean, lacquered aluminum collapsible tube which has a nozzle tip of 0.6 cm opening. Then, the tube was placed between two glass slides and clasped. Finally, 1 kg of the load was applied on the slides to record the extrudability of the emulgel and the amount of emulgel extruded from the tube was collected in the petri dish and reweighed. The amount of emulgel extruded was recorded and grades were assigned (> 90% of emulgel extruded, excellent; 80% - 89.9% extruded, very good; 70% - 79.9%, good; 50% - 69.9% extruded, fair and <50% extruded, poor (Das *et al*., 2009); (Banerjee *et al*., 2013).

3.2.14. Swelling index

One gram (1gm) blank and drug-loaded emulgel formulations were taken on porous aluminum foil to determine the swelling index and then placed separately in a 40ml beaker containing 10 ml of NaOH. Then, small amounts from the samples were taken from beakers at different time intervals (30min, 60min, and 90 min). Finally, samples were allowed to dry in room temperature and then reweighed and recorded. (Anumolu *et al*., 2011) ;(Khalil *et al*., 2011). The swelling index was estimated as per equation 2:-

$(SW)\% = [(W_S - W_0) / W_0] \times 100$……Eq 2: Swelling index

Where,

(SW) % = Percentage swelling

W_0 = initial weight of emulgel

W_S = Weight of swollen emulgel at time t.

3.2.15. Drug content uniformity

Sample of 1g from each best selected emulgels was taken and dissolved in flask containing 100 ml of phosphate buffer (pH 5.5) solution using a magnetic stirrer at 12rpm. Then, the homogenized solution was filtered using whattman filter paper No1 and a 2 ml aliquot was taken and diluted with the same buffer in 25 ml volumetric flask. Finally, the concentration was estimated spectrophotometrically at 275 nm using phosphate buffer (pH 5.5) as a blank. The drug content was calculated from the slope of the standard calibration curve of *T. serrulatus's* essential oil (Basha *et al*., 2011).

3.2.16. In vitro drug release studies

In vitro drug dissolution study was performed using glass tubes with openings on both sides. 12.1 mm inner diameter, 12.9 mm outer diameter, and 112 mm height, and the release area was (114.9 $mm^{2)}$ as a diffusion cell. Emulgel of one gram was put into the glass tube and covered with a cellulose acetate membrane (0.45 µm) pores which were immersed into a pH 5.5 phosphate buffer solution for 24hr. Then the tube was covered with a rubber band, inverted and inserted into a 1000-ml beaker containing 500 ml of phosphate buffer (pH 5.5), which was kept at a temperature of 37 ±1 ℃. An aliquot of 5 ml was taken at 30min, 1, 2, 3, 4, 5, and 6 hrs. Each withdrawn sample was replaced with an equal volume of fresh dissolution media. Finally, the sample was analyzed using a UV/Visible spectrophotometer at a wavelength of 275nm (Dima *et al*., 2016).

3.2.17. *In-vitro* antifungal activity test

3.2. 17.1. Media preparation

The susceptibility of the isolated fungal strain (candida albican ATCC) to *Thymus serrulatus* essential oil was estimated by the *in vitro* antifungal test using the disc diffusion method. To prepare the agar plate, 3.9 g of Sebourdous dextrose agar was weighed and mixed with 60ml of distilled water, followed by shaking on a shaker to make a homogenous mixture. The mixture was then boiled on hot plate and placed into the autoclave for 15minutes at 121^{o}C. Then after, 20 ml of the prepared agar media was poured into Petri dish (90mm) and then stored at room temperature to cool. After the media solidified, the fungal strain was (*candida albican* ATCC) sub-cultured and stored at 30^{o}C for 48 hr in incubator (Griffin *et al*., 2000).

3.2.17.2. Antifungal activity tests

After two days of incubation, the antifungal activity of the formulated product, F6 (*T.serrulatus* oil emulgel), positive control (Miconazol cream 0.5g), and negative control (blank emulgel of F6) were tested using the disc diffusion method. First, the sterilized Sabouraud dextrose agar of 20 ml was poured into sterilized petri dish and allowed to solidify at room temperature. Then, few amounts of colonies was taken from the sub- cultured plate and spread uniformly over the

surface of a new plate using swabs beginning from side to side to the bottom of the well. After inoculating the colonies, 3 holes which have equal distance (10 mm size) was made on the solidified media, and then filled with 0.5g of the test formulations (*T.serrulatus* oil emulgel, blank emulgel and Miconazol cream) with the help of disposable syringe. Finally, the diameter of zones of inhibition was measured by caliper and compared with the negative and positive controls. The results were recorded as presence or absence of zone of inhibition (Barry and Fuchs, 1991). The inhibitory zone diameters around the well containing the formulations were recorded as antifungal activity of the emulgel formulation. The absences of inhibition zones were recorded as zero. The test was performed in triplicate to ensure reliability of the results (Kirbaşlar *et al*., 2009).

3.2.18. Skin irritation test

Skin irritation study was performed on Albino Wister rats those weighed from 186g - 264g. The skin irritation effect of *T. serrulatus* oil emulgel, marketed Miconazol cream, standard skin irritant (formalin 8%), and emulgel without T. *serrulatus* oil was assessed. From each formulation, 0.5 g sample were applied on the backside of properly shaved skin of Albino Wister rats (four rats of both male and female) and covered with dressing gauze. After application the animals were kept under room temperature with suitable light conditions. The covering was loosely held in contact with the skin by means of a non-irritating adhesive tape. After 48 hr of exposure period, the dressing gauze and the adhesive plaster were carefully removed and the test site was wiped with tap distilled water to remove any remaining emulgel residues (Sundari *et al*., 2014). The animals skin were examined for alteration in colour, and presence of erythema and edema according to the Draize dermal irritation scoring system at intervals of 24 and 48hr as indicated in Table 3. The degree of erythema and edema was determined based on the scores shown in Table 4 to evaluate the primary dermal irritation index. Primary irritation index (PII) was calculated for the test samples according to the equation 3 (Gebrehiwot *et al*., 2015).

$$PDII = \frac{\sum(\textbf{Erythema at 24hr + 48hr}) + \sum(\textbf{Edema at 24hr + 48hr})}{1 \times 2}$$

Eq 3: Primary irritation index; Where; PII = Primary irritation index, Σ= sum of the exposure time, 24 and 48 hour = exposure time, 1= No of sites, 2= scoring interval.

Table 3: Erythema and edema scores used to determine the primary irritation index (Gebrehiwot *et al*., 2015)

Erythema	Value
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness)	4
Edema formation	**Value**
No edema	0
Very slight edema (barely perceptible)	1
Slight edema	2
Moderate edema	3
Severe edema	4

Table 4: Categories of PDII classification **(**Shukr and Metwally, 2013)

PDII	Classification
<0.5	Non - irritating
0.5-2.0	Slightly- irritant
2.1-5.0	Moderately- irritant
>5.0	Severely -irritant

3.2.19. Stability studies

A stability study was conducted for the selected *T. serrulatus* oil emulgel. The *T. serrulatus* oil emulgel was placed into a glass jar and stored under different storage conditions, 30 ± 5 ℃ / 65 ±5℃ RH, 40 ± 5 ℃ /75 ± 5℃ RH and in refrigerator at 5 - 8 ± 3°C, for 3 months in stability chamber. The change in physical appearance, phase separation, color, odor, homogeneity, rheological properties (viscosity), and drug content were monitored by taking sample at 1, 2 and 3 months at 30-days intervals. The obtained profiles were compared with the initial condition stored at room temperature (Food and Drug Administration, 2003) ;(Gabriel *et al*., 2013).

3.2.20. Data analysis

Origin Pro 8.5.1 (Origin LabTM Corporation, USA), IBM SPSS statistics 25 (ANOVA) was utilized for the statistical analysis and Microsoft Excel 2010 was used to compile the results. P values less than or equal to 0.05 at a 95% confidence interval were regarded as statistically significant. Mean and standard deviation (SD) are used to present the findings.

3.2 21. Ethical clearance

The study obtained ethical clearance from the Addis Ababa University, School of Pharmacy Ethical Review Board with the reference number ERB/SOP/458/15/2023.

4. RESULTS AND DISCUSSIONS

4.1. Authentication and extraction of essential oil

In this study, the plant material was authenticated by the Department of Biology, University of Gondar. The plant species was *T. serrulatus* (Ethiopian *thyme*) and this was one of the indigenous species found in Ethiopia. For further reference, the specimen has been recorded at the herbarium of the University with voucher number 0001/ASM/2021.

Figure 8: *Thyme serrulatus* essential oil after extraction by hydro- distillation

4.2. Essential oil analysis

The *thyme serrulatus* fresh aerial parts yielded 0.6 % v/w of essential oil, which had a light yellowish colour and a *thyme* aroma (Figure 8). The GC-MS analysis of the essential oil revealed 22 components, representing about 100% of the total detected constituents. As shown Table 5, the major components of the essential oil are thymol (36.92%), *O*-cymene (26.05 %) and carvacrol (19.15%). These EO major components are in agreement with those previously reported by Haile *et al*., (2008); Haile *et al*., (2021a); WHO, (1999). Other workers have also shown similar results for different *thyme* species from other countries Charai *et al*., (1999); Asllani and Toska, (2003). The GC/MS chromatogram profile of each compound was presented in Figure 9.

Table 5: Chemical composition of *T. serrulatus* essential oil as detected by GC/MS

Peak #	Retention Index	Retention time (min)	Compounds	Relative abundance (%)
1	976	6.974	*O*-Cymene	26.05
2	1062	7.197	ɣ-Terpinine	8.24
3	1312	12.88	Carvacrol	19.15
4	1423	12.611	Caryophyllene	0.99
5	1305	12.688	Thymol	36.92
6	1264	10.56	Benezene,2-methoxy-1,3,5trimethyl	5.69
7	1506	13.509	Beta.-bisabolene	2.97
Total				100.05

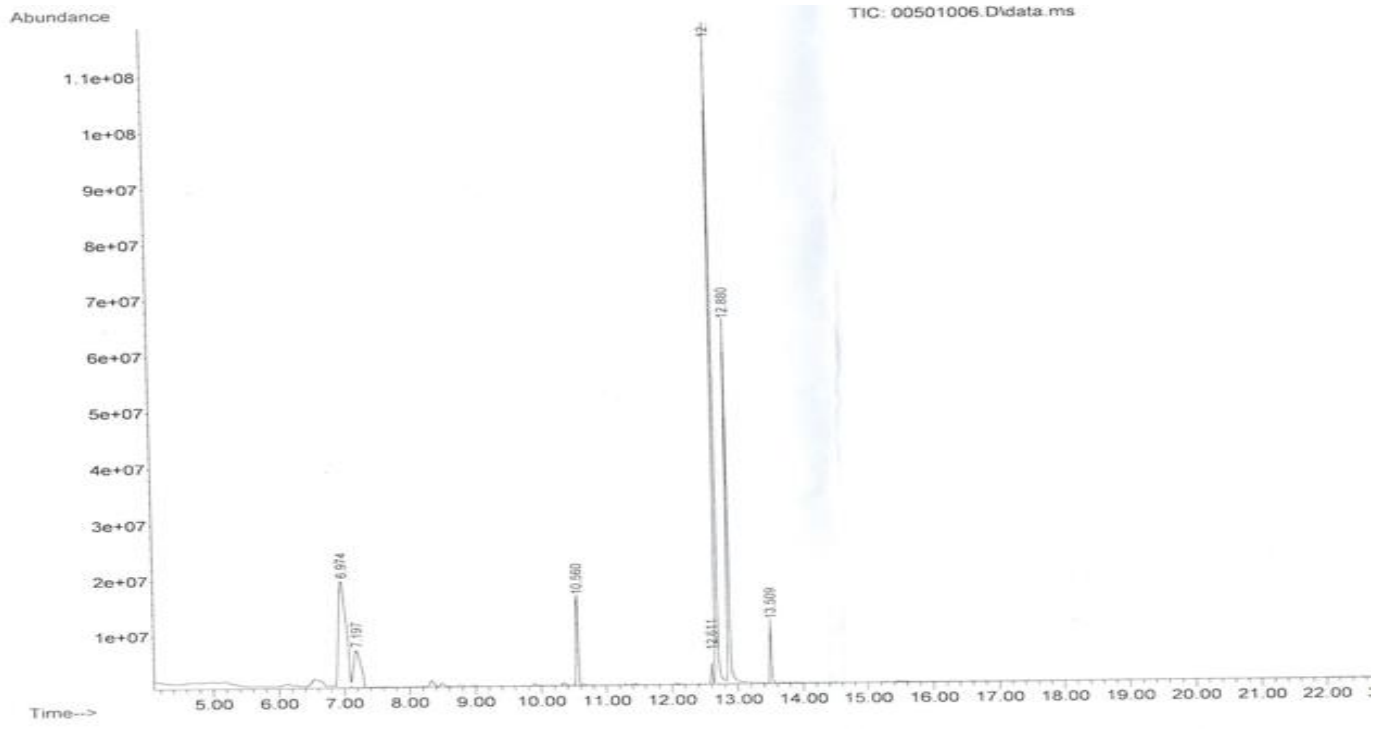


Figure 9: The GC/MS chromatogram of *thyme serrulatus* essential oil

4.3. Determination of λ max of *thyme* oil

The lambda maximum (λ) of the essential oil of *thyme serrulatus* in pH 5.5 phosphate buffer solution was found to be 275 nm as depicted in Figure 10. This value is closely related to the λ maxima of the essential oil thymol, carvacrol methyl ether, and O-cymene components.

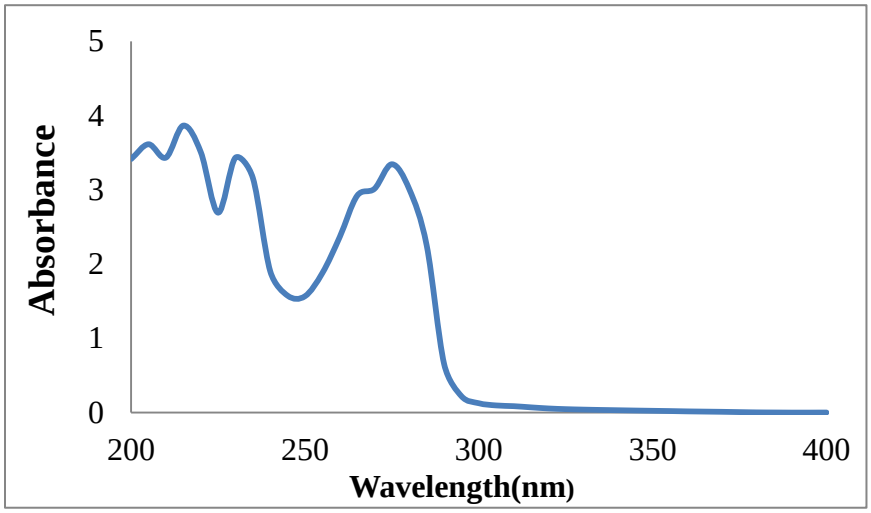


Figure 10: Ultraviolet scan of thyme serrulatus essential oil in phosphate buffer pH

4.4. Compatibility studies

One prerequisite for identifying the interaction between the compound and the polymer is a compatibility study. As Figures 11A-11E depict the FT-IR spectrum of *thyme* oil, NaCMC, and HPMC as well as the physical blend of *thyme* oil with Na CMC and HPMC. The *T. serrulatus* oil has C-H stretching around 2960.53-2729.09 cm^{-1}, strong broad O-H stretching between 3533.35 and 3359.77 cm^{-1} , C-N stretching in the region of 2354.92-2320.21 cm^{-1}, C=C bending at around 1620.09 cm^{-1}, the C-N-H stretching at around 1585.38–1515.94 cm^{-1}, C–N stretching at around 1480.09–1380.94 cm^{-1}, and C-O stretching vibrations around 1257.50-1112.85 cm^{-1}. The presence of these peaks in the physical blend indicates that there is no incompatibility between the polymers and the *thyme* oil.

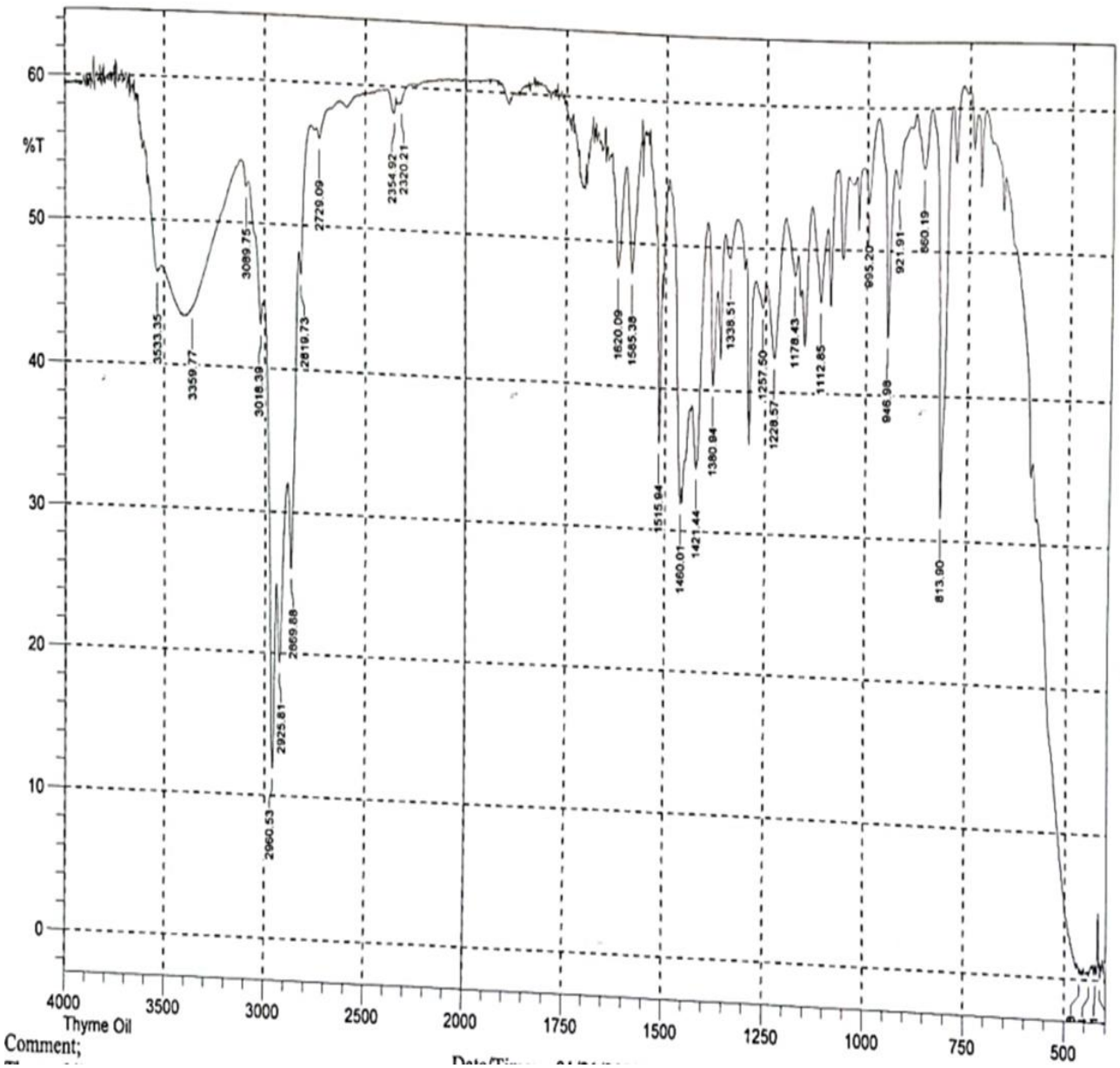
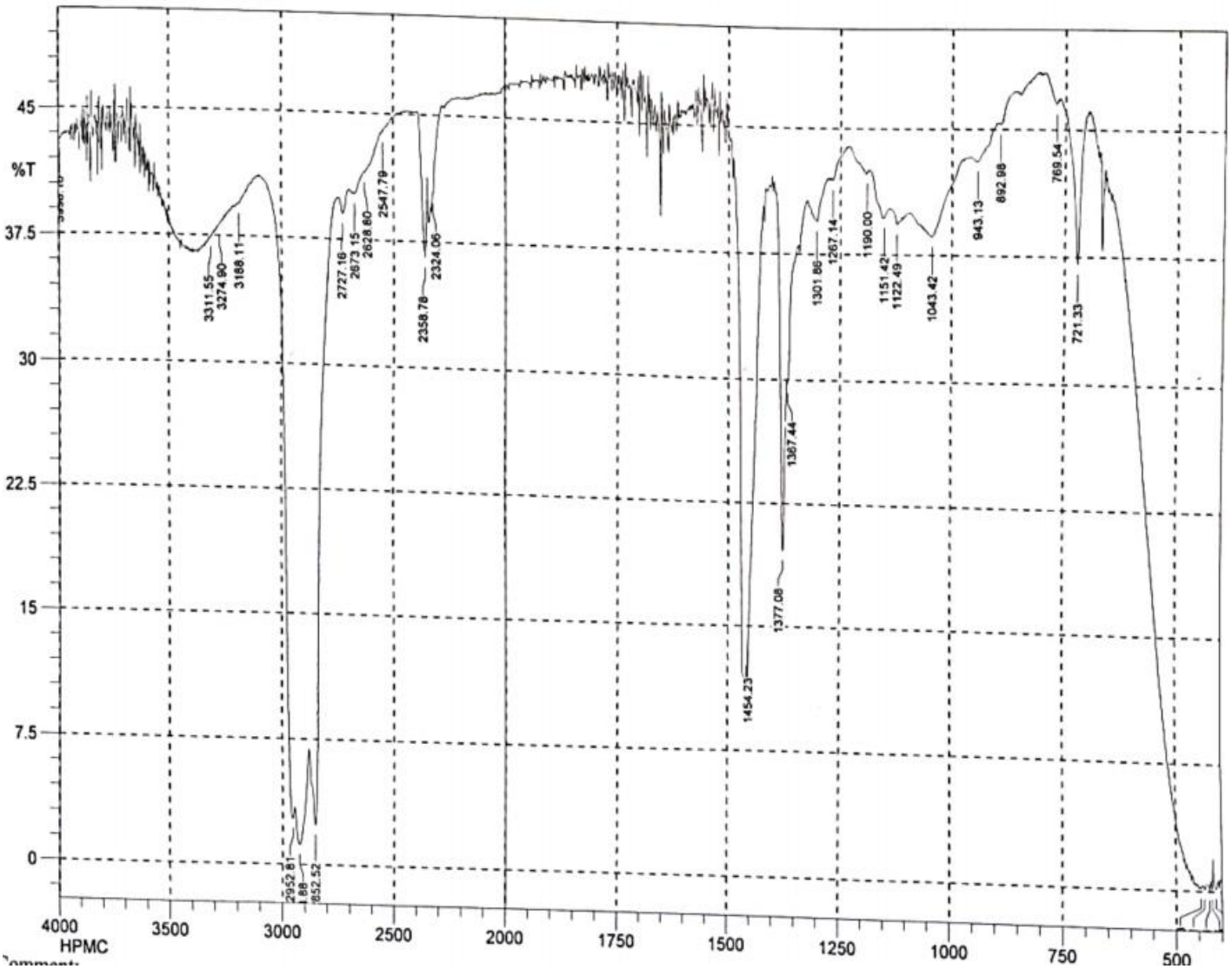
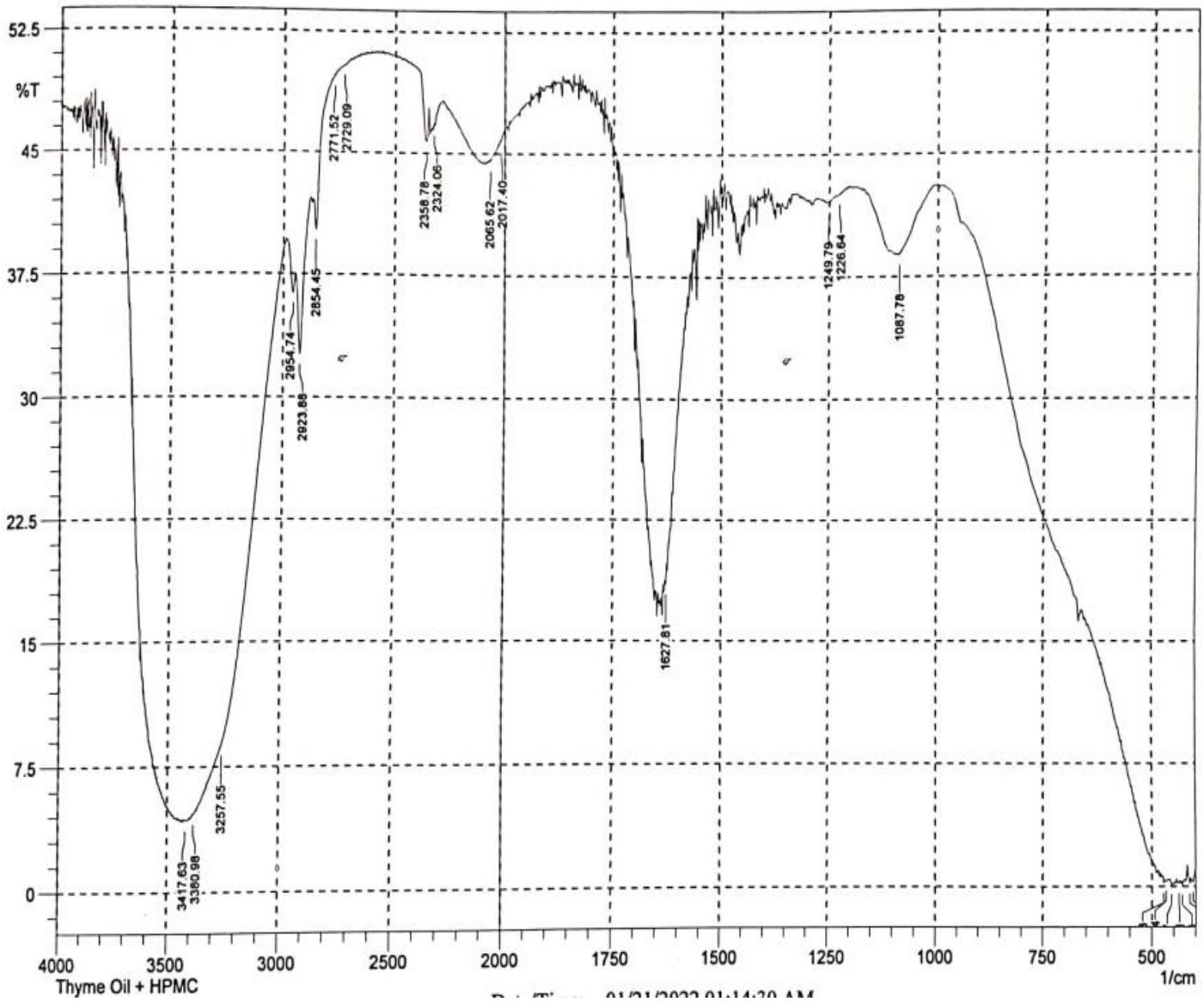


Figure 11A: FTIR spectrum of thyme serrulatus's essential oil



FTIR 11B: spectrum of HPMC



FTIR 11C: spectrum of the physical mixture of *T. serrulatus* oil and HPMC

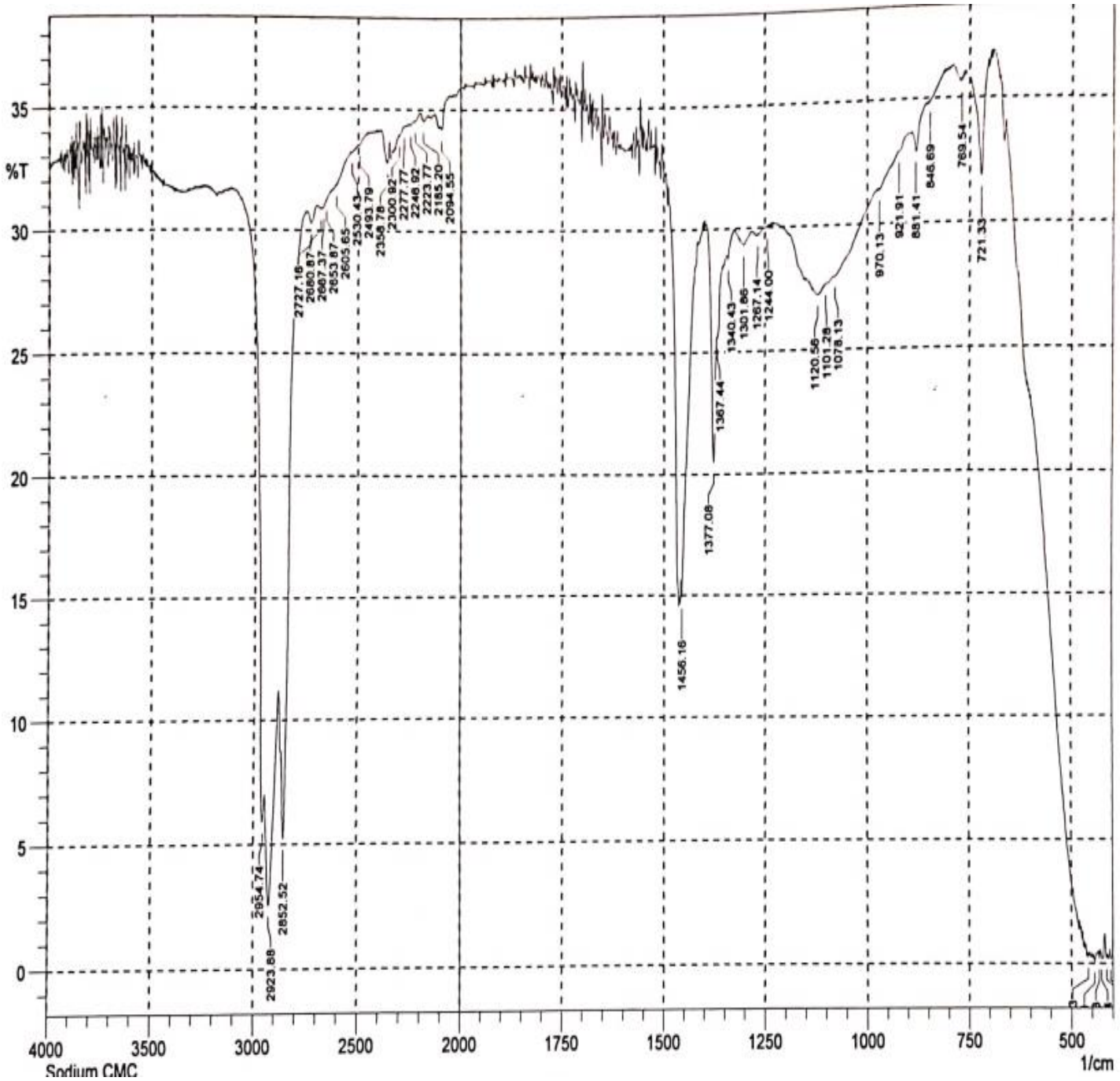


Figure 11D: FTIR spectrum of sodium CMC

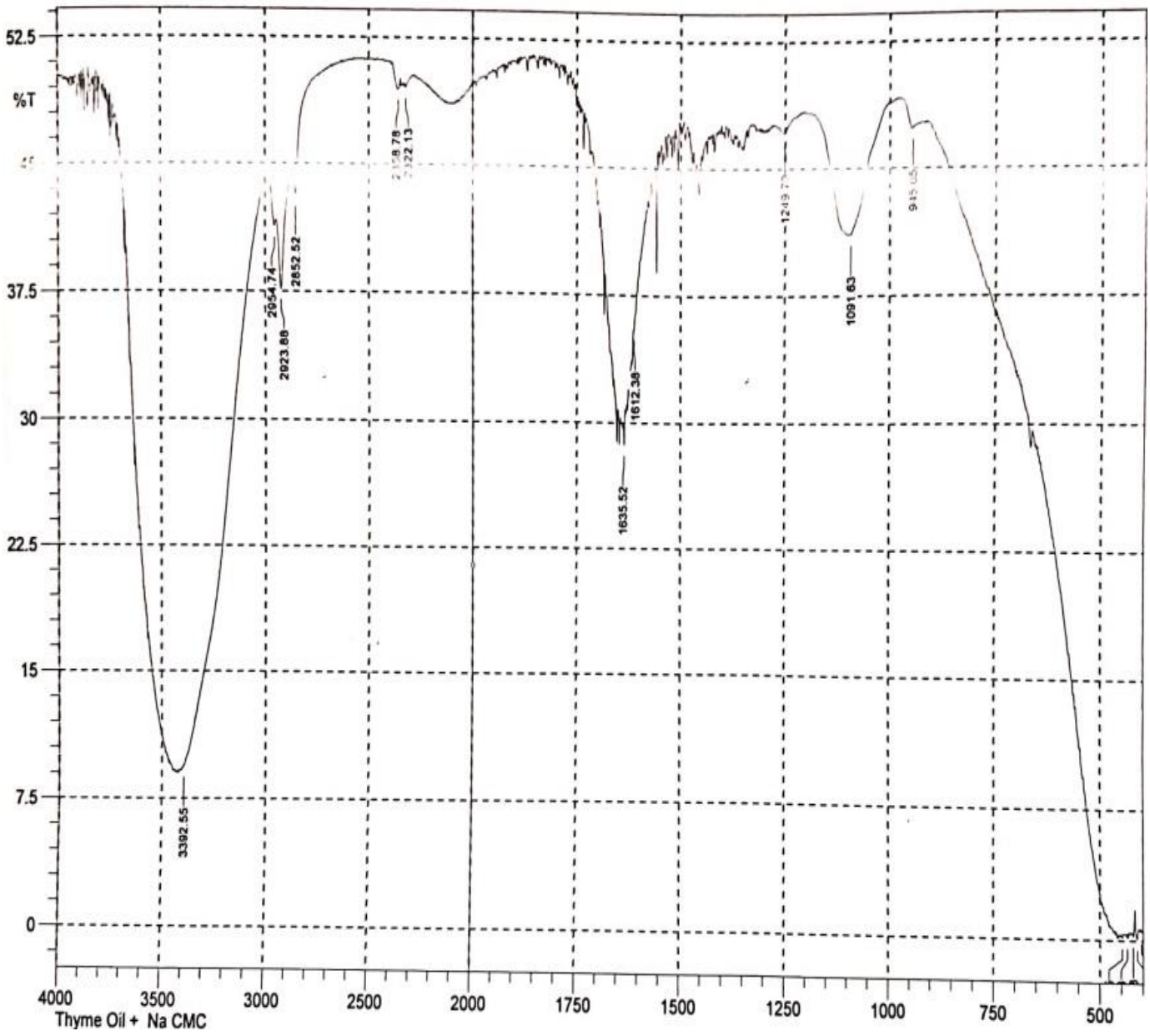


Figure 11E: FTIR spectrum of the physical mixture of *T. serrulatus* oil and sodium CMC

4.5. Preliminary studies for formulation of emulgels

4.5.1. Selection of gelling agents

From the pre-formulation trials, it was observed that NaCMC and HPMC gelling agents were favored to be incorporated into the oil in water emulsion as a gel base because they formed gel with various consistency at concentrations of 1 %, 2 %, 3 %, and 4 %. Consequently, the gelling agents in all concentrations were chosen for this study. Gelling agent carboxymethyl cellulose Sodium (Na CMC) was used in formulations from F1 to F4 with olive oil as the oily phase and in other formulations from F9 to F12 with liquid paraffin as the oily phase, respectively. It has a high-viscosity grade and provides more than 6000cps at 1% in water and was used in concentrations of 1 %, 2 %, 3 %, and 4 %.

The same concentration level (1 %, 2 %, 3 %, and 4 %) were employed in formulations from F5 to F8, which used olive oil as the oil phase, and formulations from F13 to F16, which used liquid paraffin as the oil phase, for the Hydroxylpropylmethyl cellulose (HPMC) gelling agent. It gives high -viscosity grade more than 15000cps at 1% in water.

4.5.2. Selection and composition of oil phases and emulsifiers

The selection of emulsifiers for both (Tween 80 and Span 80) combination were used at 5 % concentration. The result showed that the oil in water emulsion was excellent, physically stable and with absence of any clogging of globules, phase separation, or bubbles (Figure 13). From the required 50 g *T. serrulatus* oil emulgel formulations, level of 5% was taken by emulsifiers. In liquid paraffin as oil phase for the formation of oil in water emulsion, Tween 80 and Span 80 were found 2.9 % and 2.1 %, respectively. In the olive oil as oil phase, the required amount was 1.3% covered by Tween 80 and the remaining 3.7% was taken by span 80. The percentage composition of surfactants and oil phases are illustrated in Figure 12.

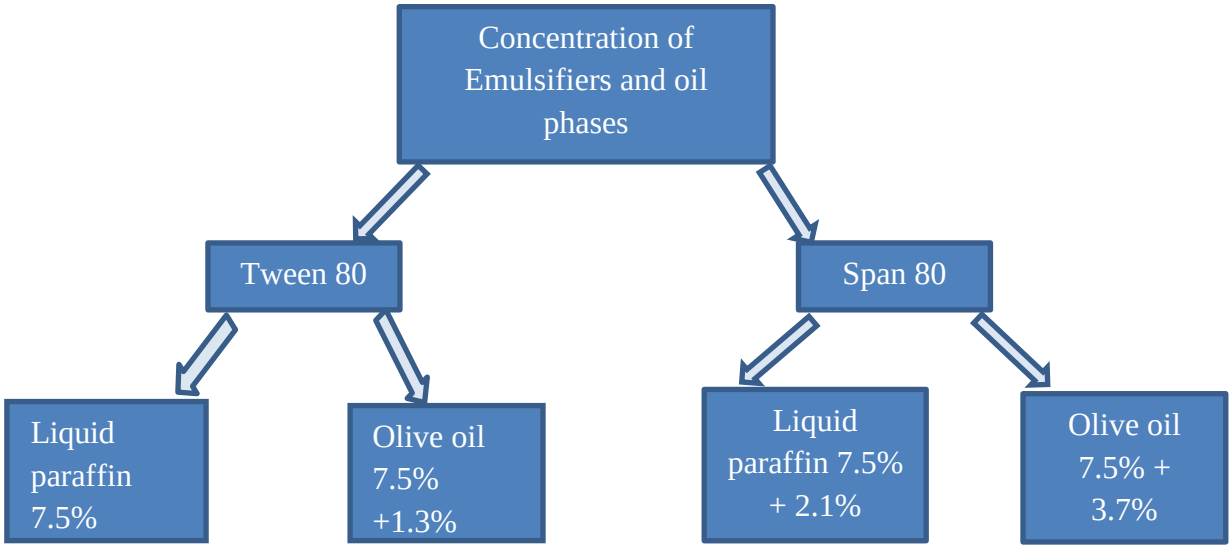


Figure 12: concentration of emulsifiers with corresponding oil phases

4.6. Evaluation of *thyme* oil emulgel formulations

4.6.1. Physical parameters evaluation

During the formulation of emulgel, the physico-chemical characterstics of gel and emulsion are considered to get stable,consistent and smooth preparation. In the current study the formulated emulgels were examined for their physical appearance, colour, homogeneity, texture, consistency and phase separation after 24 hr of preparation (Table 6). Emulgels of F1, F2 and F3 with sodium CMC as a gelling agent and olive oil as oily phases were found pale white in colour with creamy appearance at concentration of 1%, 2% and 3% gelling agent concentration respectivily. However, at 4% concentration the F4 physical appearance was very thick\viscous apperance with pale white colour. In formulations of F9, F10 and F11 with sodium CMC as a gelling agent and liquid paraffin as oil phase were found creamy appearance with pale white in colour at concentration of 1%, 2% and 3% gelling agent concentration respectivily. In contrast formulation F12 showed very viscous apperance with pale white colour at 4% NaCMC concentration. Emulgels of F5, F6 and F7 with HPMC polymer and olive oil as oily phases were found white colour with viscous creamy appearance at concentration of 1%, 2% and 3% gelling agent concentration respectivily. On the other hand, white colour with very thick appearance was seen

for formulations F8 at 4% concentration. In formulations of F13, F14 and F15 with HPMC as gelling agent and liquid paraffin as oil phase were found white viscous creamy appearance. In contrast formulation F16 showed very viscous apperance with white colour at 4% HPMC concentration. This result is supported by other findings reported by Sundari *et al*., (2014) ; Mohamed, (2004); Mounika *et al*., (2018).

As shown in Table 6, all formulations exhibited good to excellent homogeneity. The homogeneity of the formulations was related to the process of preparation and the concentration of gelling agents used. Emulgels prepared with 1% and 4% gelling agents showed good homogeneity, while those at 2 % and 3% gelling agents exhibited excellent homogeneity. The texture of all emulgel formulations were excellent without stickness, coarse partecles. This result is similar with findings reported by Singh *et al*., (2015); Shaik *et al*., (2019).

The formulations of gellified emulsion showed excellent consistency in emulgels of F3, F6, F7, F10, F11, F14 and F15; good consistency in emulgels of F4, F8, F12 and F16 and poor consistency in emulgels of F1, F2, F5, F9 and F16. These results indicated that both gelling agents (Sodium CMC and HPMC) in low concentrations (1%) gave gels with poor consistency, moderate concentration (2% and 3%) gaves excellent consistency and high concentrations (4%) gave good consistency. Moreover, the consistency was ranged from viscous to soft ones which are in accordance to the consistency of the gel base. This result is supported by other findings reported by Barry and Meyer, (1979) ; Potnis *et al*., (2014). In all formulations phase separation was not seen (Figure 13 and Figure 14). This result indicated that the amount of emulsifiers used was at sutable concentration lable (5%) with the HLB value and amount of oil phases (liquid paraffin and olive oil) as ilustrated in Table 6.

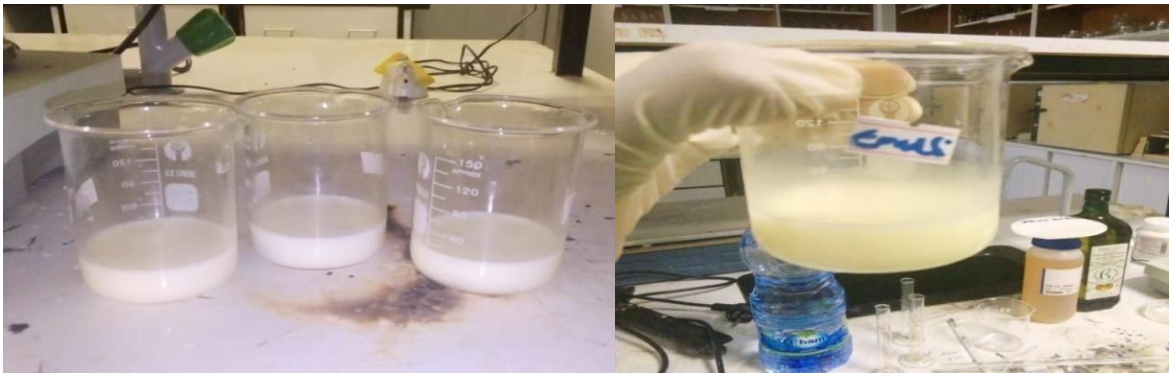


Figure 13: stable oil in water emulsion with 5% concentration composition of tween 80 and span 80

Table 6: Evaluation of all prepared blank emulgel for physicochemical properties

F. Code	Appearance/colour	Homogeneity	Texture	Consistency	Phase separation
F1	Creamy/Pale white	++	N.S	poor	No
F2	Creamy /Pale white	++	N.S	poor	No
F3	Creamy/ Pale white*	+++	N.S	excellent	No
F4	Creamy thick/ Pale white	++	N.S	good	No
F5	Creamy/White	++	N.S	poor	No
F6	Creamy/ White*	+++	N.S	excellent	No
F7	Creamy/ White*	+++	N.S	excellent	No
F8	Creamy thick /White	++	N.S	good	No
F9	Creamy/ Pale white	++	N.S	poor	No
F10	Creamy/ Pale white*	+++	N.S	excellent	No
F11	Creamy/ Pale white*	+++	N.S	excellent	No
F12	Creamy thick /Pale white	++	N.S	good	No
F13	Creamy/White	++	N.S	poor	No
F14	Creamy/ White*	+++	N.S	excellent	No
F15	Creamy /White*	+++	N.S	excellent	No
F16	Creamy thick /White	++	N.S	good	No

Note: ++ = Good ,+++ = Excellent, N.S= Not sticky, F.code = Formulation code; the sign indicated by "*"= best selected *T.serrulatus* emulgels.

4.6.1. 2.Selection of the best emulgel formulation

From a total of sixteen evaluated blank emulgel formulation batches (see Table 6), of the seven formulations (F3, F6, F7, F10, F11, F14 and F15) were selected as best emulgel formulations (Figure 18). By addition of *T. serrulatus* oil (2%v/v) to those best formulations, the following further evaluations such as, drug content uniformity, swelling index, and *in vitro* drug release studies were performed. Thus, three *thyme* oil emulgel (F6, F10 and F14) showed higher drug content and *in vitro* drug release (95.95%, 71.43% and 70.65%) respectively (Table 7). Concerning to this result, formulations were chosen as the best and the elegant *thyme serrulatus* oil emulgel formulations. Then, the last evaluation like *in vitro* antifungal, skin irritation and stability studies were conducted and have excellent stability at different storage conditions. In particular, at 30 ±5 ℃/65 ± 5% RH and at 5 ± 3 ℃ were best for these emulgel formulations.

Table 7: physicochemical evaluation of the best *T. serrulatus* oil emulgel formulations

Formulation code	Physicochemical Parameters					
	Appearance/colour	Homogeneity\ Consistency	Texture	Phase separation	Drug content (%)	*In-vitro* Release (%)
F3	Creamy /Pale white	Excellent	-	No	67.4 ±1.23	46.95 ±0.41
F6	Creamy/ White*	Excellent	-	No	95 ±0.34	95.95 ±0.65
F7	Creamy /White	Excellent	-	No	76.6 ±0.35	40.65 ±0.24
F10	Creamy /Pale white*	Excellent	-	No	90.8 ±0.32	71.43 ±0.26
F11	Creamy/ Pale white	Excellent	-	No	98.2 ±0.87	37.78 ±0.20
F14	Creamy/ White*	Excellent	-	No	101.3 ±0.53	70.65 ±0.32
F15	Creamy /White	Excellent	-	No	97.5±0.78	45.17 ±0.31

Note: the sign indicated by "*"= best selected *T.serrulatus* emulgel

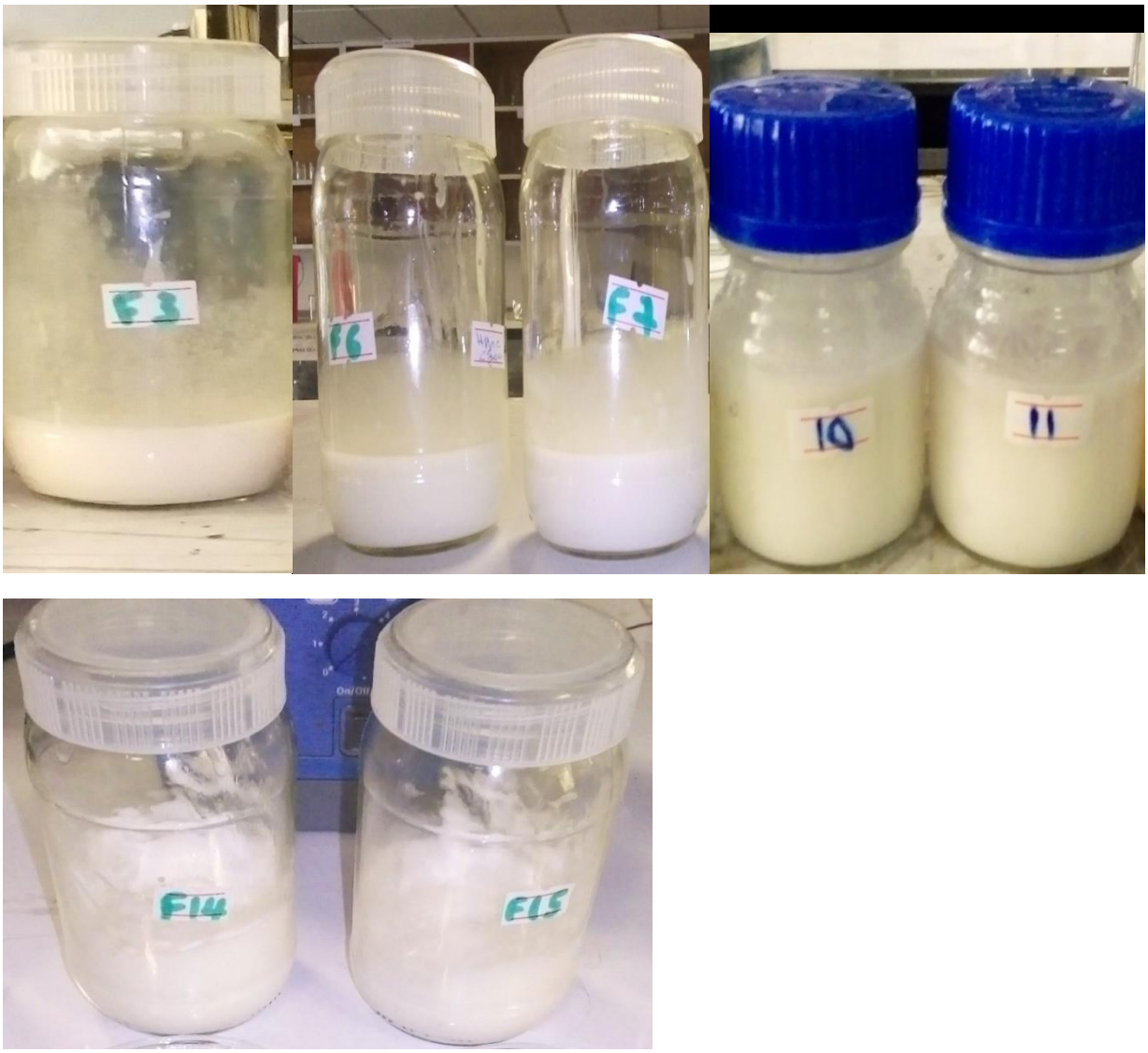
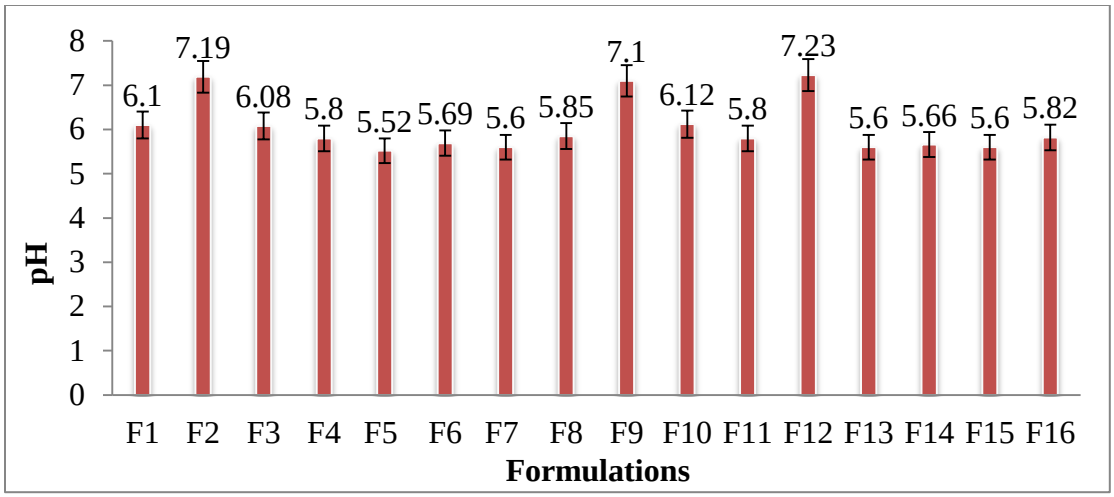


Figure 14: best selected thyme serrulatus oil emulgel formulation

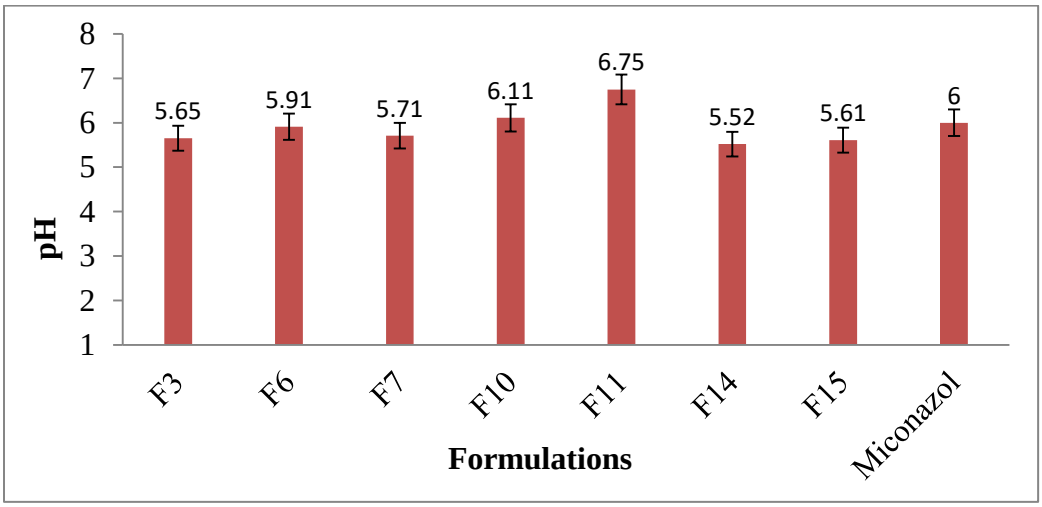
4.6.2. pH determination

In the present study, the pH of both the blank and *thyme* oil loaded emulgel preparations was found in the range of 5.52 to 7.23 (Figure 15 & 16). This pH range indicates that the prepared emulgel formulations were suitable for skin application. And the result was similar with the standard marketed topical antifungal drug (miconazol). It is also known that the normal pH of an adult skin is in the range of 5.5 - 6.5. Furthermore, it was observed that the addition of *thyme* oil on emulgel did not change the pH of the formulations.



*All values expressed as Mean± standard deviation (SD)

Figure 15: pH of all blank emulgel formulations



*All values expressed as Mean± standard deviation (SD)

Figure 16: pH of best thyme serrulatus oil emulgel formulations

4.6.3. Viscosity study

One of the crucial parameter for characterizing and evaluating emulgel formulations is the viscosity test. It exerts an impact on the drug release, extrudability, spreadability, and other physicochemical characteristics of drug delivery for topical or transdermal administration (Singla *et al*., 2012). In the present study, the result of apparent viscosity of the prepared emulgel formulations were decreased as the shear rate increased from 5 to 200 rpm (Figures 17 to 21).

This indicated that the emulgel formulations exhibited a pseudo-plastic flow property; this means naturally the gelling agents has non-Newtonian system.

It was found that the apparent viscosities of the HPMC emulgels were higher than the corresponding concentrations of the NaCMC, which indicates HPMC has a better gelling property in low concentration than the NaCMC. This result is similar with findings reported by Barry and Meyer, (1979); Mekkawy *et al*. (2013). On the other hand, the addition of *thyme* oil and type of oil phases did not have a significant effect on the viscosity of formulations (p=0.06).

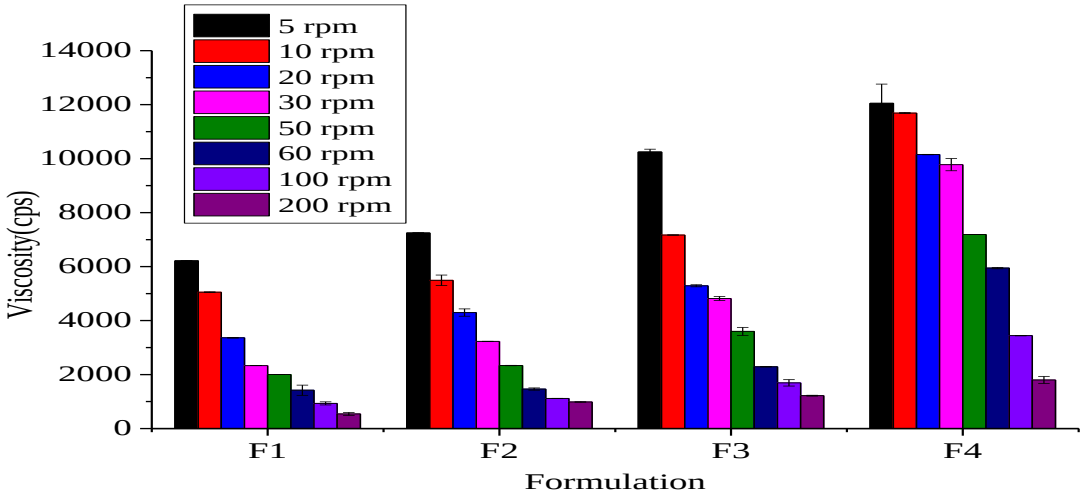


Figure 17: viscosity of F1-F4 blank emulgel formulation with NaCMC and olive oil

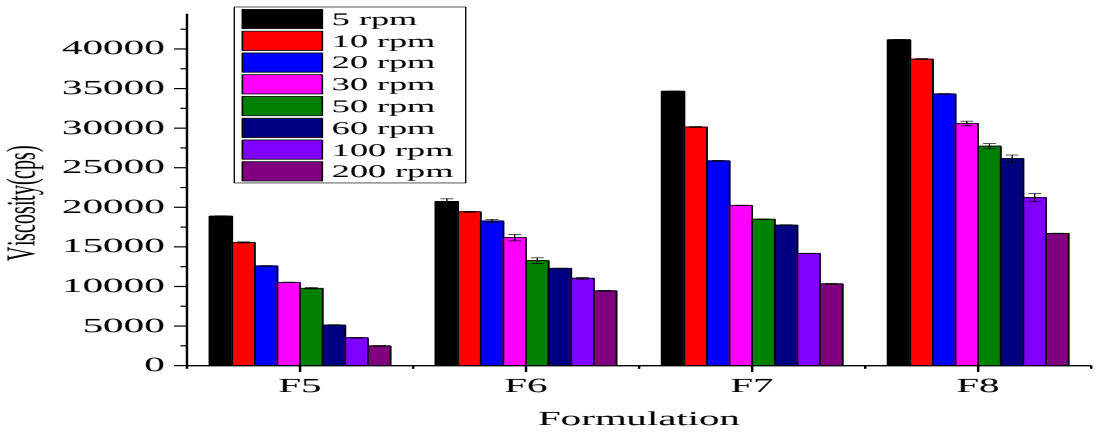


Figure 18: viscosity of F5-F8 blank emulgel formulations with HPMC and olive oil

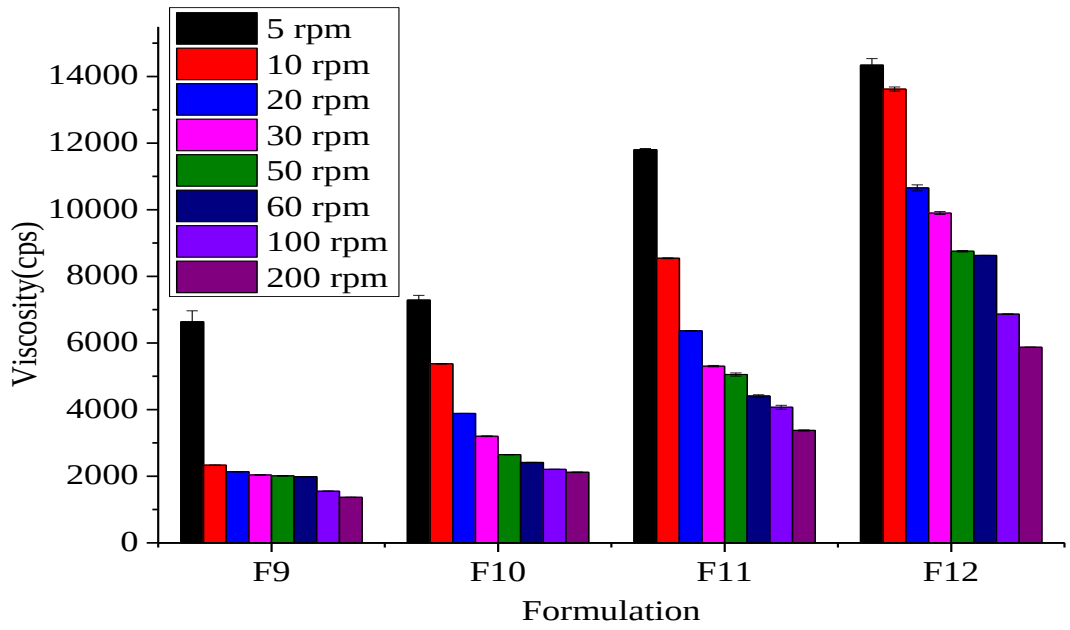


Figure 19: viscosity of F9-F12 blank emulgel formulation with NaCMC and liquid paraffin

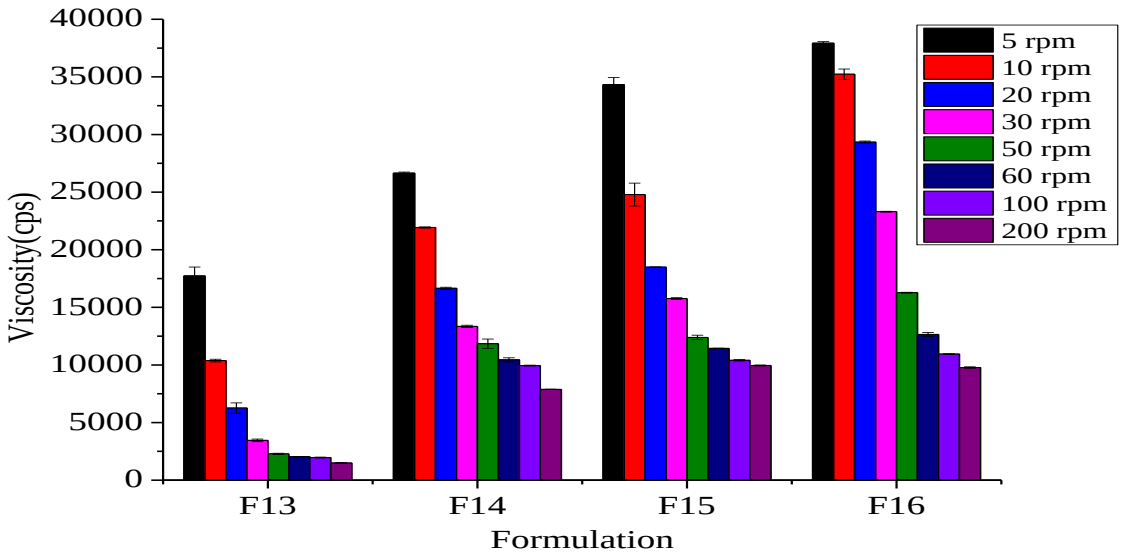


Figure 20: viscosity of F13-F16 blank emulgel formulations with HPMC and liquid paraffin

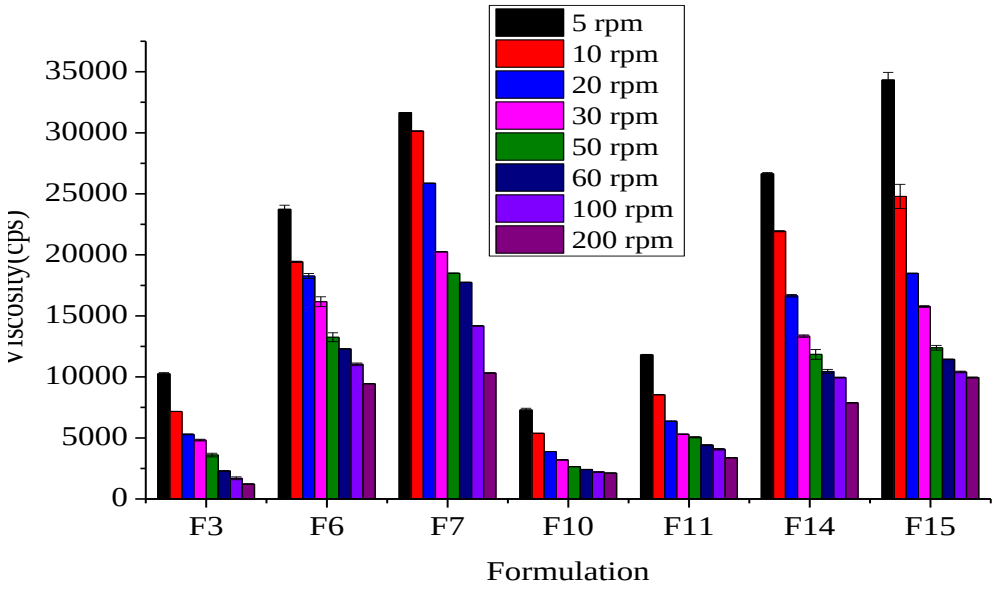


Figure 21: Viscosity of best selected *T. serrulatus* essential oil emulgel formulations

4.6.4. Spreadability test

Spreadability test indicates how easily the emulgel formulations spread on the affected area by the small amount of shear applied. In present study, the spreadability of the prepared emulgel formulation ranged from 6.5 cm -14.03 cm. This result was comparable with the result of standard marketed drugs *miconazol* cream (6.5cm) and *diclofenac* gel (7.5cm). Thus, this assures that the formulation maintains a good wet contact time when applied to the site of application (Ambala and Vemula, 2015).

The spreadability of emulgel formulations was affected by the type and concentration of gelling agents used. The spreadability of NaCMC based emulgel formulations (F1- F4 & F9- F12) was higher than formulations containing HPMC (F5- F8 & F13-F16). This may be due to the lower viscosity of the former which enables it to easily spread with modest application of shear. The result of this study is agreed with the finding of Baibhav *et al*., (2012). The oil phases also contributed to the spreadability of the emulgel preparations; this could be due to their nature of increasing emollient and solubility properties. Formulations containing virgin olive oil as the oil phase had higher spreadability (14.03cm, 12.23cm, 9.33cm and 9.25cm) than those formulations

with liquid paraffin as the oil phase. As depicted in Figure 22 and 23, addition of *T. serrulatus* oil into the blank emulgel did not result appreciable change in the spreadability (p=0.055).

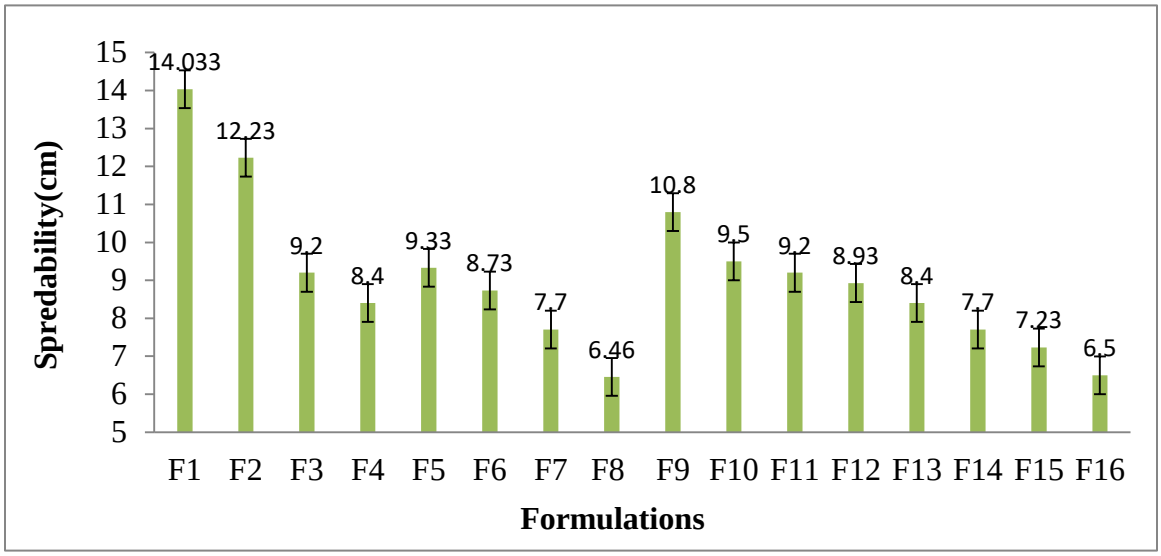
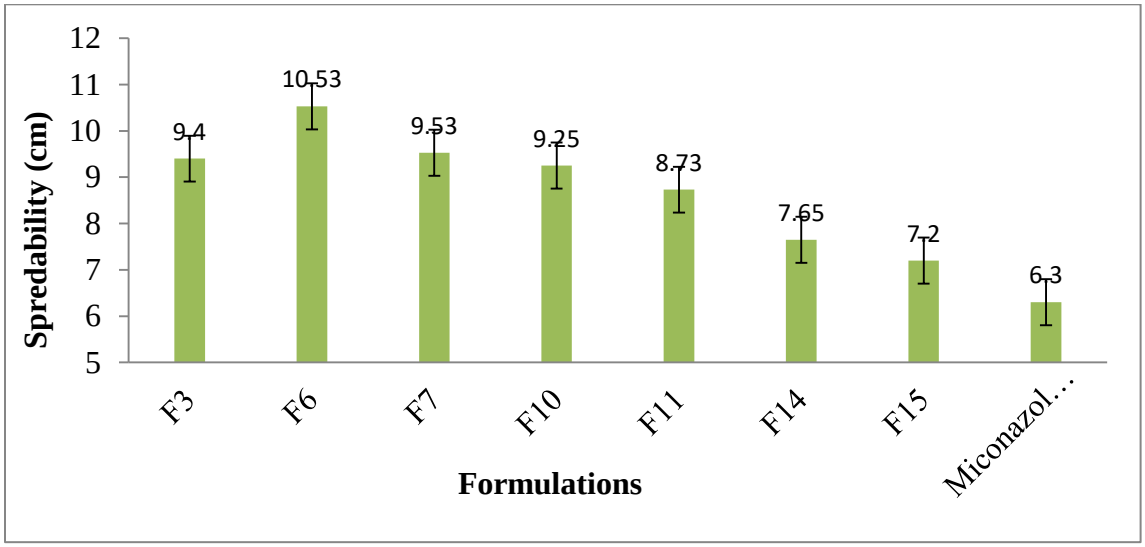


Figure 22: spreadability of all blank emulgel formulations



*All values expressed as Mean± standard deviation (SD)

Figure 23: spreadability of each best *T. serrulatus* oil emulgel formulations

4.6.5. Extrudability test

The extrusion of the emulgel from the tube is important during its application and in patient acceptance (Das *et al*., 2009). In this study the extrusion of all formulations were evaluated through application of specific load in each emulgels. Hence the extrudability of all formulations was found excellent and compatible for application (Figure 24 and 25). The result of extrudability of emulgels was as function of the concentration of NaCMC or HPMC; the extrudability was decreased while increased the concentration of both gelling agents. formulations which were contains 1% concentration gelling agent for all blank emulgels using NaCMC or HPMC gave the highest extrudability result and ranked as excellent. This is may be due to the presence of more emollient concentration. On the other hand, emulgels with 4% concentration in both gelling agent showed least extrudability and graded as poor. The concentration of gelling agent with 2% and 3% were the ranked as very good extrudability. Thus, formulations showing both excellent and very good extrudability were selected as the best formulation. This result is alien with other finding reported by Jelvehgari and Montazam, (2011). Furthermore, the compared standard topical antifungal drug (miconazol) was also show as best extrudability as *thyme serrulatus* oil emulgel formulations. The extrudability result was not significantly changed (P=0.0456) after the addition of *T. serrulatus* oil to the formulations.

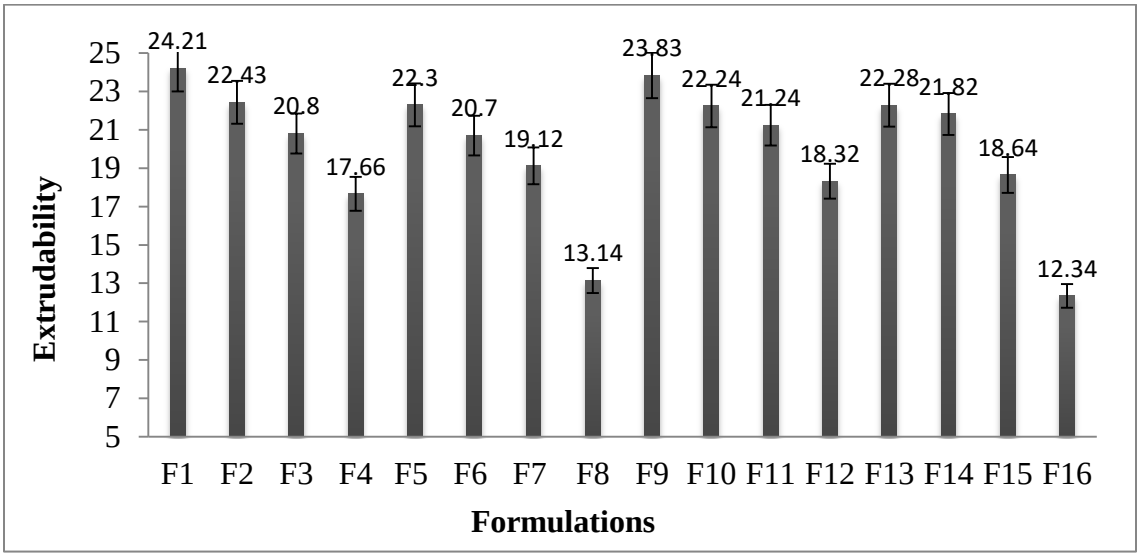
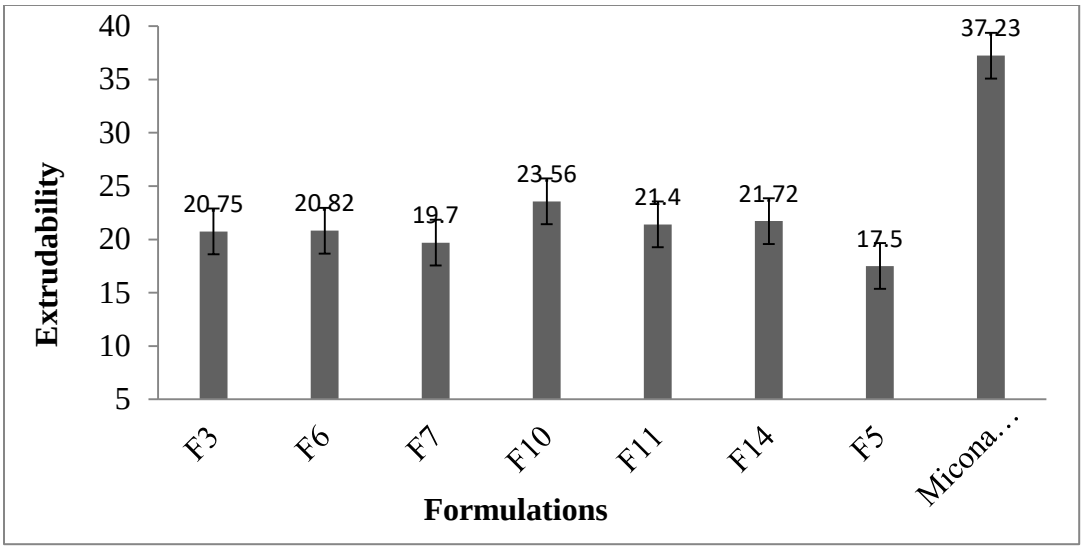


Figure 24: extrudability of blank emulgel formulations

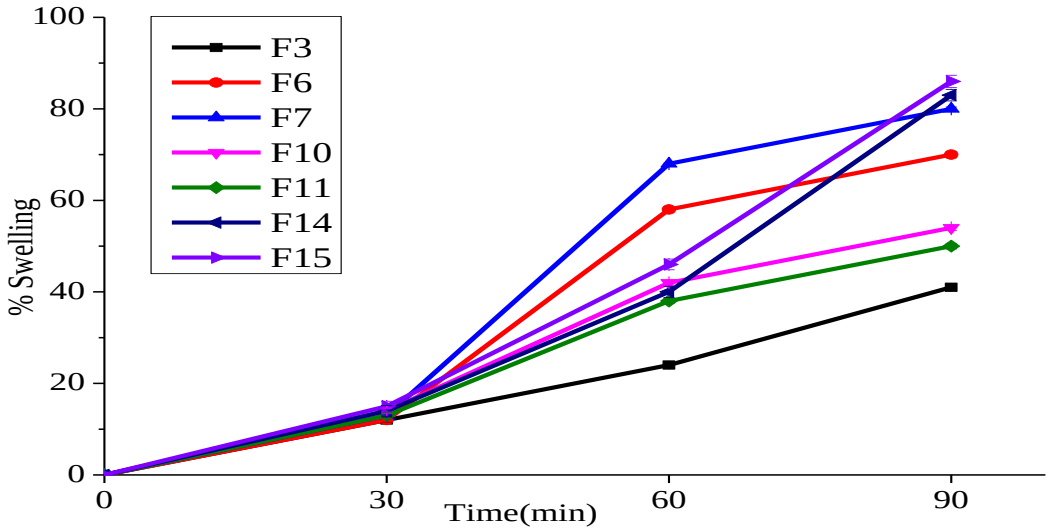


*All values expressed as Mean± standard deviation (SD)

Figure 25: extrudability of best selected *T.serrulatus* oil emulgel formulations

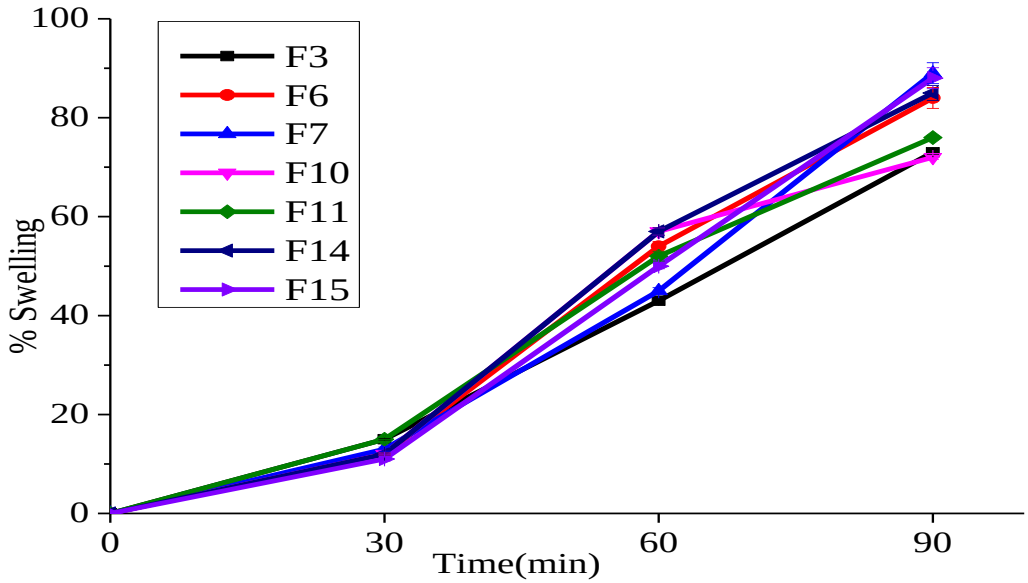
4.6.6. Swelling index

The action of the polymer in pharmaceutical preparation is enhanced when the macromolecule is completely swollen. The swelling behavior provides rheology modification, and emulsification to the topical formulations. Concentration, chain strength and the aqueous uptake nature of the polymer are main factors for swelling of the polymer (Anumolu et *al*., 2011). In the present study, the topical emulgel prepared from NaCMC and HPMC polymer in both null emulgel and *thyme* oil loaded emulgel formulations has shown a rapid increasing percentage of swelling indexes behavior as the time interval increased in contact with aqueous phase (NaOH) from 30min, 60min and 90min. This is may be due to the higher amount of hydroxyl group in each cellulose unit which responsible for the occurrence of hydrogen bonding. These results were in comparable with the findings reported by (Kumar *et al*., 2014; Abdul *et al*., 2012). The formulations with HPMC showed a maximum swelling index in comparison to the NaCMC formulations in both blank emulgel and *thyme* oil emulgel as shown in Figure 26 and 27, respectively. On the other hand, the effect of loaded *thyme* oil emulgel formulations showed slightly increased in the swelling index with compared to the blank emulgel formulations.



*All values expressed as Mean± standard deviation (SD)

Figure 26: percentage swelling index of blank emulgel formulations



*All values expressed as Mean± standard deviation (SD)

Figure 27: percentage swelling index of best *T.serrulatus* oil emulgel formulations

4.6.7. Drug content uniformity

The drug content in emulgel formulations ranged between 67.4–101.3% (Table 8). It was observed that the active agent, *thyme* oil, was present uniformly in five emulgel formulations (F6, F10, F11, F14 and F15) with 95%, 90%, 98.2%, 101.3% and 97.5%, respectively. The results of the drug content for those *thyme* oil emulgel revealed that percent drug content was within the official limit (i.e., 100 ± 10%) based on the USP. However, formulations F3 (67.4%) and F7 (76.6%) failed to meet the official limit.

Table 8: drug content uniformity of excellent *T.serrulatus* oil emulgel formulations

Formulations	F3	F6	F7	F10	F11	F14	F15
Drug content (%)	67.4±1.23	95±0.34	76.6±0.35	90.8±0.32	98.2±0.87	101.3±0.53	97.5±0.78

*All values expressed as Mean± standard deviation (SD)

4.6.8. *In vitro* drug release studies

As Figure 28 showed, the *thyme* oil emulgel formulations release profile from the seven best emulgel formulations prepared with NaCMC or HPMC as gel base. The present study showed that among the seven selected *thyme* oil emulgels, after six hours of release, F6, F10 and F14 exhibited the higher drug release rate (95.95%, 71.43% and 70.65%), respectively, than the emulgel formulations F3, F7, F11 and F15 (46.95%, 40.65%, 37.78 and 45.17%), respectively. In addition to F6 with respect to 2% gelling concentration of HPMC was showed the highest drug release. Of the seven emulgels formulations, F11 with respect to 3% concentration of NaCMC was showed the lowest amount of drug release (37.78%). In overall results of the drug release, formulations with 2% HPMC and NaCMC concentration were showed good drug release. This could be related formulations with 2% concentration of HPMC and NaCMC as gelling agents was the best in terms of percent drug release.

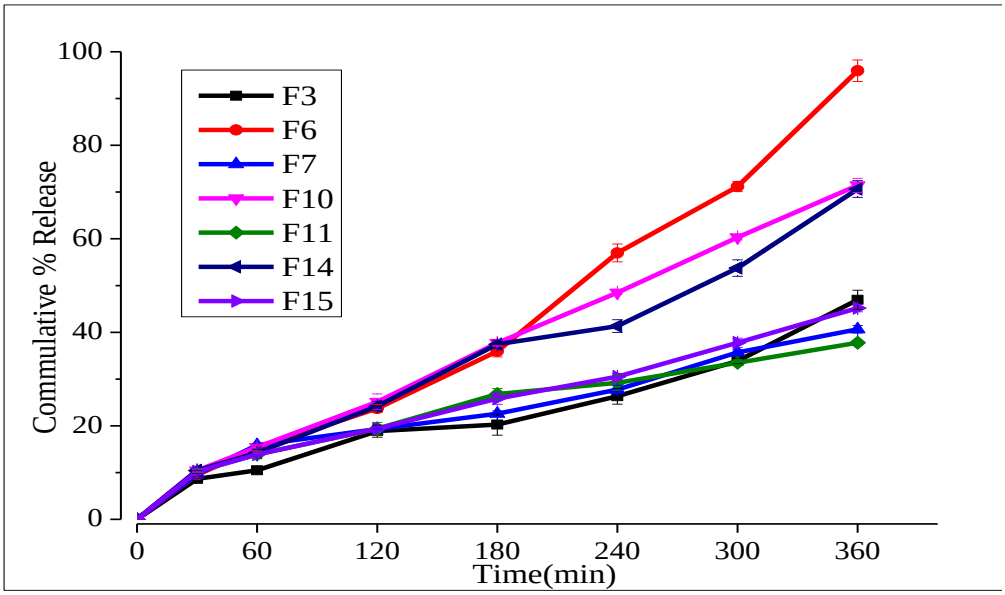


Figure 28: *In vitro* drug release of *T. serrulatus* oil emulgel formulations

4.6.9. *In-vitro* antifungal activity

Thyme serrulatus oil has excellent antifungal activities. This finding was established by previous studies which demonstrated antifungal activity of *Thymus* ssp. and their phenolic components (thymol and p-cymene) against the species of yeasts and filamentous fungi isolates, (Mekonnen *et al*., 2016 ;Nasir *et al*., 2015; Pina-Vaz *et al*., 2004). Moreover, these studies also conclude that the antifungal activity of thyme oil is related to its major components, such as thymol, carvacrol, terpene and cymene each one of these components has a specific mechanism contribute to its antifungal activity.

In the present study, the essential oil extracted from *T. Serrulatus's* leaf demonstrated a strong antifungal activity. Figure 29 depicted the zone of inhibition for *thyme* oil loaded emulgel (F6, standard commercial miconazol cream and the blank emulgel formulation (F6) in one petri dish. Accordingly, the blank emulgel base was microbiologically inert toward the tested *candida albicans* strain, whereas the *thyme* oil loaded emulgel showed a zone of inhibition of 36 mm and the miconazol cream 28 mm. These results suggest that the antifungal activity of *thyme Serrulatus's* oil loaded emulgel was greater than the commercially available product miconazol

cream. As established per clinical and laboratory standards institutes 2020 (CLSI) M23 protocol for category of zone diameter value breakpoints *candida albican* is susceptible for *thyme serrulatus* oil emulgel. The result of this study was found to be comparable with other findings which revealed the various inhibitory effect of *T. serrulatus* oil against *candida albicans*, *Aspergillus* species and *Trichophyton* species (Dagne *et al*., 1998*;* Lakew, 2011; Mekonnen *et al*., 2016). The study reported by Šegvić Klarić *et al*., (2007) was indicated that essential oil of *T. spathulifolius* with thymol content of 36.5% showed strong antifungal activities against *Aspergillus* species*, Cladosporium* species, and *Trichoderma* species.

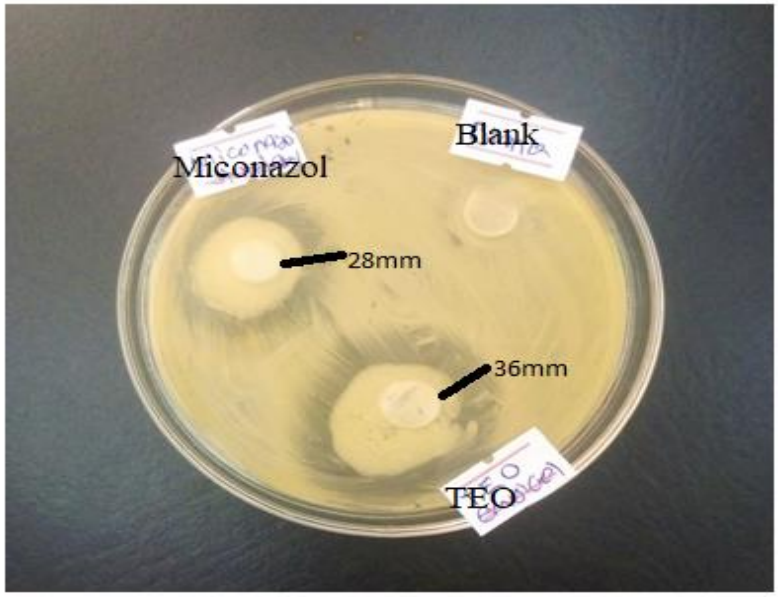


Figure 29: inhibition zone of *T.serrulatus* oil emulgel, blank emulgel and miconazol cream on *candida albican* ATCC

4.6.10. Skin irritation test

The skin irritation test for best selected emulgel formulations (F6, F10 and F14), as indicated on Figures 30B and 30C there was no signs of irritation and dryness on the skin of rats for the blank and *thyme serrulatus* oil loaded emulgel formulations after application and investigation for 24hrs and 48hrs. According to the Draize dermal irritation scoring, the primary dermal irritation index of the blank emulgel, and *T. serrulatus* oil emulgel was PDII=0 this means that there is no edema, erythema and ulceration occur. As shown in Figure 30D, the result was comparable with the standard commercial antifungal drug (miconazol cream) PDII value = 0, after monitoring of the irritation signs for 24hrs and 48hrs. Redness and erythema was observed on the rat in which standard skin irritant formaldehyde (8%) solution applied and the primary dermal irritation index value was 4; this result tells the formaldehyde was moderately – irritant (Figure 30A). Therefore, the current study indicated that the local *T. serrulatus* oil emulgel formulation do not cause irritation up on application of the skin and can be safe and suitable topically applied antifungal drug. This result was similar with the research work reported by Dires *et al*., (2018) in *T. serrulatus* topical base preparation.

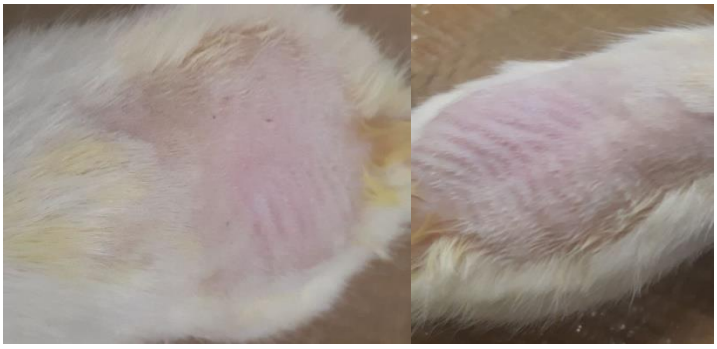


Figure 30A: skin irritation study on rat after application of standard skin irritant

Figure 30B: skin irritation study on rat after application of *T.serrulatus* oil emulgel formulation

Figure 30C: skin irritation study on rat after application of blank emulgel

Figure 30D: skin irritation study on rat after application of a standard miconazol cream

4.6.11. Stability studies

The present study showed that none of the selected *thyme serrulatus* emulgel formulations undergo any variation in odor, color, physical appearance, consistency, homogeneity, spreadability and viscosity when evaluated and inspected at different time intervals of every 30-days at 1, 2, and 3 months. Based on the physico-chemical stability emulgel formulations stored at 30 ± 5 ℃/ 65 ± 5% and at 5 ±3 ℃ temperature conditions was showed comparable result with formulations stored at room temperature during inspection.

Furthermore, there was no change in pH and drug content uniformity result of any emulgel formulations even though they exposed to humidity and heat. Besides, there was no any presence of air bubbles, phase separation, particles, crystals and formation of precipitation in the formulations during the periods of stability study. Therefore, those two storage conditions (30 ±5 ℃/65 ± 5% RH and 5 ±3 ℃) are favorable for storage of *thyme serrulatus* oil emulgel and formulations were found to be stable.

However, for the formulations (F6, F10 and F14) stored at 40 ± 5℃/75 ± 5% RH conditions, the physicochemical characteristics were not changed for a month and drug content was same throughout the stability period whereas change in colour to more pale, decrease in pH to more acidic, decrease in apparent viscosity and increase in spreadability was seen after a month of storage. Based on this outcome 40± 5 ℃/75±5% RH temperature condition was not a suitable storage condition for *thyme* oil emulgel formulations to store more than a month. This result is closely similar with the research work by Bounatirou *et al*., (2010). Tables 9 to 11 illustrated the results of F6, F10 and F14 stability study after three months of storage, respectively.

Table 9: stability studies for F6 *T.serrulatus* oil emulgel formulation after three months under various storage conditions.

Formulation code	Periods Stability studies	Storage condition	Physical test appearan	pH	Spreadability(cm)	*Thyme* Content (%)	Viscosity (cps.)
F6	1st Month	30 ℃/65% RH	None	5.48±0.02	8.4±0.6	95.72±0.53	20734±332
		40 ℃/75%RH	None	5.54±0.01	9.4±0.9	94.21±0.34	10678±32
		5℃	None	5.8±0.06	9.22±1.2	95.4±0.67	22660±341
	2nd Month	30 ℃ /65% RH	None	5.6±0.04	9.34±1	95.43±0.37	21876±338
		40℃/75% RH	Changed	3.78±0.05	13.34±0.9	94.34±0.51	10345±27
		5℃	None	5.7±0.02	9.32±0.9	95.46±0.47	23480±341
	3rd Month	30 ℃ /65% RH	None	5.6±0.05	9.3±0.4	95.12±0.37	22567±339
		40 ℃/75%RH	Changed	4.0±0.09	15.45±0.4	94.32±0.52	9876.4±35
		5℃	None	5.9±0.08	10±0.6	95.32±0.31	23678±346

Table 10: stability studies for F10 *T.serrulatus* oil emulgel formulation after three months under various storage conditions.

Formulation code	Periods Stability studies	Storage condition	Physical test appearanc	pH	Spreadability (cm)	*Thyme* Content (%)	Viscosity (cps.)
F10	1st Month	30 ℃/65% RH	None	5.58±0.02	8.7±1.2	97.72±1.23	7734±152.1
		40 ℃/75% RH	None	5.54±0.03	9.5±1.19	96.54±2.1	7337.4±121
		5 ℃	None	5.55±0.01	9.0±1.15	97.63±1.2	7553.6±151
	2nd Month	30 ℃ /65% RH	None	6.0±0.03	9.82±1.15	97.2±2.4	7288±141.8
		40℃/75% RH	Changed	3.15±0.02	15.12±1.3	95.32±1.4	7231±139
		5 ℃	None	6.3±0.04	10.6±1.18	97.45±1.12	7655.4±161
	3rd Month	30 ℃ /65% RH	None	5.82±0.05	10.5±1.2	97.45±1.23	7654±161
		40℃/75% RH	Changed	3.32±0.02	16.11±1.4	94.12±2.31	6789±332
		5℃	None	5.7±0.04	11.4±1.14	97.65±3.1	7865.3±167

Table 11: stability studies for F14 *T.serrulatus* oil emulgel formulation after three months under various storage conditions.

Formulation code	Periods of Stability studies	Storage conditions	Physical test appearance	pH	Spreadability(cm)	*Thyme* Content (%)	Viscosity (cps.)
F14	1st Month	30℃/65% RH	None	5.6±0.03	7.64±0.6	99.87±0.98	26643±97
		40℃/75%RH	None	5.5±0.04	7.77±0.7	96.98±0.86	23765±47.2
		5℃	None	5.52±0.02	7.32±0.9	99.98±0.65	26885±100
	2nd Month	30 ℃/65% RH	None	5.9±0.04	9.9±0.9	97.56±0.73	26432±95
		40℃/75%RH	Changed	3.73±0.03	10.12±1	96.76±0.62	20345±34
		5 ℃	None	6.1±0.03	8.9±0.7	98.67±0.51	27899±99
	3rd Month	30℃ /65% RH	None	5.72±0.05	9.9±0.9	98.56±0.54	33475±114
		40℃/75%RH	Changed	3.26±0.03	15.7±0.8	95.23±0.32	20675±37
		5 ℃	None	5.6±0.02	9.2±0.6	97.54±0.43	34568±123

5. CONCLUSIONS

In this study, *T.serrulatus* provided a percentage yield of 0.6 % essential oil. According to the GC/MS analysis, the main components of *T.serrulatus* essential oil are thymol (36.92%), O-cymene (26.05%), carvacrol (19.15%), and -terpinine (8.24%). NaCMC and HPMC can be added to the aqueous phase of the emulsion at concentration of 2% and 3%, resulting in better physico-chemical characteristics, spreadability, and extrudability and showing shear thinning pseudo plastic behavior of *T.serrulatus* oil emulgel formulations. The formulations' pH ranged from 5.52 to 7.23, which is an acceptable pH range. The drug release of *T. serrulatus* oil emulgel was satisfactory after 6 hours. The topical application of *T. serrulatus* emulgels to the skin caused no irritation. Furthermore, *T. serrulatus* essential oil emulgel formulations demonstrated potent antifungal activity against Candida albican, a common cause of skin infection. The *T. serrulatus* oil emulgels also showed excellent stability profile for at least three months under various storage conditions. As a result, it is concluded that *T. Serrulatus* oil may be a promising candidate for the development of a natural alternative phyto-therapeutic agent to be delivered topically as an emulgel dosage form for the treatment of *Candida albican* skin infections.

6. SUGGESTIONS FOR FURTHER WORK

This study suggests the following for further works:

- *In vivo* and *in vitro* correlation for drug release.
- Further *in vivo* studies should be performed to explore different skin parameters like melanin, hydration level and sebum.
- Specific studies in experimental animals (*in vivo)* are recommended to determine the safety and effectiveness of TEO in the treatment of fungal infections and other diseases.
- Further studies are needed to pinpoint the chemically bioactive components present in the extract which are causing for the antifungal activity seen in this study.
- Further chemical isolation, toxicological study and clinical trial are recommended for this *T. serrulatus* species of Ethiopian *thyme*.

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7. ANNEXES

Annex 1: Ethical clearance

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Addis Ababa University

School of Pharmacy
Ethical Review Committee

ቀን Date: January 10, 2023

ቁጥር Ref. No.: ERB/SOP/458/15/2023

To: **Atsede Solomon**
School of Pharmacy

Re: Ethical Clearance

It is to be recalled that you submitted a research proposal entitled "**Comparative Evaluation of Essential Oils from Local Thyme in Topical gel and Emulegel Formulation for the Treatment of Skin Infection".** The committee thoroughly reviewed the proposal based on its operational guideline and found that, it fulfills all the ethical requirements stipulated in the guideline. This is, therefore, to inform you that the proposal is ethically approved for implementation.

With best regards,

Shemsu [illegible]ier (PhD)
Chairperson, ERC
School of Pharmacy
College of Health Sciences
Addis Ababa University

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