Formulation and Evaluation of Herbal Shampoo from Seed Extract of *Trigonella Foenum Graecum* for Antifungal and Antibacterial Activity

Thesis Submitted to Office of Graduate Studies in Partial Fulfillment of the Requirement of the Degree of Master of Science in Applied Chemistry (Industrial Chemistry)

By

Birtukan Deme

Adama, Ethiopia

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APPROVAL SHEET

We, the undersigned, members of the Board of Examiners of the final open defense by Birtukan Deme have read and evaluated her thesis entitled "Formulation and Evaluation of herbal shampoo from seed extract of *Trigonella foenum graecum* for antibacterial and antifungl activity and examined the candidate. This is, therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the Degree of Master of Science in applied Chemistry (Industrial Chemistry).

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Declaration

I hereby declare that the work which presented in this research entitled as "Formulation and Evaluation of Herbal Shampoo from Seed Extract of *Trigonella Foenum Graecum* for Antifungal and Antibacterial Activity" in partial fulfillment of the requirement for the award of the Degree of Masters Science in Applied Industrial chemistry authentic record of my original work under the supervision of Dr. Milkyas Endale, Dr. Tegene Desalegn ,School of Applied Natural Sciences, Adama science and Technology University.

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TABLE OF CONTENT

CONTENTS **PAGE**

List of Tables

List of Figures

List of Acronyms and Abbreviations

BHA	Butylated Hydroxyanisole
BHT	Butylated hydroxyl-toluene
DEA	Diethanolamine
EDTA	Ethylene diamine tetra acetic acid
IAR	International Agency for Research on Cancer
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
NDELA	Nitrosodiethanolamine
SDA	Sabouraud Dextrose Agar
SLS	Sodium lauryl sulfate
STPP	Sodium Tri-Poly Phosphate

Abstract

Shampoo is one of the most common components of hair treatment. The primary function of shampoo is aimed at cleansing of the hair necessitated due to accumulated sebum, dust, scalp debris etc. Various shampoo formulations are associated with hair quality, hair care habit and specific problems such as treatment of oily hairs, dandruff and for androgenic alopecia. Synthetic preservatives and detergents have sometimes been the cause of adverse effects among consumer. An alternative approach in reducing the adverse effects of synthetic ingredients uses herbal extracts whose functionality is comparable to that of synthetic ingredients. In this study shampoo was formulated with anionic surfactant enriched with different proportions of fenugreek seed extract and evaluated its physicochemical properties and Antimicrobial activity using Disc Diffusion method. Fenugreek extract containing trigonelline and trigonellic acid is used as a hair growth stimulant. The herb also a good source of lecithin which is a natural emollient helps to strengthen and moisturize hair and keeps the dandruff away. The pH of the formulated shampoos was in the standard range of 6 to 8. Evaluation of antibacterial activity revealed shampoo enriched with 5% and 10% ethanol extract showed promising activity against S. aureus, *E. feacalis, P.aeroginosa, E. coli and shampoo enriched with water extract of Trigonella foenum showed promising activity against Staphylococcus aureus whereas shampoo enriched with 5,10 and 20% water and ethanol extracts of Trigonella foenum showed promising antifungal activity against Candida albicans. Further research is required to study herbal shampoo quality especially on the conditioning performance and to identify the constituents which are responsible for the performance, also to prove the safety, stability and efficacy of this formulation to make it suitable as a commercial product.*

Keywords: Anionic surfactant, herbal shampoo, *Trigonella foenum graecum*

1. Introduction

1.1. Background

Hair is one of the external barometers of internal body conditions. It is a filamentous biomaterial consisting of the cuticle, the cortex and the medulla, all held together by the cell membrane complex (Robbins, 2002). Hair-care products may be defined as the preparation which are meant for cleansing, modifying the texture, changing of the color, giving life to the stressed hair, providing nourishment to the hair and giving the healthy look to the hair. There are various types of hair: normal hair, oily hair, dry hair that vary from person to person. The problems of hair: hair falling, white hair, dandruff and split end hair etc. The reasons of hair problem are tension**,** scalp infection**,** hormones disturbances, lower vitamin, food, minerals**,** and large chemical shampoo use.

The term 'shampoo' is believed to have originated from India, where it referred to a head massage, often with pleasant smelling oils. During the nineteenth century, the word came to mean the application of soap to the hair in order to clean it. Now days it may be described as a cosmetic preparation meant for the washing of hair and scalp, packed in a form convenient for use. Shampoos are primarily being products aimed at cleansing the hair and scalp (Ashok and Rakesh, 2010). Its primary function is of cleansing the hair of accumulated sebum, scalp debris and residues of hair-grooming preparations. Moreover, shampoo also has other functions that include lubrication, conditioning, bodybuilding, prevention of static charge builds up, medication and so on. Finally, the complete shampoo formulation must be medically safe for long-term usage (Nand *et al*.,2006). Shampoo formulation composed primarily of chemicals called surfactants that have the special ability to surround oily materials on surfaces and allow them to be rinsed away by water.

Dandruff is a common disorder affecting the scalp condition caused by yeast *Pityrosporum*. Dandruff cannot be completely eliminated but can only be managed and effectively controlled. Symptoms of dandruff mainly include Presence of fragments, Itching of the scalp, and Redness around the scalp. The synthetic shampoo contains cationic, anionic and non-anionic surfactant mix in this surfactant having good foaming character but its toxic and caused irritation of eye. In hard water the surfactants leave a deposit of sodium, calcium and magnesium salts on the hair shaft. So these synthetic shampoos are found to have side effects like drying effect on the hair. These shampoos leave the hair too dry to handle (or) comb.

A more radical approach in reducing the synthetic ingredients is by incorporating natural extracts whose functionality is comparable with their synthetic counter parts. Herbal drugs or their formulations are viable alternative to synthetic drugs. Natural botanicals may be used in their crude form or they may be extracted or derivative to make suitable for use in cosmetics. A wide range of active principles of various plants including vitamins, hormones, phyto-hormones, bioflavonoids, enzymes, tannins, essentials oils are being used in cosmetics formulations. The awareness and need for cosmetics with herbs is on the rise, primarily because it is believed that these products are safe and free from side effects.

The current investigation was focused on formulation of a self-preserving shampoo using fenugreek seed with emphasis on safety and efficacy. Selection of *Trigonella foenum* graecum seed extract was based on its traditional use as antimicrobial and antiseptic activity. Fenugreek extract containing trigonelline and trigonellic acid is used as a hair growth stimulant. Bioactive compounds isolated from fenugreek seeds include saponins (fenugreekine, diosgenin), alkaloids (trigonelline, gentianine, carpaine), amino acids (4-hydroxyisoleucin, arginine), flavonoids (Badhe *et al*., 2015). The herb also contains *lecithin*, a natural *emollient* known to strengthen and moisturize hair. Shampoo was formulated containing different proportions of fenugreek plant extract. Evaluation of physicochemical property and performance test were performed and examined for its antimicrobial activity.

1.2 Statement of problem

A shampoo is a cleaning aid for the hair and is counted among the foremost beauty products. As the scalp is one of the most absorbent part of the body, products applied to the scalp go directly to the blood, without being filtered in any way (Surupsing *et.al*.,2017). So it is very important to know and understand the effects of ingredients used in shampoo formulations. Most of the commercially available shampoos are loaded with chemicals that are hazardous to skin and health (Arora *et al*., 2001). Dandruff is a common embarrassing scalp disorder affecting a large chunk of population.

Currently, treatment options for the management of dandruff include therapeutic use of antidandruff shampoo containing Zinc pyrithione, Selenium sulphide, Salicyclic acid, Imidazole derivatives, Sulphur Coal tar, etc (Faergemann *et al*., 1996). However, these agents have certain limitations, either due to poor clinical efficiency or due to compliance issues.

The anti-dandruff shampoo only slow down the scalp flaking and have their own disadvantages like loss of hair, increased scaling, itching, irritation, nausea, headache, vomiting, photosensitivity. Furthermore, these drugs are unable to prevent reoccurrence of dandruff which is the commonest problem (Ravichandran *et al*., 2004).

Synthetic ingredients in shampoo caused various conditions such as scalp irritation, hair loss and severe hair damage. Common to most synthetic ingredients based shampoos is an ingredient called a surfactant, which has ability to reduce surface tension of water. The result is that, as the hair is rinsed, the sebum is washed away. Cocamidopropyl betaine is an amphoteric surfactant which is frequently used in cosmetic products especially shampoos. Cases of contact allergy due to it are reported increasingly. Di-ethanol amine (DEA) is a common ingredient in bath products. DEA and any combinations of DEA, including the widely used cocamide DEA has been found to pose a serious health risk to consumers. The ill effects of DEA were reported to be a potential carcinogen, and that in even small doses, repeated use of DEA increases the risk of cancer. Nitrosodiethanolamine (NDELA) is a recognized carcinogen. It is reported that when SLS comes into contact with alkalonamides it becomes contaminated with NDELA (Schothorst *et.al*, 2001).

1.3 Significance of the study

The shampoo sector is probably the largest unit sale among the hair care products since shampoos are one of the cosmetic products used in daily life. Synthetic preservatives and detergents have sometimes been the cause of adverse effects on consumers. An alternative approach in reducing the adverse effects of synthetic ingredients uses herbal extracts whose functionality is comparable to that of synthetic ingredients. Herbal extracts formulations are viable alternative to synthetic drugs. This project is important for formulation of a self-preserving and anti-dandruff shampoo having low concentration of the detergent using seeds extracts of *Trigonella foenum graecum* as an ingredient, which is locally available and cheap.

1.4 Objectives

1.4.1 General objective

The main objective of this study was to formulate and to evaluate herbal shampoo from seed extract of *Trigonella foenum graecum* for antifungal and antibacterial activities of prepared formulations

1.4.2 Specific objectives

- To carry out extraction on the seeds of *Trigonella foenum graecum* with water and ethanol.
- To formulate herbal shampoo enriched with different extract concentrations of *Trigonella foenum graecum*
- To evaluate the physicochemical parameters visual inspection, pH, percent of solid contents, foam volume and stability, dirt dispersion for the formulated herbal shampoo and shampoo from market
- To evaluate the antifungal activities of formulated shampoos and shampoo from market against *Candida albicans*
- To evaluate antibacterial activity of formulated shampoo and shampoo from market against *Staphylococcus aureus, Escherichia coli, pseudomonas aeroginosa, Entrococcus feacalis and Klebsiela pneumonia.*
- To compare the shampoo formulation with the leading brands of antidandruff shampoos available in the market for its effectiveness in terms of viscosity, foam height/stability, pH, and antimicrobial activity.

2. Literature Review

2.1 History and significance of *Trigonella foenum*

The plant *Trigonella foenum-graecum* commonly known as Fenugreek is an aromatic herb. Locally it is known as Abesh given below in figure 1. Fenugreek (*Trigonella foenum*-graecum L.) is one of the most ancient medicinal plants belonging to family Leguminosae/Fabaceae. Fenugreek is widely cultivated in China, India, Egypt, Ethiopia, Morocco, Ukraine, Greece, Turkey, etc. Various parts of fenugreek, mainly its leaves and seeds have been widely used in the Indian food. It has several cosmetic uses including antidandruff property (Trease and Evans, 2002).

Fig 1. Seed of T*rigonella foenum* graecum (Photo taken by Birtukan, march, 2010 E.c)

2.1.1 Phytochemistry of *Trigonella foenum*

Trigonella foenum-graecum is the unique source of various types of compounds having diverse chemical structures. Several compounds have been isolated from fenugreek seeds, this include saponins (fenugreekine, diosgenin), alkaloids (trigonelline, gentianine, carpaine), amino acids (4-hydroxyisoleucin, arginine) and flavonoids (Sumayya *et al*., 2012). The phenolic compounds isolated from seeds of *Trigonella foenum-graecum* is coumarin, scopoletin, chlorogenic acid, caffeic acid and p-coumaric acid. Seeds have been containing pyridine (trigonelline and gentianine), piperidine (carpaine) and steroidal (choline) group of alkaloids in smaller quantities.

Terpenes and Terpenoids that present in it contain sabinene, 3-carene, menthol, α-pinene, β-pinene, β-terpineol, cineol, anethol, β-terpinyl acetate, carvone,1-p-menthen-8-yl acetate, 1-pentanol,1-hexanol,2-methyl-2-butene-1-ol, 2-methyl-2-butenal,2- pentylfuran, formic acid, propanoic acid, γ-butyrolacetone, 5-alkylated γ-lactones, 3-amino-4,5-dimethyl-2(3H)-furanone (Badhe *et al*., 2015). The Fenugreek seeds have Lecithin, a natural emollient which helps in strengthening and moisturisation of hair. It also keeps the dandruff away and keeps the hair free of lices. Further, these plant materials serve as good antimicrobial agents. (Nasroallah, 2013). The chemical composition of fenugreek seeds contains various components like water, crude fiber, crude fat, starch, minerals, and crude protein. The presence of pharmacologically active phytochemical in methanolic and acetonic extracts *Trigonella foenum* were recently reported (Mandegary *et al*., 2012).

The seed of the fenugreek plant contains many active compounds with pharmaceutical applications. fenugreek seeds are very effective in curing dandruff, hair thinning, dull hair, reversing baldness & hair fall. Being a rich source of protein helps in getting healthy hair growth & acts brilliantly on all the hair related concerns. *Trigonella foenum graecum* is a potent dihydrotestosterone (DHT) blocker and rich source of multivitamin B which plays major role in hair stimulant property. DHT is synthesized in adrenal glands, hair follicles and prostate by action of enzyme 5α-reductase, which plays a major role in occurrence of alopecia. Blocking this DHT synthesis helps in preventing hair loss and treating alopecia (Kalyari and Apurva., 2016).

2.2 Soaps and shampoo

Cleansing agents like soaps and detergents are able to make oily and greasy substances more water soluble by reducing the surface tension between the two. Soaps and detergents contain a type of molecule known as a surfactant. A surfactant molecule contains two parts: a hydrophilic polar head group and a hydrophobic non-polar tail group. The polar head group is attracted to other polar molecules, such as water, and repelled by non-polar molecules such as oils. Conversely, the non-polar tail is attracted to oils and repelled by polar molecules. A surfactant molecule will therefore position itself on the interface between an oil and water. When a surfactant is added to water that contains some oil, the oil becomes surrounded by the surfactant, with its polar head groups pointing outwards towards the water. This forms an emulsion, causing small clusters of oil molecules to become more water soluble, allowing the two layers to mix and the oil and dirt to be rinsed away with water.

Soaps are not suited to washing hair. They can cause irritation to the scalp and leave a thin film behind, making hair appear dull. Hair needs to be at a mildly acidic pH (about pH 4–5) in order to be at its strongest. At this pH all the non-covalent forces that hold the hair strand together are maintained. Soap has a rather basic pH (approximately pH 8.5), which can disrupt some of the covalent interactions such as disulfide bonds, thereby weakening the hair.

In fact, substances that are highly basic (pH 12 and above) destroy all the non-covalent interactions holding the hair together, causing it to dissolve completely. Continual use of soap on hair decreases the number of covalent interactions, thereby increasing the amount of weakening and damage. The calcium and magnesium ions in hard water react with fatty acids in soap to form insoluble lime soaps, which are ineffective at cleaning.

2.3 Herbal shampoo

Herbal shampoo is a cosmetic preparation which uses herbs from plants and it is meant for washing of hair and scalp just like a regular shampoo. It is an alternative to the synthetic shampoo available in market. Herbalists today, believe in helping people build their good health with the help of natural sources (Shivanand *et al*, 2010). Herbal extracts because of some special properties such as hair fortifying and anti-hair loss effects in addition to their fewer side effects, lower costs and being more available are also now increasingly used in shampoos.

Herbal shampoos are rich in herbs that fight scalp infections, strengthen the roots, condition and revitalize the hair and induce hair growth with significantly no side effects observed when compared with synthetic shampoo. Herbal products are cost effective. Proper use of herbs for anti-dandruff condition can reduce the side effects and irritation potential of chemicals to a large extent.

2.4 The action of shampoo on hair

The original prime function of the shampoo is to clean the hair. There are three types of hair soil to be dealt with. These are oily soil or sebum, soluble soils, and insoluble particulate soils. All three types of soil require being wetted, thus surface tension of the water is reduced by the shampoo surfactant allowing full contact with the soils surface. Any soluble soil is then removed in the aqueous medium. A process known as roll-up, i.e. the displacement of the soil by the detergent solution, removes oily soil or sebum.

Insoluble particulate soils tend to be removed by electrostatic repulsion between the soil and the hair fiber assisted by repulsion between the surfactant molecules adsorbed onto the hair fiber and those dissolved onto the soil. In the process of soil removal, the detergent micelles make contact with the lipid surface for a finite time during which they take up an increment of lipid. This is assimilated to form lipid–detergent co-micelles which detach and float away into the bulk aqueous solution.

2.5 Ideal characters of shampoo

An ideal shampoo should possess following properties (Preethi *et al*., 2013)

- It should effectively and completely remove the dust, excessive sebum, dandruff etc.
- It should leave the hair non dry, soft, lustrous with good manageability.
- It should produce a good amount of foam.
- It should be easily removed by rinsing with water.
- It should impart a pleasant fragrance to the hair.
- It should not cause irritation to skin or eye

2.6 Surfactant

Surfactants (or 'surface active agents') are organic compounds with at least one lyophilic ('solvent-loving') group and one lyophobic ('solvent-fearing') group in the molecule. If the solvent in which the surfactant is to be used is water or an aqueous solution, then the respective terms 'hydrophilic' and 'hydrophobic' are used and given below in figure 3.

In the simplest terms, a surfactant contains at least one non-polar group and one polar (or ionic) group**.** They act through the weakening of the physicochemical adherence forces that bind impurities and residues to the hair. Surfactants dissolve these impurities, preventing them from binding to the shaft or the scalp.

The cleansing ability of a shampoo depends on how well it removes grease as well as the type and amount of surfactants used (Shapiro and Maddin, 1996). In the bulk aqueous phase, surfactants form masses, such as micelles, where the hydrophobic tails form the core and the hydrophilic heads are immersed in the surrounding liquid.Surfactants form the 'heart' of most shampoo formulations and perform many different roles in these systems.

Their primary function is to remove soils, such as sebum and solid particulates, from the hair, but they also are important for foaming, building product viscosity, suspending actives and the solubilisation of fragrances. In addition to this, they have to be selected and blended to be as mild to the skin, hair and eyes as possible.

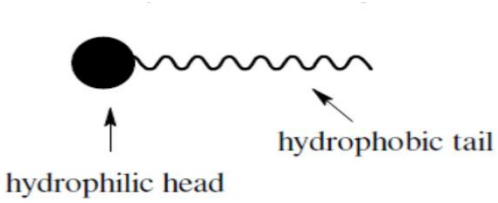

Fig 2. Schematic of a surfactant molecule

2.6.1 Classification of surfactants

Surfactants are classified according to hydrophilic polar group as anionic, cationic, amphoteric (zwitterionic) and nonionic (Bouillon, 1996).

A. Anionic surfactants

Anionic surfactants are characterized by a negatively-charged hydrophilic polar group. The best known anionic surfactants are sulfated fatty alcohols, alkyl sulfates, and their polyethoxylated analogues, alkyl ether sulfates. They possess an outstanding cleansing ability and produce rich lather. A number of anionic surfactants such as alkyl ether carboxylate, acyl peptides, alkyl sulfosuccinate, and olefin sulfonate, which are well tolerated by the skin, and are used together with other anionic and amphoteric surfactants to optimize shampoo properties. Although very good in removing sebum and dirt, anionic surfactants are strong cleaners and may cause an increase on electrical negative charges on the hair surface and increase frizz and friction. In order to minimize damage, other surfactants called secondary surfactants such as nonionic and amphoteric surfactants are added to the formulation (Abraham *et al*., 2009).

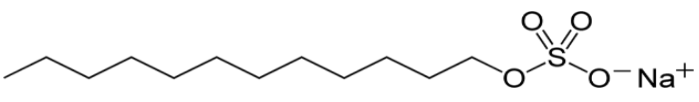

Fig 3. Structure of sodium lauryl sulfate

B. Cationic surfactants

Cationic surfactants carry a net positive charge on the hydrophilic portion of the molecule in water. Its usage in shampoo formulation is more limited than that of surfactant classes. they are also not as effective detergents due to their ability to strongly bind to hairs negatively charged surface and not effectively removed during rinsing. They are quaternary ammonium compounds which are characterized by a positively-charged hydrophilic polar group. Given its amino acid structure, the keratin found in human hair has an excess of negatively-charged acid groups. The positively-charged quaternary ammonium compounds of cationic surfactants thus attach by salt bonds to the negatively charged hair and remain after rinsing.

Due to its high level of cysteine, damaged hair contains a higher number of negatively charged acid groups and thus absorbs more quaternary ammonium compounds than intact hair. Because of this quality, quaternary ammonium compounds are used as conditioning agents for damaged hair. Although they make hair easier to comb and manage, and they have anti-static properties, they are poor cleansers and do not lather well. They are also potentially strong irritants, and are thus only used with less irritating nonionic surfactants in shampoos designed for chemically-treated or very dry hair. Their incompatibility with anionic surfactants limits their use in other shampoo formulations. All are examples of quats, so named because they all contain a quaternary ammonium ion. They tend to neutralize the negatively charged net of the hair surface and minimize frizz. They are often used as shampoo's softeners.

C. Amphoteric surfactants

Amphoteric (zwitterionic) surfactants contain both negatively and positively charged hydrophilic polar groups. At low pH values they behave as cationic agents and at higher pH values as anionic agents, and are thus classified between cationic and anionic surfactants. Amphoteric surfactants form complexes with anionic surfactants and reduce their tendency to attach to proteins.

In combination with anionic surfactants, amphoteric surfactants are mainly used to optimize anionic-based shampoos: amphoteric acetates/diacetates in particular demonstrate excellent skin and mucous membrane tolerability, have a conditioning benefit on the hair, and are suitable for use in mild shampoos. Examples include betaine, sulfonate betaine, amphoteric acetate/diacetate. structure of coco betaine shown below (figure 5)

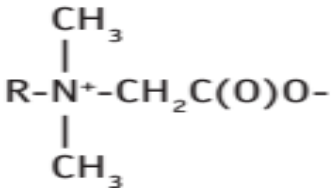

Fig **4.** Structure of coco betaine

D. Nonionic surfactants

Nonionic surfactants differ from other surfactants in that they do not possess a charged polar group and are hence compatible with all other surfactants. They are also among the mildest form of surfactant; they make good cleansers with dispersion and emulsification properties, but lather poorly. In combination with alkyl ether sulfates or amphoteric surfactants, they serve to enhance tolerability in very mild cleansers such as baby shampoos. Examples of nonionic surfactants are fatty alcohol ethoxylates, sorbitan ether esters, and alkyl polyglucosides. Nonionic surfactants have covalently bonded oxygen-containing hydrophilic groups, which are bonded to hydrophobic parent structures.

The water-solubility of the oxygen groups is the result of hydrogen bonding. Hydrogen bonding decreases with increasing temperature, and the water solubility of nonionic surfactants therefore decreases with increasing temperature. Many long chain alcohols exhibit some surfactant properties (Abraham *et al*., 2009).

2.7 Scalp infections and dandruff

The common skin infections in humans is generally due to fungal infections. Most fungal parasites are however, generally opportunistic pathogens and are members of the normal skin flora. Mycotic infection of the skin may be categorized into superficial and deep fungal infections. *Malassezia furfur (Pityrosporum ovale),* a lipophilic fungus, affects the hair and causes diseases called dandruff (Ranganathan *et al*., 2001) and also called pityriasis versicolar, tinea circinata, seborrheic dermatitis. However recent studies show that a closely related species, *Malassezia globosa,* is the major cause of dandruff, and *M. furfur,* is a secondary pathogen which proliferates in number extensively, along with *M. globosa,* in the affected area.

Dandruff is a pathological condition, causing rusty white flakes of skin to separate and fall from the scalp. Also the flakes of dandruff and peeling of skin, is observed beyond the scalp region, in the eye corners, eyelashes, eyebrows, regions in the ears and corners of nose and also near cheekbones; all courtesy to the same pathogenesis. People who suffer from dandruff have over active sebaceous glands, which make their scalp oily. Often though opposite to common belief, dandruff activity is higher in males, due to the effect of male hormone testosterone which is associated with increasing sebaceous gland secretions. There is reportedly no complete cure for this disease and temporary reservation is conventional treatment. *Malassezia furfur* is pleomorphic yeast like fungus. It is referred to as *Pityrosporum orbiculare* and *P. ovale* depending on the morphology of the cells.

Being a lipophillic yeast, Malassezia metabolises sebum components and releases oleic acid as an end product which initiates an immune-signaling pathway, attracting neutrophils and other phagocytes. This results in cytokine release, inflammatory response (hence the term 'dermatitis'), release of ROS (Reactive Oxygen Species). This kind of immune reaction causes local epidermal cell damage, and in response to it and also triggered by excess oleic acid release, hyper-proliferation of epidermal cells occurs causing erroneous differentiation process and irregular keratinization. So instead of healthy cells, degenerate cells clump and cell debris accumulate seen as flakes of dandruff.

dandruff is commonly aggregated by changes in humidity, truma (scratching), seasonal and emotional stresses. dandruff may improve in summer (as UV rays from sunlight counteract p.ovale) and it may worse in winter. pityrosporum organism are linked to T-cell depression and they inhibit cell division & thus reduce sealing by decrease in epidermal turnover. Dandruff may cause various symtome seborrheic dermatitis, psoriasis fungal infection, or scalp and sorness, itching infestation of head lice.

Dandruff is clinical condition caused by *Malassezia (Pityrosporum)* species is of great cosmetic concern all over the world. *Pityrosporium ovale* is strongly suspected to play a role in the manifestation of the seborrheic dermatitis (Hay and Graham, 1997). Dandruff is known to be controlled by fungi static ingredients in Antidandruff shampoos. herbal formulation has growing demand in the world market (Agarwal *et* al., 2009).

The natural remedies are more acceptable in market because it's safe and fewer side effect antidandruff shampoo and nutritional shampoo containing vitamin, amino acids proteins hydrolysate (Ravichandran *et al*., 2005).

2.7.1 Management of dandruff/antidandruff agents

Different types of formulations like shampoos, creams, lotions, emulsions, hair oils and other cosmetic formulations are available in the market that are used to control dandruff. These formulations include therapeutic use of anti-dandruff agents that are classified into three groups according to their mechanism of action.

a) Fungicidal substances: For example, zinc pyrithione ((ZnPTO), imidazoles.

b) Cytostatic substances: For example, tar, selenium sulphide, octopirox.

c) Keratolytic substances: For example, salicyclic acid, Sulphur compounds.

Fungicidal substances

These compounds are a group of drugs with etiological action, inhibiting the growth of *Malassezia furfur*. Ketoconazole, an imidazole derivative with wide fungicidal spectrum is effective against many fungi both *in vivo* and *in vitro* (Warner *et al.,* 2001*).*

Other imidazoles that are effective against *Malassezia* isolate are bifonazole, fluconazole, metronidazole, climbazole, econazole and miconazole (Prabhamanju *et al.,* 2009). Of all the imidazoles, ketocanozole has become the leading contender among treatment options of dandruff.

Cytostatic substances

Substances from this group act through regulation of the excessive speed of epidermal cells division inhibiting exaggerated peeling of the scalp area, thus eliminating the scales. This group includes: tar, selenium sulphide and octopirox. The action of these substances is however restricted only to the period of actual use, meaning that after cessation of treatment relapses of the disease are quite often noticed. Their action is only symptomatic and lack of etiological effect associated with insignificant influence on the population of *M. furfur* (Maleszka and Zatuga, 2002).

Keratolytic substances

Keratolytic formulas, whose action relies on removal of scales of epidermal -keratinized layer include: salicyclic acid, tars and Sulphur derivatives. Shampoos containing salicyclic acid are found to be effective in achieving reduction of a population of *Malassezia*.spp.

Salicyclic acid is a beta hydroxyl acid keratolytic agent that is useful in removing scaly and hyperkeratotic skin, it decreases cell-to-cell adhesion between corneocytes.

2.7.2 Disadvantages of antidandruff agents of synthetic origin

Common way to control dandruff is to use agents mentioned above like imidazoles, selenium sulphide, coal tar, zinc pyrethion, salicyclic acid etc. either alone or in combinations (Faergemann *et al.,* 1996). These agents are commonly available either in the form of ointments, lotions, gels or as ingredients of antidandruff shampoos. Currently available treatment options have various limitations due to either poor clinical efficacy, or compliance issues. Also, these drugs are unable to prevent reoccurrence, which is the most common problem (Ravichandran *et al.*, 2004). Moreover, side effects associated with them cannot be neglected. Like the most widely used antimycotic shampoos in dandruff therapy are products with ketaconazole and zinc pyrithione. However, the risk of developing yeast species resistance to the imidazole agents has to be taken under consideration while treatment. It must be however remembering that widespread use of imidazole derivatives in long term can cause increased resistance to *Malassezia* yeasts to the formulas used and make therapy more difficult.

Antidandruff products such as keratolytic and cytostatic agents work only symptomatically, and often reoccurrence of dandruff after stopping treatment is observed (Adamski and Deja, 2006). Continuous use of anti-dandruff shampoos based on synthetic ingredients make hair brittle and causes dryness of the scalp. It is also reported that continuous use of one product loses its effectiveness over time (Bulmer, 1999). Coal tar based anti-dandruff shampoos not only stain colored or grey hair and make the scalp more sun sensitive but also carcinogenic. There is also evidence that coal tar itself is mutagenic, carcinogenic in animals and phototoxic as reviewed by the WHO‟s, International Agency for Research on Cancer (IARC).

2.7.3 Herbal anti-dandruff shampoos

Herbal anti-dandruff shampoos are the cosmetic formulations which contain herbal ingredients such as plant extracts and essential oil (Swati *et* al., 2012). These herbal shampoos are generally used to remove the dandruff, to add natural color to the hair, to remove the extra oil content of the hair, for the healthy growth of the hair, to remove the dust, dirt and scales of the scalp, to prevent hair falling, to impart softness and smoothness to the hair shaft etc.

Prabhamanju *et al*. (2009) had conducted comparative study on the efficiency of chemical and herbal antidandruff ingredients and their performance in market shampoos. The study concluded good antidandruff activity of commercial shampoos with tea tree oil, rosemary oil, henna and lemon, in spite of their higher MIC than the synthetic ingredients. In another study, Ravichandran et al. (2004) had reported an excellent action of antidandruff shampoo containing the extracts of *Rosmarinus officinalis*, *Vetiveria zizanioides*, *Nigella sativa*, *Santalum album*, *Ficus bengalensis*, Citrus limon and oil of *Melaleuca leucadendron*.Sagar *et al.*, (2005) had formulated of liquid cream antidandruff shampoo using active extract of *Tridax procumbens* and formulation was evaluated using various parameters that proved its efficiency and safety. Kaffir lime is a well-known herb used in cosmetic industry because of its aromatic oil. It possesses antidandruff property (Suksringam, 1984 and Jaripakorn, 2001).

Prasart *et al*. (2005) had formulated a good kaffir lime antidandruff shampoo at low cost. The efficiency of the formulated shampoo was compared with the commercial shampoo and found better performance of the formulation. Mainkar *et a*l. (2001) had focused in their study, the method of formulation of completely natural shampoos, their evaluation and comparison with commercial herbal shampoos. Attention is drawn to the fact that due to ambiguous definition of the word Natural cosmetics are available in the Indian market. The authors emphasized on the fact that it is up to the cosmetic chemists themselves to promote and encourage the development and use of truly natural cosmetics. They formulated an herbal shampoo in the laboratory and evaluated the same. The results gave an idea about the standards that should be met by shampoo formulated in the laboratory to be comparable with the commercial shampoo (Mainkar and Jolly, 2000).

Nasrin *et al*. (2007) had formulated an herbal shampoo using total saponins of *Acanthophyllum squarrosum*. The main goal of this study was the elimination of synthetic materials like alkanolamides that are often used for the formation of stable foam, but because of producing nitrosamines they are potentially carcinogenic compounds. The formulation was evaluated for organoleptic, physicochemical, rheological properties and its stability. It was concluded that the formulation was chemically and physically stable. Mohamad *et al*. (2009) had formulated and evaluated herbal shampoo powder with antidandruff property using *Ocimum sanctum* and *Azadiracta indica* as antidandruff agents along with *Acacia concina*, *Trigonella foenum* graecum, *Lawsonia inermis*, *Hibiscus rosa sinesis*, *Sapindus laurifolia* as other ingredients of shampoo powder.

3. Materials and method

3.1 Reagents and chemicals used in the experiment

- Solvents: ethanol, water
- Surfactant: to remove environment dirt, styling products, sebum, and skin scales from the hair and scalp. sodium lauryl sulfate
- Conditioning agents: Leave the hair soft and smooth after sebum removal by the detergent. In this project Seed extracts of *Trigonella foenum* was used
- Preservatives: formaldehyde
- Sequestering agents: prevent scum from forming on the hair and scalp in the presence of hard water. In this project citric acid was used.
- Viscosity modifiers: methyl cellulose, sodium chloride
- Stabilizers: citric acid
- Other group of ingredients: It includes perfume (strawberry perfume), dyes (pink)

3.2 Instruments and apparatus

The apparatus and instruments used in this research were electrical grinder, pH meter, Thermometer, filter papers, funnels, analytical balance, evaporating dish, graduated cylinder, Viscometer, Spatula, rotary evaporator, glass rod and beaker.

3.3. Experimental procedure

3.3.1 General procedure of extraction

Seeds of *Trigonella foenum graecum* were collected from Debre Zeit Agricultural Research Center. Seeds (500g) of *Trigonella foenum* were extracted with1.5L ethanol (99.9%v/v) for 72hrs with occasional swirling to ensure thorough extraction, the extraction mixture was filtered and concentrated by rotary evaporator. The concentrated ethanol extract was dried in hood and weighed. The produced powder was weighed and stored in the fridge until needed for further analysis (5%). The marc left was further extracted with 1.5L distilled water for 72hrs with occasional swirling to ensure thorough extraction.

3.3.2 Formulation of herbal shampoo

Sodium lauryl sulfate 120g (12%) was dissolved in distilled water. Then 5g of citric acid was added into the beaker containing solution of sodium lauryl sulfate followed by addition of 5g viscosity modifiers Methyl Cellulose(CMC) and Sodium chloride (2.2%) were added to adjust the viscosity of shampoos and 0.5mL Formaldehyde was also added as preservative. The pH of shampoo was adjusted using 0.6g of sodium hydroxide followed by addition of perfumes 5mL or 0.5%, dye (pink) and seed extracts (2-20%) of *Trigonella foenum graecum* in different proportions given in Table 1 below.

Table 1. Composition of formulated herbal shampoos

S. No	Ingredients	Quantity of ingredient taken for different formulation in 100mL shampoo						
		BD-2W	BD -5W	BD-10W	BD-20W	BD-5E	BD-10E	BD-W
1	Sodium lauryl sulfate	12g	12g	12g	12g	12g	12g	12g
2	Citric acid	0.5g	0.5g	0.5g	0.5g	0.5g	0.5g	0.5g
3	Methyl cellulose	0.5g	0.5g	0.5g	0.5g	0.5g	0.5g	0.5g
4	NaCl	2.4g	2.4g	2.4g	2.5g	2.4g	2.5g	2.2g
5	Formal aldehyde	0.05mL	0.05mL	0.05mL	0.05mL	0.05mL	0.05mL	0.05mL
6	Strawberry perfume	0.5mL	0.5mL	0.5mL	0.5mL	0.5mL	0.5mL	0.5mL
7	NaOH	0.06g	0.06g	0.06g	0.06g	0.06g	0.06g	0.06g
8	Distilled water	90mL	90mL	90mL	90mL	90mL	90mL	90mL
9	Fenugreek seed extract	2g water extract	5g water extract	10g water extract	20g water extract	5g ethanol extract	10g ethanol extract	without plant extract

N.B

BD -5E …… Shampoo with five percent ethanol extract of fenugreek

BD -10E ……..... Shampoo with ten percent ethanol extract of fenugreek

BD - 2W ……..... Shampoo with two percent water extract of fenugreek

BD – 5W …… Shampoo with five percent water extract of fenugreek

BD - 10W ……. Shampoo with ten percent water extract of fenugreek

BD -20W ……...Shampoo with twenty percent water extract of fenugreek

BD –R…….... Rotana shampoo from market

BD-W …….... Shampoo without plant extract

3.4 Evaluation of quality formulated shampoo

To evaluate the quality of prepared formulations, several quality control tests including visual assessment and physicochemical controls were performed (Ashok and Rakesh, 2010)

3.4.1 Physical appearance/visual inspection

The formulation prepared was evaluated for the clarity, color, odor foam producing ability Homogeneity and Appearance (Aghel *et al*., 2007).

3.4.2 Determination of pH

PH meter was calibrated using solution of pH 4,7 and 10. The pH of shampoo solution was measured by using pH meter at room temperature 25°C (Mainkar and Jolly, 2000).

3.4.3 Determination of percent of solid contents

4 g of shampoo was placed in a clean, dry and weighed evaporating dish. The dish and shampoo were weighed again to confirm the exact weight of the shampoo. The liquid portion of the shampoo was evaporated by placing the evaporating dish on the hot plate. The weight and thus, percent of the solid contents of shampoo left after complete drying was be calculated.

3.4.4 Dirt dispersion test

Two drops of shampoo were added to 10 mL of distilled water taken in a large test tube. To this solution, one drop of India ink was added and the test tube was stoppered, the mixture was shaken gently ten times. The amount of ink in the foam indicated by the rubric such as None, Light, Moderate or Heavy (Ali and Kadhim, 2011).

3.4.5 Test to evaluate foaming ability and foam stability

Foaming ability was determined using cylinder shake method. Briefly, 50 mL of the 1% formulated shampoo solution was placed into a 250 mL graduated cylinder; covered with one hand and shaken 10 times. The total height of the foam content after 1 min of shaking was recorded for one hour.

3.4.6 Viscosity evaluations

The viscosity of the shampoos drops; this is a favorable property which eases the spreading of the shampoos on hair. The viscosity of the shampoos was determined by using Brookfield Viscometer. The temperature and sample container’s size was kept constants during the study.

3.4.7 Antifungal and Antibacterial activity tests

Antibacterial activity tests were carried out using Disc diffusion method against *Staphylococcus* aureus, *Escherichia* coli (ATCC 25922), *Pseudomonas* aeruginosa (ATTC 27853), *Klebsiella pneumonia* (ATTC 700603) and Enterococcus *feacalis* (ATTC *29212).* Antifungal activity test was also carried out against *Candida albicans (ATCC7596)* for the formulated shampoo.

The antibacterial and antifungal activity test were also carried out on shampoo products obtained from market. 20ml of sterile MHA (maintained at 45-50^{o}C in a molten state), and SDA poured into sterilized Petri dishes separately and set aside. After solidifying, 20 microliters of test bacteria were spread on MHA plate and 20 microliters of fungus were spread on SDA. Then previously prepared filter paper discs were placed onto the surface of the HMA and SDA plate at equal distance from each other and 15 mm from edge of plate separately as described by (Clutterbuck *et al.*, 2007). Each disc was pressed down to ensure complete contact with the agar surface and the discs were placed with a dispensing apparatus, they distributed evenly. Ordinarily, the discs were placed on each plate. Each plate was inverted and placed in an incubator set at 37^{0}c for 24 hours and 72 hours for bacteria and fungus respectively after the discs are applied for bacteria and fungus. The antimicrobials susceptibility was evaluated by measuring zone of inhibition using plastic ruler in mm.

3.5 Statistical Analysis

The results obtained in the present investigation were subject to statistical analysis like Mean(x) and Standard Deviation (SD).

4. Results and Discussion

4.1 Physical appearance and pH of shampoo

A shampoo like any other cosmetic preparation should have good appealing physical appearance. The formulated and marketed shampoos were evaluated for physical characteristics such as color and odor as given in Table 2.for formulation of shampoo pink color dye was used as colorant, but this pink color was changed as different proportion seed extract of *Trigonella foenum graecum* was added and form different colored shampoo.

The pH of shampoos has been shown to be important for improving and enhancing the qualities of hair, minimizing irritation to the eyes and stabilizing the ecological balance of the scalp. The current trend to promote shampoos of lower pH is one of the ways to minimize damage to the hair. Mild acidity prevents swelling and promotes tightening of the scales, there by inducing shine. Acidic solutions cause the cuticle (outer layer) of the hair to shrink and lay flatter on the shaft of the hair. Basic solutions cause the cuticle to swell and open up. The shampoos pH values should be between 6 and 8 (Gholamreza *et. al* ,2011). The pH values of formulated shampoos were measured and the values were in the range 6.25 – 7.3 and The pH of shampoo from market was 7.95 falling within the ideal pH range for shampoo which is between 6 and 8 given below in Table 2.

Table 2. Evaluation of formulation for physical appearance and pH

Number of sample	Formulation	Physical Appearance	pH
1	BD -2W	Light pink	6.35±0.2
2	BD-5W	Pink	7.3±0.4
3	BD -10W	Pink	7±0.7
4	BD -20W	Brown	7.2±0.05
5	BD -5E	Orange	6.6±0.57
6	BD -10E	Orange	6.25±0.35
7	BD –W	Pink	6.6±0.4
8	BD –R	Light green	7.95±0.07

The physical apperance of the formulated shampoos and shampoo from market are given in figure 5 below

Fig 5. Formulated shampoo from water extracts and ethanol extracts of *Trigonella foenum graecum* and shampoo from market

4.2 Evaluation of shampoo for Foaming ability and foam stability

Foaming or lathering is very important to the consumer and therefore, it is considered as an important parameter in evaluation of shampoo. The foam stability of formulated shampoo after one-hour range between 5.8-8.3 given in Table 3. The foaming stability of a shampoos increase as percent of plant extract increase this is because of there is the activity of extract of *Trigonella foenum graecum* in foaming ability and foam stability of shampoo due to it contains foam forming agent called saponin.

Table 3. Evaluation of shampoo for foaming ability and foam stability

Time (min)	Foam height (cm)							
	BD-2W	BD-5W	BD-10W	BD-20W	BD-5E	BD-10E	BD-W	BD-R
1	24	24	24	24	24	24	23.8	24
5	21.6	22.7	21	20.7	22.6	22	21.9	22.7
10	19.2	19.9	20.2	17	20	20	18.5	19
20	14.2	14.2	14.8	14	14	15	14.5	16.2
30	10.7	11	12.2	11.7	11.5	12	12	13.4
40	8.8	9	10	9.6	10.1	10	9.6	12
50	6.9	50	8.5	8.8	9.4	9.2	7.8	11.2
60	5.8	6.4	6.8	7.1	8.3	7.9	6.4	9.5

4.3 Viscosity measurement

Shampoos must have a good consistency in order to facilitate their use, low enough to ensure a facile removal from the package and facile spreading to the hair. To increase the viscosity of shampoo the thickening agent sodium chloride was used. To the total of one thousand milliliter shampoo twenty-two gram of sodium chloride was added before addition of plant extract of fenugreek. After addition plant extract, viscosity of shampoo decrease since plant extract was added by dissolving with water. So it needs adjustment by addition of sodium chloride. The viscosity of shampoo with different *Trigonella fooenum graecum* seed extracts given below in Table 4.

Table 4. Evaluation of shampoo for viscosity

Number	Formulation	Amount of NaCl added after addition of plant extracts(gram)	Viscosity(cps)
1	BD -2W	0.2	2031.33
2	BD-5W	0.3	1844.66
3	BD -10W	0.3	1597
4	BD -20W	0.4	1264.66
5	BD -5E	0.2	1585
6	BD -10E	0.4	1190.33
7	BD –W	-	2680.66
8	BD –R	-	1702.33

4.4 Dirt dispersion and percent of solid content

Dirt dispersion is an important criterion for evaluation of cleansing action of shampoo. Shampoo that cause the ink to concentrate in the foam is considered poor quality, the dirt should stay in water. Dirt that stays in the foam will be difficult to rinse away. It will redeposit on the hair. Therefore, the dirt should stay in the water portion for achieving better cleansing action. All eight shampoos showed similar results given below in Table 5. These results indicate that no dirt retained in the foam so, prepared and marketed formulations are satisfactory.

Good shampoos usually have 20% -30% solid contents as it is easy to be applied and rinse out from the hair (Khaloud *et al*., 2014). If it doesn't have enough solid it will be too watery and wash away quickly, similarly too many solids will be hard to work into the hair or too hard to wash out. The result of percent of solids contents is tabulated in Table 5 below and were found in the range 9.1 – 13.65. There are different factors that affect values of solid contents like temperature of hot plate at which solvent evaporated, time of evaporating the solvents.

Table 5. Evaluation of formulation for percent of solid contents and dirt dispersion

Number	Formulation	Dirt dispersion (presence of ink in foam)	Percent of solid contents (w/w %)
1	BD -2W	Light	10.75
2	BD-5W	Light	11
3	BD -10W	Light	12
4	BD -20W	Light	13.65
5	BD -5E	Light	10.45
6	BD -10E	Light	10.5
7	BD –W	Light	9.1
8	BD –R	Light	11.75

4.5 Antimicrobial susceptibility and resistance patterns

The antibacterial and antifungal activity of formulated herbal shampoo and shampoo from market were evaluated.

4.5.1 Antibacterial activity

The antibacterial activity of the herbal shampoo revealed a promising activity for shampoo enriched with 5%, 10%, 20% water extracts of *Trigonella foenum* against *S. aureus*, whereas shampoo enriched with 5% and 10% ethanol extract of *Trigonella foenum* showed promising activity against S. aureus, *E. feacalis, P.aeroginosa,* and *E. coli* given below in Table 6.The zone of inhibition of shampoos against *S.aureus* were given below in figure 6.

Table 6. Evaluation of antibacterial activity and zone of inhibition (in mm) at 5 microliters for shampoo

Number of sample	Code of sample (5micro liter)	*S. aureus*	*E. feacalis*	*P.aeroginosa*	*K.pneumoni .a*	*E.coli*
1	BD-2W	12±0.57	6	6	6	6
2	BD-5W	15±1.7	6	6	6	6
3	BD-10W	15.6±0.2	6	6	6	6
4	BD-20W	16±1.7	6	6	6	6
5	BD -5E	21.6±1.17	14±1.7	17±0.57	8±0.32	13±1
6	BD-10E	17±0.01	16±0.7	15±0.1	6	12±0.6
7	BD –W	28±0.01	14±0.1	6	6	6
8	BD –R	13.3±0.02	6	6	6	6
9	Positive control	20	14	14	18	23

N.B. Ceftriaxone drug 5 µg/disc was used as positive control

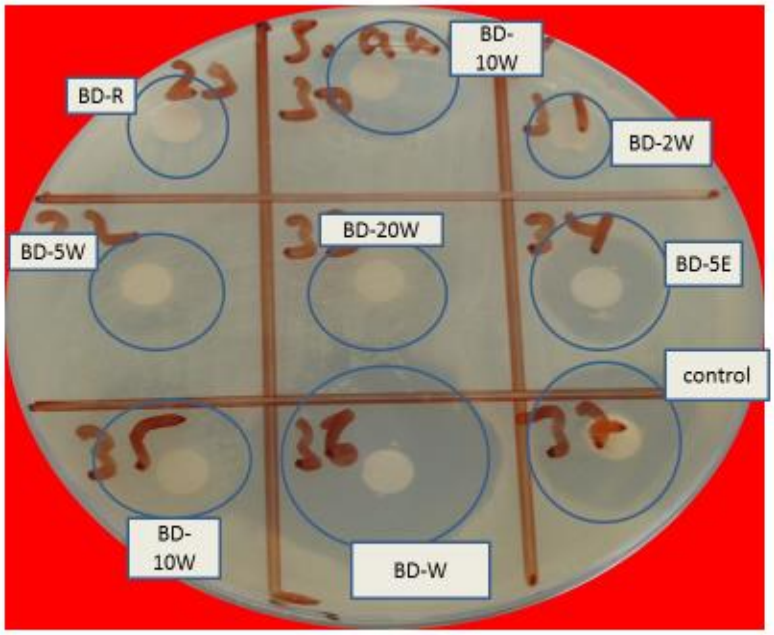

Fig 6. Antibacterial activity of formulated shampoo and shampoo from market against *Staphylococcus aureus*

4.5.2 Antifungal property

Dandruff is a common disease caused by *Malassezia* species especially *Malassezia furfur*. The lipolytic activity of these organisms induces hydrolysis of human sebum tri-glycerides in to free fatty acids that cause both hair loss and scalp. The antifungal activity of shampoos enriched with 2% and above water and ethanol seeds extracts of *Trigonella foenum* revealed promising activity against *Candida albicans* given below in Table 7. Of these, shampoo enriched with 10% and above water and ethanol extracts showed better activity when compared to the Nystatin as standard drug for fungus.

Table 7. The antifungal activity of shampoo against model strain *candida albicans*. (Mean ± SD)

S.No	Formulation Code	Zone of inhibition against *Candida albicans* (mm)
1	BD -2W	12.5±0.05
2	BD-5W	16±0.02
3	BD -10W	16.5±0.2
4	BD -20W	16±0.1
5	BD -5E	15.5±0.05
6	BD -10E	17±0.03
7	BD –W	16±0.18
8	BD –R	12±1
9	Control	13

N.B. Nystatin was used as positive control(5µL)

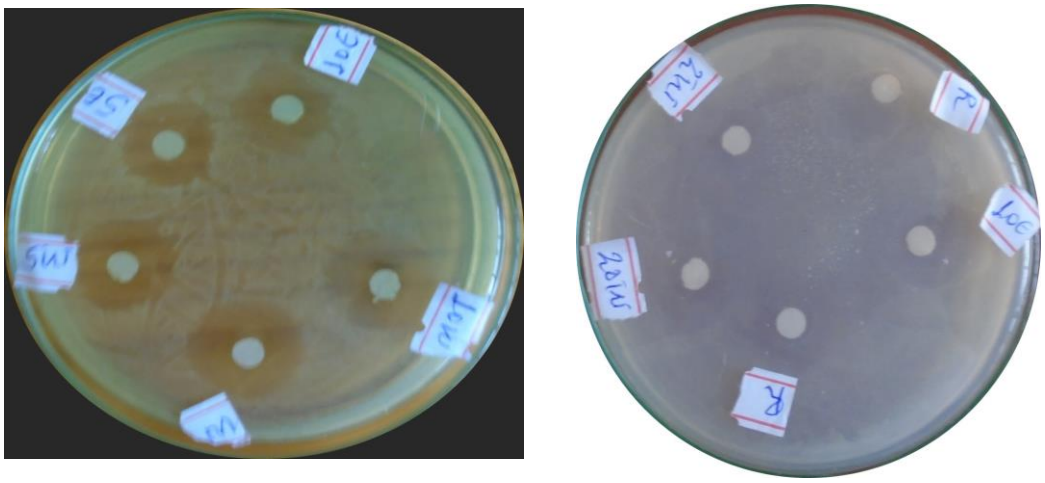

Fig 7. Antifungal activity of formulated shampoo and shampoo from market against *C. albicans*

5. Conclusion and Recommendation

Several tests were performed to evaluate and compare the physicochemical properties and antimicrobial activity of formulated shampoo with shampoo from market. The observed results demonstrate that the formulated herbal shampoo inhibits bacterial growth of different bacterial strains at different composition of plant extracts. The antibacterial activity of the herbal shampoo revealed a promising activity for shampoo enriched with 5%, 10%, 20% water extracts of *Trigonella foenum* against *S. aureus*, whereas shampoo enriched with 5% and 10% ethanol extract of *Trigonella foenum* showed promising activity against *S. aureus*, E.*feacalis*, *P.aeroginosa*, and *E. coli*. The sample taken from market which is rotana shampoo showed promising activity against *S. aureus* only with zone of inhibition shows 13±0.02 and fails to inhibit bacterial growth for most strains of microorganisms when compared to that for the formulated herbal shampoo in this project. The antifungal activity of shampoo enriched with 2% and above water and ethanol seeds extracts of *Trigonella foenum* revealed promising activity against *Candida albicans*. Of these, shampoo enriched with 10% and above water and ethanol extracts showed better activity when compared to Nystatin standard.

Further research is required to study herbal shampoo quality especially on the conditioning performance and to identify the constituents which are responsible for the performance, also to prove the safety, stability and efficacy of this formulation to make it suitable as a commercial product

6. Reference

Abraham, L.S, Moreira A.M, Moura L.H, Dias M.F. (2009). Hair care: A medical overview: Part 1. *Surg Cosmet Dermatol*: pp. 130-6.

Abraham, L.S, Moreira A.M, Moura L.H, Dias M.F. (2009). Hair care: A medical overview: Part 2. *Surg Cosmet Dermatol*: pp.178-85.

Adamski, Z. and Deja, M. (2006). The treatment of dandruff of the scalp. Aesthetic Dermatology **2**: pp. 49-56.

Aghel N., Moghimipour B. and Dana R.A. (2007),'Formulation of an herbal shampoo using total saponins Acanthophyllum squarrosum,' *Iranian Journal of Pharmaceutical Research*,**6**(3): pp. 167-172.

Arora P, Nanda A, and Karan M. (2001),'Shampoos based on synthetic ingredients visa-vis Shampoos based on herbal ingredients', *International Journal of Pharmaceutical Sciences Review and Research,* **7**(1): pp. 42- 46.

Ashok Kumar, Rakesh R.M. (2010), 'Evaluation of Prepared Shampoo Formulations and to Compare Formulated Shampoo with Marketed Shampoos', *International Journal of Pharmaceutical Sciences Review and Research*, **3**(1): pp.120-126.

Badhe,N, Tekawade S, Shirode L and Lale S (2015), 'formulation and evaluation of herbal hair tonic', *World Journal of Pharmaceutical Research*, **4**(10):pp.1801-1808.

Bouillon C. (1996), Shampoos. *Clin Dermatol* **14**: pp.113-121.

Bulmer, A. and Bulmer, G. (1999). The antifungal action of dandruff shampoos. *Mycopathologia* **147**(2): pp. 63-65.

Clutterbuck A, Cochrane C, Dolman J and Percival S (2007). Evaluating antibiotics for use in medicine using a poloxamer biofilm model. *Ann. Clin. Microbial. Antimicrob*. **6**: pp. 476.

Deshmukh S, kaushal B and ghode S. (2012), 'Formulations and evaluation of herbal shampoo and comparative studies with herbal marketed shampoo', *International journal pharma and bio sciences*, **3**(3): pp. 638-645.

Faergemann, J., Jones, T.C., Hettler, O. and Loria, Y. (1996), 'Pityrosporum ovale (Malassezia furfur) as the causative agent of Seborrhoeic dermatitis: New treatment Options, *British Journal of Dermatology* **134** (46): pp. 12-15.

Gholamreza D.N,Fariba,Pavam K,Ehsan M and Javad J. (2011), 'Formulation of herbal conditioner shampoo by using extract of fenugreek seeds and evaluation of its physicochemical parameters,' *African Journal of Pharmacy and Pharmacology* , **5**(22): pp. 2420-2427.

Hay, R.J and Graham, Brown R (1997), 'Dandruff and Seborrheic Dermatitis: causes and management,' *Clin Exp. Dermatol*, **22**: pp. 3-6.

Jaya P. P, Padmini K., Srikanth J., Lohita M., Swetha K., Vengal R. P. (2013),'A Review on Herbal Shampoo and Its Evaluation', *Asian Journal of Pharm Ana*, **3**(4): pp.153-156.

Kalyani B and Apurva,D (2016),springer briefs in green chemistry, the chemistry and application of sustainable natural hair products ,India pp. 25-35.

Khaloud A.B, Shah A, and Khan (2014), 'Formulation, evaluation and comparison of the herbal shampoo with the commercial shampoos, *journal of basic and applied science* **3**: pp.301-305.

Mainkar A.R and Jolly C.I. (2001), 'Formulation of natural shampoos,*'International Journal of cosmetic science* **23** (1): pp. 59-62.

Mainkar A.R., and Jolly C.I. (2000), 'Evaluation of Commercial herbal shampoo,*' International Journal of Cosmetic Science*,**22** (5): pp. 385-391.

Mandegary A, Pournamdari M, Sharififar F, Pournourmohammadi S, Fardiar R and Shooli S. (2012), Alkaloid and flavonoids rich fraction of Fenugreek seeds (Trigonella fenumgraecum L.) with antini ciceptive and anti-inflamatory effects. *Food and chemical toxicology*, **50**(7): pp. 2503-2507.

Mohamed, H.S., Abirami, A., Jayaprakash, S. and Karthikeyini, K. (2009). Effects of Ocimum sanctum and Azadiracta indica on the formulation of Antidandruff Herbal Shampoo Powder. *Der Pharmacia Lettre* **1**(2): pp. 68-76.

Moradi N.K, Didarshetaban M.B, Pour HRS. (2013).' Fenugreek (Trigonella foenum-graecum L.) as a valuable medicinal plant'. *International Journal of Advanced BiolBiomed Res*, **1**(8): pp.922-931.

Nanda, S., Nanda, A. and Khar, R.K. (2006). Shampoos. In: Cosmetic Technology, 1st ed. Birla Publications Pvt. Ltd., Delhi. pp. 354-378.

Nasrin, A., Eskandar, M. Azadeh, R.D. (2007), 'Formulation of an Herbal Shampoo using total Saponins of Acanthophyllum squarrosum,'. *Iranian Journal of Pharmaceutical Research*, **6**(3): pp. 167.

Nasroallah, M. K, Mohamad B. D, Hamid R, Saeid P (2013),'Fenugreek (Trigonella foenum-graecum L.) As a Valuable Medicinal Plant,' *International journal of Advanced Biological and Biomedical Research*, **1**(8): pp .922-931.

Oleszek W, and Bialy Z. (2006). 'Chromatographic Determination of Plant Saponins,'*Journal of Chromatography A*, **1112**(1): pp.78-91.

Prabhamanju, M., Gokul, S.S. and Babu, K. (2009). Herbal vs chemical actives as antidandruff ingredients - Which are more effective in the management of dandruff? - An overview. *Ethanobotanical leaflets* **13**: pp. 1373-1381.

Prasart, F.T. and Chonlada, W. (2005). 'Development of antidandruff shampoo from Kaffir lime that is the By-Product of Food Industry,' *Kasetsart Journal: Natural Science* **39**: pp. 725- 729.

Ranganathan S, Gogul Shangar S and Ranjith M.S. (2001), 'Fungal disease of the skin', *The Hindu Magazine.*

Ravichandran, G., Bharadwaj, S.V. and Kolhapure, S.A. (2004), Evaluation of the clinical efficacy and safety of Antidandruff Shampoo in the treatment of dandruff. The Antiseptic **201** (1): pp. 5-8.

Ravichandran, G; Bharadwaj, S.V and Kolhapure, S.A (2005), Evaluation of the clinical efficacy and safety of Anti Dandruff Shampoo in the treatment of dandruff, *The Antiseptic,* **102**: pp.1-5.

Robbins, C. R. (2002). Chemical and Physical Behavior of Human Hair. pp .2611

Sagar, R. and Dixit, V.K. (2005), 'Formulation and Evaluation of Herbal Antidandruff- shampoo', *Nigerian Journal of Natural Products and Medicine* **9**: pp. 55-60.

Samy R.P, Gopalakrishnakone P. (2010). Therapeutic potentials of plants as anti-microbials for drug discovery. Evidence-Based Complementary and Alternative Medicine; **7**(3): pp. 283-294.

Schothorst R.C, Stephany R.W. (2001), 'Occurrence of Nitrosodiethanolomine (NDELA) in cosmetics from Dutch Market,' *International Journal of Cosmetic Science,* **23** (2): pp. 109-114.

Shapiro J, Maddin S. (1996), Medicated shampoos, *Clin Dermatol*, pp.123-8.

Shivanand P, Nilam M, Viral D. (2010),'Herbs play an important role in the field of cosmetics', *International Journal of Pharm Tech Research,* **2**(1): pp .632-9.

Suksringam, B. (1984). Spices using for Herbs. Amorn Press, Bangkok, **2**: pp. 104-200.

Sumayya, A.R., Sivagami S and Nabeelah A. (2012),'Screening and biochemical quantification of phytochemicals in fenugreek (Trigonella foenum-graecum),' *Research Journal of Pharmaceutical, Biological and Chemical Sciences,* **3**(1): pp. 165- 169.

Surupsing M. Vlavi, Akash D. Patil, Harishchandra M. Yeowle, Vipul H. Jain and Pawar S.P (2017).'Formulation and Evaluation of Herbal Shampoo Powder', *International Journal of Pharma and Chemical Research,* **3** (3): pp.492.

Trease, G.E. and Evans, M.C. (2002). Text Book of Pharmacognosy, 15th ed. Balliere Tindall, London, pp. 189-210.

Warner, R.R., Schwartz, J.R., Boissy, Y., Dawson, T.I. and Obio, R. (2001). 'Dandruff has an altered stratum corneum ultrastructure that is improved with pyrithione shampoo,' *Journal of American Academy of Dermatology,* **45**: pp. 897- 903.