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SCHOOL OF CHEMICAL AND BIO ENGINEERING

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PRODUCTION AND CHARACTERIZATION OF HARBAL SHAMPOO FROM ZIZIPHUS SPINA CHRISTI L. (GEBA) PLANT

By:

Gebrihans Haile (GSR/4298/12)

Advisor:

Dr. Lemma Dendena Tufa (Asso.Prof.)

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This is to certify that the thesis prepared by Gebrihans Haile, entitled: "**PRODUCTION AND CHARACTERIZATION OF HERBAL SHAMPOO FROM ZIZIPHUS SPINA CHRISTI L. (GEBA) PLANT"** and submitted in partial fulfillment of the requirement for the degree of Master of Science in Process Engineering complies with the regulations of the University and meets the accepted standards with admiration to originality and quality.

Approved by the Examining Committee:

Dr. Lemma Dendena	_____	_____/_____/_____
Advisor	Signature	Date
Dr. _____	_____	_____/_____/_____
Internal examiner	Signature	Date
Dr. _____	_____	_____/_____/_____
External examiner	Signature	Date
School Chairperson	_____	_____/____/_____
(PG coordinator)	Signature	Date

Declaration

I hereby declare that the work presented in this research entitled "**PRODUCTION AND CHARACTERIZATION OF HERBAL SHAMPOO FROM ZIZIPHUS SPINA CHRISTI L. DESF. (GEBA) PLANT** "has not been submitted in any form for another degree, diploma, or a verdict at any university or other institution of the tertiary education. Whenever contributions of others are engaged, every effort exists achieved toward designate this obviously, with due allusion to the literature and discussions. Information taken from issued and unissued work of others has been acknowledged in the text and a list of references is given. The work was under the help of Dr. Lemma Dendena instructor at Addis Ababa University, School of Chemical and Bio-Engineering.

Name: Gebrihans Haile

Signature: _____

Date of Submission: ____/_____/_____

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Abstract

The aim of this study was the formulation of herbal shampoo using Ziziphus Spina Christi L. Three herbal shampoos were produced which contain 10%, 15% and 20% of Ziziphus Spina Christi extract as a surfactant. Sample collection, per treatment, preparation, moisture content determination, extraction and optimization, herbal shampoo production, and characterization of the produced shampoo was the major activity of this research. Central composite design was used to determine, and optimum saponin extraction parameters. Extraction temperature (100-121℃) and time (15-40 min) have been selected as the major study parameters. Extraction time was the most significant parameter that affecting saponin yield. The quadratic model was found to be accurate enough with a predicted R^2 of 0.9858 which was in close agreement with the Adjusted R^2 of 0.9806. A temperature of 121 ℃ and an extraction time of 40 min were found to be the optimum conditions and the validation experiment resulted in an optimum saponin yield about 6.24%. FTIR results show that N-H stretch group at 3450 cm^{-1}, 1411cm^{-1} for the C=C group, organic nitrates at 1634.5 cm^{-1}, and C–H group at 712.23 cm^{-1}. The evaluation of the physicochemical test was carried out and compared with aloe vera and organza shampoo. It was observed that many characteristics of the formulated shampoos were in the standard range, and no significant differences regarding their stability at different temperatures, times, and all are physically stable. Formulation (F3) showed all of the ideal properties of the shampoo and showed similar properties to the two reference shampoos and was selected as the best formulation. The research is essential in terms of ensuring the production of an alternative form of herbal shampoo with a wide range of cosmetic applications, as well as having a substantial impact on meeting customer needs and reducing the need for foreign currency. Further research is required to determine cleaning effectiveness, conditioning performance, and toxicity test.

Keywords: *Ziziphus Spina Christi*, extraction, optimization, formulation, characterization

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List of abbreviations

ANOVA	Analysis of variance
ASP	Alkali -surfactant-polymer
CAPB	Cocamidopropyl Betaine
CCD	Central Composite Design
CMC	Critical micelle concentration
CMC	Carboxyl methylcellulose
DOE	Design of expert software
EDX	Energy Dispersive X-Ray Spectroscopy
EOR	Enhanced oil recovery
F1, F2, F3, F4	Formulation 1,2,3,4
FG	Foenum-graecum
GC-MS	Gas chromatography-mass spectroscopy
GGRE	Glycyrrhiza glabra root extract
1HNMR	Proton Nuclear Magnetic Resonance
HPLC-MS	High-performance liquid chromatography and mass spectrometry
HS	Herbal shampoo
IOC	Indian Oil Corporation
LPRE	Low-pressure refluxing extraction
ME	Maceration extraction
Mt	Montmorillonite
NTA	Nitrilotriacetic acid
pH	Power of Hydrogen
PS	Quillaja Bark saponin
RA	Ruscus aculeatus
RE	Refluxing Extraction
RSM	Response surface method
SD	Standard deviation
SE	Soxhlet extraction
SEM	Scanning Electron Microscope
SLS	Sodium Lauryl Sulphate
SZJ	Semen ziziphus jujube
TGA	Thermal Gravimetric Analysis
TJS	Total jiaogulan saponins
TT	Tribulus Terrestris
UAE	Ultrasound aided extraction

1. Introduction

1.1. Background

The phrase 'shampoo' is a word that comes from the Hindi language term 'champana,' which meaning 'to push.' Shampoos now account for half of all hair product sales, demonstrating the importance of shampoo in the market, community, and personal lives of individuals. The shampoo is the most successful cosmetic for washing hair and removing sebum and diseases related to it. Practitioners have consistently expressed their opinions on shampoo performance. Shampoos are primarily used as hair cleaners and are based on a complex system of surfactants. A shampoo's function, however, extends much beyond solubilizing sebum and removing pus. The shampoo is a product made up of surfactant as the major ingredient and other components that work to boost the shampoo's effectiveness. The intention of using shampoo is to eliminate dirt that is build up on the hair without stripping out much of the sebum (Moghimipour et al., 2021).

Shampoo has very extended history preliminary from ancient times. The term shampoo appeared within the English from India throughout the colonial era since 1762. In India herbs and their extracts had been used as shampoos in past. Effective early shampoo was made by way of boiling Sapindus with dried Indian gooseberry and multiple other herbs, the use of the strained. Throughout the untimely degrees of shampoo in Europe, English hair stylists boiled shaved cleaning soap in water and herbs to deliver the hair shine and fragrance. Marketed shampoo was obtainable from the turn of the 20th century. In 1927, liquid shampoo was created via German inventor Hans Schwarzkopf in Berlin, whose call is hooked up a shampoo brand offered in Europe. revolutionary shampoo because it's far diagnosed in recent times turned into firstly offered inside the 1930s with Drene, the primary shampoo the use of insincere surfactants in its locations of soap. Early shampoos applied in Indonesia had been formed from the husk and straw of rice. The husks and straws were overcooked into ash, and therefore the ashes are cosmopolitan with water to make lather (Babajanzadeh et al., 2019).

Shampoos are either synthetic or herbal shampoos. Herbal shampoos have evolved as alternate to synthetic shampoos since they're considered safe and therefore the ingredients utilized in such shampoos are used traditionally for several years around the world. There are several medicinal plants that are reported to possess beneficial effects on hair and are utilized in

formulation of shampoo in their powdered form or extracts or derivative form. To maintain healthy hair without fear the side effects of chemicals contained in synthetic shampoos are merit of herbal shampoo. Natural botanicals may be used of their crude form or they will be extracted, purified or spinoff to render them extra suitable to be used in cosmetics. Water, viscosity, fragrances, color, functional additives, and a surfactant are all found in a typical shampoo. Surfactants are amphipathic substances containing hydrophilic and hydrophobic sections that ideally partition at the interface between liquid phases of varying degrees of polaritys. Due to the coexistence of hydrophilic and hydrophobic parts in the same molecule, this property decreases the surface tension of liquids by causing specific, preferential interactions at surfaces and interfaces (Rodríguez-López et al., 2017).

Shampoo with essential oils is a simple approach to improve its effectiveness; however, be careful to choose oils that do not irritate or to which you may be allergic, as shampoos tend to get into one's eyes. When essential oils are used in shampoos, they must be diluted to the proper concentrations so that they do not irritate the eyes. Rosemary essential oil is usually a good supplement for hair treatment. Rosemary oil has a light, fresh herbal scent is clear in color, and has a watery consistency (Brilhante, 2018).

Solvents are the principal element in all shampoo preparations, accounting for 60-80 percent of the whole solution. It helps to dilute the cleaning ingredients, resulting in less irritation. It facilitates the application of the shampoo mixture to the hair and scalp. They can help with formulation stability, evaporation rate regulation, cooling, product application, skin feel, viscosity, and film-forming qualities. Water has been extensively used as a solvent for shampoo manufacture in several distinct works of literature (Dhingra, 2019).

Herbal shampoos have evolved as an alternative to synthetic shampoos since they are considered safe and the ingredients used in such shampoos have been used traditionally for many years around the world. Several herbal plants are reported to possess a positive impact on hair and are used in the formulation of shampoo. Natural shampoo is used to maintain healthy hair without worrying about the side effects of chemicals contained in synthetic shampoos (P. Arora et al., 2011; Of & Liquid, 2020).

1.2. Statement of the problem

The demand for cosmetics, particularly hair shampoos, is increasing from time to time as young people become more worried about their hair, style, moisturize, nourish, and prevent dandruff and other unpleasant feelings by using shampoos. Ethiopia has a limited number of cosmetics factories, thus it imports a variety of shampoo formulas from Arab countries at a significant cost to meet demand. Shampoo keeps hair silky and smooth, easy to rinse, minimum skin and eye irritation, gives a thick and creamy feeling, is less toxic, biodegradable, and repairs damaged hair are shampoos advantage. The majority of synthetic shampoos on the market contain one or more ingredients such as ammonium lauryl sulfate, sodium lauryl sulfate, sodium Laureth Sulfate, diethanolamine, dimethicone, formaldehyde, lanolin, ether chain, ethylene oxide, and petroleum, Polyethylene glycol, propylene glycol, glycol, nitrogen, triethanolamine (Sakthivel & Nagarajan, 2020). Shampoos commonly include sodium lauryl sulfate, which can cause irritation to the eyes and skin and may potentially be carcinogenic. This can induce headaches, dizziness, and pain in the eyes, nose, throat, and lungs with prolonged exposure. A subsequent ether chain, which is a cheap cleaning chemical, will result in significant scalp irritation as well as hair loss. SLES, which is based on nitrogen, is potentially carcinogenic and can cause irritation, skin rashes, and other allergy symptoms. SLES inhibits new hair development and prolongs the hair loss period. The presence of SLES in hair may aggravate hair follicles and cause scalp-related issues, which are the same disadvantages of shampoo (Terreessaa, 2016).

There is no research conducted concerning the production of shampoo from this plant in Ethiopia and there are only a few research outputs in the open literature related to the production of shampoo from the Ziziphus plant. Sodium lauryl sulfate was used in its formulation composition. To solve these problems Ziziphus Spina Christi L coming from feedstock could be used to produce alternative herbal shampoo. Converting Ziziphus Spina Christi L to shampoo with locally available raw materials reduces both the health effects of synthetic shampoo and the scarcity of natural herbal shampoo on the market. The Ziziphus Spina Christi L. plant was utilized to make shampoo in this study, along with a suitable organic chemical that would not hurt users since the Ziziphus Spina Christi L plant prevents dandruff, balding, and hair loss. Women traditionally applied Ziziphus Spina Christi to their bodies and hairs to make their hair shine and their skin soft without sunburns.

1.3. Objective

1.3.1. General objectives

The aim of this research is production and characterization of herbal shampoo from Ziziphus Spina Christi L. (GEBA) pant.

1.3.2. Specific objectives

- To extract and characterize saponin from Ziziphus Spina Christi L raw material
- To investigate the temperature and time effects of the yield
- To produce, and characterize herbal shampoo using saponin extract and compare it with commercial herbal.

1.4. Scope of the study

This was an experimental study to demonstrate the possibility of producing herbal shampoo from Ziziphus as a raw material. Sample collection, sample preparation, moisture content analysis, saponin extraction using distilled water as a solvent, saponin purification, quantitative, qualitative, and Fourier Transform Infrared Spectroscopy (FTIR) analysis of the extracted saponin, formulation, and evaluation of the herbal shampoo are all covered in this thesis. During the procedure, standard processes and testing methodologies are used.

1.5. Significance of the study

The research is important in terms of ensuring the production of an alternative form of saponin (biosurfactant) that has numerous applications in the cosmetics industry. The study is significant because it adds to previous information by introducing saponin as a shampoo ingredient. The leaf of the Ziziphus plant that can be used for the production of saponin is a nonedible feedstock that can be cultivated from mountains, hells, and unsuitable lands can be harvested within few days. This study is a means of production and characterization of Ziziphus leaf herbal shampoo by utilizing cheap raw material and easy process within cost-effective methods. So the study supports the development of long-term usage of renewable resources for production of valuable products and also they used to promote the usage of the Ziziphus leaf plant for herbal liquid shampoo production.

2. Literature review

2.1 Overview of Ziziphus plant

Plants are currently used in cosmetics as a source of biologically active chemicals. The Ziziphus plant is the most crucial source of biologically active chemicals called biosurfactants, which is the main component of shampoo. The plants are evergreen trees and shrubs distributed across the world, tropical and subtropical regions (Alzahrani et al., 2019).

The natural range of the species extends from Sudan and Ethiopia to North Africa, the Middle East, and the Eastern Mediterranean Near East. The two indigenous Ziziphus plant species present in Ethiopia's arid land area are Ziziphus-Spina Christi and Ziziphus-abyssinica. Ziziphus Spina-Christi is a multipurpose natural plant that belongs to the Rhamnaceae family. It is a spiny shrub or small tree that thrives in arid environments and is known as a thermophilic tree (Zait et al., 2018).

Table 1: Ziziphus Spina-Christi plant botanical classification.

Species	Ziziphus spina- Christi
Genus	Ziziphus
Family	Rhamnaceae
Order	Rhamnales
Class	Dicotyledonae
Subphylum	Angiospermae
Phylum	Spermatophyte
Kingdom	Plantae
Domain	Eukaryota

Source: Chemical Study and Antioxidant activity of Siddir (*Ziziphus Spina*) Roots Extracts

Southern Ethiopia, Afar, lowland Tigray, Gonder, Wello, Shewa, Gamo-Gofa, Bale, Adama, and Harergé regions are also home to this species. The species matures into a tree, but due to focused grazing during the dry seasons and destruction for fencing material and firewood, it frequently becomes a bush. The tree can grow from five to fifteen meters tall, with a stem diameter of up to sixteen centimeters. Its bark is light grey, cracked, and scaly; the trunk is

twisted; the crown is thick; the shoots are pale, flexible, drooping, and the thorns are in pairs, one straight and the other curled (Huang et al., 2017).

Leaves are simple, alternating, narrowly varied in length from one to nine centimeters and width from one to three and a half centimeters, glabrous above, densely pubescent beneath, with sthree basal, visible veins going up to the apex and approximately five centimeters to one centimeter long petioles. The flowers have a fragrant scent and are found in dense clusters in the leaf axils. They are little, greenish-yellow, sub-sessile flowers with five m in sepals measuring two mm in length and five petals measuring one and a half mm in length. The spines are light brown in color and are joined in pairs, with one of each pair being up to eight mm long and straight, while the other is shorter and somewhat bent. Branch range in color from yellowish to pale yellow (Asgarpanah & Haghighat, 2012; Hegazi, 2019).

Figure 1: Leaf (A), flower (B), and fruit (C) of Ziziphus Spina Christi plant

Ziziphus species contain cyclopeptide, isoquinoline, alkaloids, flavonoids, saponin, terpenoids, and glycosides. The primary components of Ziziphus Spina-Christi leaves were dodecaacetylprodelphinidin B_3, A flavonoid, C-glycoside, three′, five′-di-C-beta-d-glucosylphloretin, cardiac glycosides, polyphenols, geranyl acetate (fourteen percent), methyl hexadecanoate (ten percent), methyl octadecanoate (nine and point nine percent), farnesyl acetone C (nine and point nine percent), hexadecanol (nine and point seven percent), ethyl octadecanoate (eight percent), and spinanine-A (a 14-membered cyclopeptide alkaloid of the amphibine-B type) (Ads et al., 2018).

The leaves are put to good use to treat headaches, bone pain, fever, pain, abscess treatment, and superficial wound dressing, as well as dandruff, ulcers, asthma, and as an analgesic. In the treatment of eye infections, little branches are employed. One of the most common sources of saponin is Ziziphus spina-christi. Saponin is a bio surfactant found in a variety of plants and is one of the most significant used as natural detergents for centuries (Asgarpanah & Haghighat, 2019).

Saponin gets its name from the Latin word "sapo," which means "soap," because it has remarkable foaming and emulsifying capabilities. Saponins are high-foaming agents, making them ideal for applications requiring natural surface active chemicals (Info, 2018). Saponin in the plant is responsible to reduce the amount of glucagon, a hormone that is responsible for mobilizing into the circulation, and so lowering blood glucose levels in an indirect pathway. It is also utilized for hypotension, anticancer, and anti-inflammation (Article & Elbossaty, 2020; Taylor, 2007).

According to Nguyen et al., (2020) Saponin biomolecules are split into two groups: triterpenoid and steroid glycosides, which differ in structure depending on the number of sugar units attached. Saponins have long been thought to be anti-nutritional compounds, same literature indicated that saponin stereoisomers show a antioxidative, antidiabetic, neuro-protective, and as anticancer agents (Fuchs & Weng, 2011).

The glucosidal compounds' saponin has an aglycone branch (27-carbon steroid or 30- carbon triterpene). The gene, or sapogenin, is a portion of the aglycone that is classified into three groups: a-triterpene saponins, b-steroidal saponins, and c-steroids-alkaloids saponins. Saponins are classified mostly based on their surface activity. This group of herbal chemicals provides a cooling and cleaning effect, and its foams are water resistant. Saponins are recognized for their bitter taste, fishy smell, and hemolytic properties. The trees are traditionally used for skin and hair treatments (to treat skin diseases, increase hair length, and strength). Its leaves are traditionally boiled in water, combined with lemon, and used as a shampoo to retain hair softness, and skin purity(Desai et al., 2009).

2.2. Extraction of saponin (bio surfactant) from plants

According to Azmir et al., (2013) conventional and green are the two developed saponin extraction method. Conventional methods including maceration, Soxhlet, and reflux extractions are based on a huge number of chemical solvents. Green techniques based on water as the solvent, such as ultrasonic, microwave, and accelerated solvent extractions, have various advantages, including shorter extraction times with higher efficiency, use of fewer chemical solvents, lower energy consumption, and pollution prevention.

Mohaddes-kamranshahi et al., (2019) use green extraction methods to extract saponin from Iranian Ziziphus Spina-Christi leaves using autoclave, microwave, and bain-Marie heating extraction methods, and solvents methanol, ethanol, and water. Extraction variable, the temperature of 121°C, pressure 15 psi, and a period of 15 minutes. The findings indicate that autoclave extraction of saponin from Ziziphus Spina Christi leaves using water solvent is the best green extraction method with the highest yield. The study suggests that extending the extraction time increased saponin output.

Yu-fen Chen et al., (2010) investigate the foam properties and detergent ability of the saponins from the defatted seed meal of C. oleifera using boiling water extraction, Ross and Miles, and a modified version of the Thompson method. This study uses sebum removed from 0.5% sodium lauryl sulfate (SLS) for the states of detergent ability, and foamability. The findings show that total extracted saponins content were all less than 46% and saponin concentration of C. oleifera defatted seed meal is more than that of other traditional Chinese medications. The study also found that saponin from C. oleifera shows excellent foam properties and moderate detergency. The study suggests that results are useful for the implementation of saponin from the C. oleifera in the detergent and cosmetic fields.

Motamedi et al., (2014) study on evaluation and comparing the antibacterial activity of methanolic and ethanolic extracts of Ziziphus Spina Christi as well as subsequent structural changes in affected bacteria using Mixer centrifuged at three thousand five hundred rpm, and twenty min time. This study uses ethanolic, methanol, and methanol- distilled water extraction solution. The findings show the presence of beutic acid and ceanothic acid, cyclopeptides, saponin glycoside, flavonoids, lipids, protein, free sugar, and mucilage. The study also found that

hydroalcoholic extracts yielded are suitable for fighting bacterial pathogens which its resistance to present antibiotics is an increasing alarm and it might be a life in next future threatening pathogen in both communities and hospital-acquired infections. The study recommends that further studies are needed to find the bioactive constituents of this plant and to be used in cosmetics

Jiang et al., (2007) separated and purified saponins from semen Ziziphus jujube (SZJ) and investigated sedative and hypnotic properties using the Soxhlet extractor, silica gel column chromatography, thin-layer chromatography, and high-performance liquid chromatography techniques. This study uses petroleum benzin solvent, and animal test compares their sedative and hypnotic effects. The findings show that saponins from SZJ comprised two compounds called compounds I and II. According to the study, both medicines had a significant impact on walking time when compared to the control group.

The primary ingredients of total jiaogulan saponins (TJS) produced from tetraploid jiaogulan were examined using D-101 macroporous resin column chromatography, 2D NMR, and HR-MS analyses. This study used 95% ethanol extraction solvent. The study resulted in the identification and isolation of nine saponin components from tetraploid jiaogulan leaves (Liu et al., 2016).

According to Edewor & Owa, (2016) quantitative determination of the saponin content, phytochemicals, and types of saponin present in leaf extracts of Cassytha filiformis using colorimetry via a UV spectrophotometer, standard extraction method. The experiment employed n-hexane as a solvent, methanol as a solvent, and ginsenoside as a standard. The measurements were taken at a wavelength of five hundred fifteen nm. The butanol fraction was subjected to gas chromatography-mass spectrometer analysis. The findings show that n-hexane extract was devoid of all tested phytochemicals, whereas the methanol extract contained saponins, steroids, tannins, and glycosides. The study also found that the total saponin content of the methanol extract is 73.47µg ginsenoside, and Cassytha filiformis leaves are high in steroidal saponin.

According to Ezeabara, (2014), the saponin content of various sections of six citrus species was determined using water bath extractor, double extraction gravimetric method. The study used 20% aqueous ethanol solution, extraction temperature (55℃) and extraction time (90 min). The finding relieved that saponin was found in all parts in different levels of the Citrus species.

The study also found that saponin that occurred in these species was triterpenes. The study recommends that to determine at what level saponin becomes toxic to human and farm animals; and ascertain side effects, if any. Estimation of total saponin, and secondary metabolites from chlorophytum borivilianum Sant. et. Fern plan in vitro cultures using soxhlet, cold maceration method, Electron spray ionization-mass spectroscopy, and Gas chromatography-mass spectroscopy techniques. This study uses petroleum ether, ethanol (85%), and methanol solvent. The total saponin concentration of extracts was determined using a vanillin sulphuric acid assay. The presence of key classes of phytochemicals was shown by the phytochemical screen of the ethanol extract. The study also found that a high percentage of saponin content in the roots, β-Sitosterol, and Taraxerone were also revealed by GC-MS (Borivilianum et al., 2018).

Saponin was extracted from the basis of Acanthophyllum glandulosum below subcritical water state, and therefore the consequences of root pulvilio and solution pH on saponin concentration as measured by foamability and antioxidant activity were investigated using response surface methods With ten gram of root powder and a pH of four, saponin with the maximum foam height, concentration, and antioxidant activity was extracted. The extraction response variables' expected and experimental values were determined to be non-significant. Response Surface Methodology was used in the study to show that the models produced were appropriate. Furthermore, greater R^2 values were obtained for foamability and antioxidant activity, as well as substantial p-values indicating lack-of-fit response, confirming the models' acceptable fitness. The saponin extract also had a bactericidal effect, indicating that it might be used as a natural antibacterial component. RSM was used to construct models, optimize the extraction process, and estimate saponin concentration with specific ranges for the selected extraction factors, according to the findings. Other natural sources of saponin can be extracted using this extraction process (Najjar-tabrizi et al., 2020).

The study used ambient temperature aqueous extracts using macerates extraction to saponin from the purported saponin-rich plant parts, which were produced and spray-dried under equivalent conditions using benzoate of soda and potassium sorbate as preservatives and drying aids to test forty-five plants from different families that were reported to be rich in saponins for their surface activity and foaming properties. The extraction of fifteen selected plants was likewise done using hot water decoction for fifteen min. Only three of the plants tested were

allowed to drop the surface tension of their solutions by more than twenty mN/m at a dry extract mass level of one percent. The surface dilational rheology responses of the adsorption layers that spontaneously developed on the surface of these extracts ranged from null to very high, with the surface dilational elasticity modulus exceeding a hundred mN/m for five plants. The elastic contribution dominated the surface dilational response in all cases, which is typical of saponins and other biosurfactants. Nearly all of the extracts were able to foam. Based upon the overall characteristics, the best saponin potential sources for surfactant applications in natural cosmetic products and household products are Saponaria officinalis L, Avena sativa L, Aesculus hippocastanum L, Chenopodiumquinoa Willd, Vaccaria hispanica, Rauschert, and Glycine max Merr (Góral & Wojciechowski, 2020).

The review provides collective data on the use of plant saponins in plant crops and food storage for insect pest control. Using water-alcohol solvent systems, saponins are extracted. Ethanol as a solvent to be extracted is preferred over methanol. Ethanol and methanol are beneficial for saponin extraction in combination with water. Methanol is commonly utilized as a solvent for saponin extraction at room temperature. As an extraction condition, ethanol requires a high temperature and could degrade the levels of saponins. The large-scale extraction and formulation of saponins from plants have been observed to be required for field trials. Saponins are highly polarized non-volatiles and thermolabile compounds, and extra care is needed in the exercise of the special structural elements of the aglycons. An important area requiring more attention is the development of economically viable and affordable methods for extracting and purification of saponins from plants (Singh & Kaur, 2018).

To get the highest possible emulsification index and the optimum average droplet size by the Taguchi method, saponin has been extracted from glycyrrhiza glabra using a soxhlet technique and has to optimize its effective saponin production parameters. The study evaluated the temperature of the extraction, the first fraction of the solvent volume, the second fraction of the solvent, and the n-butanol fraction. The temperature and the volume fraction of n-butanol were shown to be the most efficient parameters in extraction (Hajimohammadi et al., 2017).

The extraction of saponin from lerak (Sapindus rarak) and its ability to solubilize reactive using extraction of maceration and ultrasound aided extraction (UAE). The study demonstrated

that, when the process was implemented using the method of ultrasound extraction, the excessive saponin yield was obtained. In addition, FTIR tests showed that saponin extract had functional groups that were similar to pure saponin. The study uses 30℃, 10 mL/g solvent to solvent, and 40 min extraction times. This yield was about twice the maceration-based extraction. The result of the UAE was a better extraction time, a lower temperature, and a lower solvent requirement against maceration extraction (ME). The investigation of saponin extracts' surfactant characteristics also confirmed that saponins extracted from lerak pericarp were oleanine triterpenoids(Aryanti et al., 2021).

Based on a two-level factorial design microwave-assisted extraction of saponin, phenolic, and flavonoid components from Trigonella foenum-graecum seed. The study utilizes five microwave-assisted extraction parameters such as irradiation time, microwave strength, ethanol concentration, feed-to-solvent ratio, and a two-level factor design for checking. The main factor for reaching a high extraction yield was evaluated in each factor. Results showed that ethanol concentration was the major contributing factor that affected fenugreek seed extract, except for the feed-to-solvent ratio within the appropriate levels, bioactive seed extraction can be enhanced with microwave-assisted extraction parameter. The GC-MS analysis has shown that bioactive compounds known as steroid-saponins are present. Furthermore, an increase in water content in the solvent will lead to the reduction of fenugreek seed bioactive compounds. The study suggests that MAE-extracted fenugreek seed was good for food and drug use (Akbari et al., 2019).

Determination of saponin content from Tribulus Terrestris L, using ultrasound-assisted (UAE), refluxing (RE), low-pressure refluxing (LPRE), and soxhlet (SE) extraction techniques were developed. Parameters of extraction for UAE (base of the solvent: methanol, ethanol, isopropanol and acetonitrile; solvent/solid ratio: 50-400 mL/g, concentration in isopropanol and acetonitrile: 30% to 100%; time of extraction: 15-120 min), RE (base of the solvent: isopropanol and acetonitrile; ratio of the solvent to solid: 50%-400 mL/g, concentration of isopropanol: 30% to 80%) and SE (extraction time: 4, 8, 12,1%) for UAE. The most important factors for both the compounds studied were the organic solvent percentage and extraction time. HPLC-MS were analyzed for protodioscin and dioscent content to quantify the extracts from T. Terrestris obtained by UAE, RE, LPRE, and SE methods. The study founding that RE, LPRE, and SE allow about 1.2 times higher extraction performance compared with UAE (Sarvin et al., 2018).

The structural modification, extraction, and online isolation of saponins with an extensive variety of polarity from Panax notoginseng by one extraction–separation operation with three stages was successfully achieved using accelerated solvent extraction incorporate with high-performance counter-current chromatography. The upper phase of the solvent system of ethyl acetate–n-butanol–water was utilized as both the accelerated solvent extraction solvent and the high-performance counter-current chromatography stationary phase extracted at 60℃ in the first stage. The target moderate polar compounds were eluted with the matching lower phase of the solvent system of ethyl acetate–n-butanol–methanol-water and stationary phase extracted at 115 ℃ were employed in the second stage. At last, the upper phase of the n-hexane–n-butanol–methanol-water solvent system was used as both the accelerated solvent extraction solvent and the high-performance counter-current chromatography stationary phase extracted at 135℃. The target low polar chemicals were eluted with the solvent system's corresponding lower phase, and more than nine pure chemicals were effectively separated using seven different solvent systems in one extraction–separation procedure (Zhang et al., 2013).

A study conducted on tested non-traditional extraction techniques for the picky extraction of saponins from unripe yerba mate fruits, using a moderate electric field, ultrasound-assisted extraction, and pressurized liquid extraction. For pressure liquid extraction on the fruits, several solvent flow rates range 1.67–2.78 10^4 kg /s) were used. For both extraction procedures, the statistical method of response surface method proved effective in revealing response patterns. The pressurized liquid extraction was shown to be more selective for saponins than ultrasound and electric fields in this investigation. Ultrasonic extraction yields were less than electric field extraction under ideal conditions. Despite its modest extraction yields, pressured liquid extraction proved highly selective for saponin(Garcia et al., 2018).

The aglycone and a sugar moiety are the two primary components of saponin, according to this review. Monodesmosidic saponin has one sugar moiety, while bidesmosidic saponin has two sugar moieties. The aglycone or genin, or sapogenin itself, was classified as a triterpenoid, steroid, and alkaloid glycosides based on the number of carbon atoms, O_2, and N_2 found in the molecule. Traditional saponin isolation methods include maceration in organic solvents and Soxhlet. Incumbent on the foam production properties, a dry or wet test was used to establish the presence of saponin in the plant material. The total saponin content can be determined via a

series of n-butanol solvent extractions (Mohamed et al., 2019). The microwave-assisted extraction rate is affected by extraction duration, temperature, solvent-to-material ratio, and solvent type. Microwave-assisted extraction yields the most triterpenoid saponins in five minutes, whereas other extraction procedures take many hours and offer lesser yields. The study also discovered that five minutes at 90°C was the best temperature for microwave-assisted extraction (Yi Chen et al., 2007).

According to Schreiner et al., (2021), three saponin-rich extracts from different sources were evaluated as emulsifiers: Tribulus Terrestris (TT), Trigonella foenum-graecum (FG), and Ruscus aculeatus (RA), and their performance was compared to Quillaja Bark saponin (PS). FTIR, solubility investigations, CMC assays, and emulsifying properties were all used to characterize the product. Solubility analyses for all compounds revealed great solubility in water and low solubility in apolar solvents, which is consistent with their emulsifier properties. PS > TT > FG > RA were the extracts that performed best in terms of saponin content. The creation of single-phase systems in the region of low oil and high extract concentration is indicated by the pseudo-ternary diagrams used to map emulsion composition zones. Apart from the RA extract, gel samples were created, which are technologically fascinating options for a variety of applications. The best performance was found in the TT extract, which was used instead of PS.

Szaniawska & Miller, (2021) conducted a systematic study on natural surfactants isolated from Sapindus mukorossi and Sapindus trifoliatus soap nuts that were poured in 500 mL Milli-Q water and incubated for 15 minutes at 25 °C in a shaker (100 rpm). Their qualities were investigated in terms of surface tension decrease and wettability. Natural surfactants have been shown to lower surface tension and improve wettability on a hydrophobic polytetrafluoroethylene surface. Sp. extracts in their natural state trifoliatus have greater surface characteristics than Sp. trifoliatus. mukorossi is classes of mukorossi. As a result, soapnuts could be a suitable source of biosurfactants for domestic use.

Saponin was isolated from lentils using Soxhlet extraction, and the surface tension of saponin and synthetic surfactants was measured using the Du Nouy ring method at 20 °C in distilled water at various doses. The foaming power of the 0.6 percent crude saponins solution, SLS solution, and Tween 80 solution, respectively, was determined to be 80 percent, 93.87

percent, and 97.56 percent. It was discovered that crude saponins with a concentration of 0.6 percent had a moderate foaming capacity. Saponin was also compared to synthetic surfactants such as sodium lauryl sulfate and Tween 80 in terms of surface-active characteristics. Surface tensions of 0.6 percent saponin, SLS, and Tween 80 solutions were measured and found to be 37.39 mN/m, 26.15 mN/m, and 37.18 mN/m, respectively (Meshram et al., 2021).

The production of biosurfactants produced from the soapnut for prospective use against synthetic surfactants is done by mixing the soapnut solution with deionized water to get the necessary concentration. Energy Dispersive X-Ray Spectroscopy (EDX), confocal microscope, and Scanning Electron Microscope (SEM) examination were used to determine the chemical composition, surface topography, and functional group analysis of the produced surfactant, respectively. Variations in foaming stability as a function of time are also investigated, and the results are compared to synthetic surfactants. The result shows that the surfactant is free of the element or functional group. In the case of soapnut, the comparison reveals exceptional foam stability (Panda et al., 2020).

Triterpenoid saponin called glycyrrhizin, and nonionic biosurfactant were extracted from Glycyrrhiza glabra root extract (GGRE). The performance of GGRE was intermittently assessed for inhibiting montmorillonite (Mt) hydration, through a variety of experiments. To make a comparison, TX-100 (a nonionic synthetic surfactant) and KCl were also employed. The inhibition mechanism depends on the adsorption of the hydrophilic group through hydrogen bonding on Mt's surfaces and orientation of the hydrophobic group toward the aqueous phase by which a hydrophobic shell be made on Mt's surfaces. Cost-effective, biodegradability and low toxicity are an advantage of GGRE (Moslemizadeh et al., 2017).

To explore the natural surfactant features, a non-ionic surfactant was recovered from the soapwort plant utilizing ultrasonic extraction and saponin purification processes. Proton Nuclear Magnetic Resonance (1HNMR), FTIR, and Thermal Gravimetric Analysis (TGA) techniques were used. The critical micelle concentration (CMC) of this natural surfactant was calculated using pendant drop surface tension measurements. Water-oil, contact angle, and alkali-surfactant-polymer (ASP) slug injection studies were used to illustrate the use of surfactant in plant extracts in the enhanced oil recovery (EOR) process. Various salinities have different

effects on surfactant efficiency in lowering the interfacial tension and contact angle. Surfactants are employed in a variety of oil-related applications. The application of plant surfactant in EOR via the ASP injection technique was the focus of this research. Other surfactant applications for injection in various circumstances based on chemical water in the EOR process can, however, be developed (Nowrouzi et al., 2020).

2.3. Formulation and evaluation of herbal shampoo.

According to Badi & Khan, (2014), ten percent aqueous gelatin solution, extracts of Acacia concinna, Sapindus mukorossi, Phyllanthus Emblica, Ziziphus Spina-Christi, and Citrus aurantifolia were mixed in various quantities, and produce herbal shampoo. Citric acid was used to alter the pH. visual inspection, pH, wetting time, percent of solid contents, foam volume, and stability, surface tension, detergency, dirt dispersion were performed to work out the physicochemical properties of both prepared and marketed shampoos. The herbal shampoo composition was clear and inviting. After five minutes, it demonstrated strong cleansing and detergency, low surface tension, tiny bubble size, and strong foam stability. The percent solid contents of the homemade shampoo and commercial shampoos were likewise comparable. The designed shampoo performs as well as commercially available shampoo in terms of conditioning. To improve its quality and safety, however, more research and development are required.

Dash et al., (2017) were tasked with developing and testing a comprehensive herbal shampoo made entirely of traditional plant ingredients. Hibiscus rosa-Sinensis, Azadirachta indica, Trigonella foenum-graecum, Phyllanthus Emblica, Sapindus mukorossi, Acacia concinna, and fresh Aloe vera juice were among the ingredients in the shampoo. Color, clarity, pH, skin irritation, percentage of solid components, dirt dispersion, foaming ability and foam stability, wetting time, and conditioning performance were all investigated using the prescribed protocols. The shampoo exhibited the optimum properties of a shampoo that provides good conditioning.

Ritha fruits, Liquorice stolons, Bengal gram seeds, Brahmi leaves, Greengram seeds, Banana roots, Pomegranate seeds, Hibiscus leaves, Marigold flowers, and Lemon fruits were used to make herbal shampoo, which was then formulated into four numerous formulations (F_1, F_2, F_3, and F_4) and stability tested against marketed Dove shampoo. The investigation data was

analyzed, it was discovered that formulation four of anti-dandruff herbal shampoo contains all of the desirable characteristics of an ideal shampoo, and it was safer, more effective, and less expensive than synthetic Dove anti-dandruff shampoo. It was clear that the development of a stable, effective anti-dandruff herbal shampoo that could commercially replace the existing synthetic shampoo was necessary (Revansiddappa et al., 2018).

Formulating and assessing the physicochemical characteristics of an herbal shampoo comprising olive leaf extract. The ethanolic extract of olive leaves was used to create the herbal shampoo. Three formulations (F_1, F_2, and F_3) were created with the same amount of olive leaf extract (one percent w/w). Several tests were carried out, including visual examination, pH, amount of active component, and foamability. The physiochemical parameters of the prepared herbal shampoo were also determined by stability testing. The shampoo's constituents were all deemed to be safe, and the physiochemical examination yielded optimal results. During six months of storage at various temperatures (48°C, 40°C, and ambient temperature), stability experiments revealed a stable homogeneous look. Formula three provided the best stability, particularly for the olive leaf extract (Jaraiseh & Hanania, 2018).

Vijayalakshmi et al., (2018) Prepare and design an herbal shampoo, as well as examine its physiochemical function, with a focus on safety, efficacy, and the substitution of safe natural constituents for dangerous synthetic components. Emblica Officinalis, Hibiscus rosa-Sinensis, Acacia concinna, Sapindus Indica, Eclipta prostrate, Aloe barbadensis, and Cassia auriculata extracts in various amounts were used in the creation of shampoo. Visual assessment, wetting time test, pH, assurance of solid contents, physical phenomena, detergency, dirt dispersion, conditioning performance, foam volume, and stability were all used to evaluate organoleptic, physicochemical, and performance tests. The cleanser that was generated was clear and attractive. It has good foam stability, detergency, cleansing ability, tiny bubble size, low surface strain, and effective conditioning execution. The prepared shampoo passed the physicochemical test with flying colors.

Two herbal shampoo powder formulations were created using some typical traditional medications used for hair treatment traditionally in the Bundelkhand area (M.P.) of India. The preparations were examined for organoleptic, powder characteristics, foam test, and physical

evaluation utilizing Behera, amla, neem tulsi, shikakai henna, and Brahmi. Because the selected substances have been used as single medications or in combination for a long time, the current research will assist to establish a standard formulation and assessment parameters, which will undoubtedly aid in the standardization of quality and purity of herbal powder shampoos of this type (Dubey et al., 2004).

Saad & Kadhim, (2014) Formulate a self-preserving shampoo with a low detergent content utilizing Ziziphus Spina cristi leaves, with a focus on safety and efficacy; this will eliminate the risk provided by chemical additives. Ethanol (70%) was used to extract the samples. The yield percentage was 3.34 percent. To create a transparent shampoo foundation, four samples were created, labeled F-0, F-1, F-2, and F-3. F1, F2, and F3 were made by combining 5, 10, and 20% w/w of Ziziphus Spina extract with ten %, five %, and free of sodium Laureth sulfate, respectively. 100mL of pure water was used to finish the volume. F-0 was used as a control sample (without plant extract). Organoleptic, physicochemical, and performance testing were carried out, and the results were compared to an herbal marketed product. The results indicate that F_2 and F_3 created clear shampoos with average pH values of 5.59-6.25, which were suitable for maintaining the acidic mantle of the scalp. They produced stable foam, reduced surface tension, improved cleaning and wetting, and exhibited pseudoplastic rheological behavior. The foam volume was comparable, and the formulae had better detergency and foaming properties than the commercial herbal formula (p 0.05) (Saad & Kadhim, 2011).

A cost-effective herbal shampoo with no chemical side effect was prepared using different step. The first step was to choose a few well-known, locally available plants. The saponin concentration was measured to ensure a good lathering effect without the use of chemicals. Albizia Amara, Azadirachta indica, Acacia concinna, Sapindus laurifolius, and Vigna radiata showed very high saponin content and hence had good foaming index, hemolysis impact, and crude saponin content, according to the study. Three of them were kept as lathering herbs, and four more herbs were added as important ingredients, all of which are recognized to be helpful for hair care. Three more herbs were added to each of the seven shampoos to see which had the best overall effect and anti-lice property. When Emblicaofficinalis, Centellaasiatica, Ocimum sanctum, Ecliptaalba, Hibiscus rosa-sinensis, Acacia concinna, Sapindus laurifolius,

Albiziaamara, Azadirachta indica, and Lawsonia inermis were extracted in petroleum ether, they demonstrated good anti-lice properties (Rao, 2016).

Design and evaluate a pure herbal formulation was made by combining extracts of Azadirachta indica, Sapindus mukorossi, and Phyllanthus Emblica in a transparent soap base; herbal shampoo was made by combining extracts of Acacia concinna, Sapindus mukorossi, and Phyllanthus Emblica is a Methylcellulose base; and herbal face wash gel was made by combining extracts of Aloe vera. The physicochemical parameters of formulations such as Physical evaluation, pH, foaming ability, and foam stability were determined. The conclusions indicated that the formulation has a pH level that is approximately identical to that of the skin and that the foaming index is outstanding. Further formulations were tested for ocular and skin irritation in animal models (rabbit and Gunia pig), and the results revealed that the animals were not irritated (Joshi & Devi, 2019).

Herbal shampoo (HS) herbal extract made from a combination of amla, reetha, shikakai, nagarmotha, bhringaraj, Brahmi, aloe vera, lemon juice, amla, reetha, shikakai, nagarmotha, bhringaraj, Brahm Extract of herbs (ten percent) In a beaker filled with an aqueous medium, place dried amla, reetha, shikakai, nagatmotha, bhringaraj, and brahmi, heating up to the beginning of a boil using the microwave method, sift, and set aside to cool. To make them more transparent, squeeze in some lemon juice and stir till colorless. Glycerin and CAPB are all added and stirred gradually to avoid foaming. Methylparaben and sodium benzoate are preservatives that have a polarizing effect. Pour Aloe Vera, along with a small amount of cocamono, mockup with water in it for a tiny proportion, and increase the thickness with cocodi to get the HS product. The resulting HS is thick and semi-transparent, with excellent foaming ability and fluidity. After drying, the percentage of solid components of HS is 0.05g. The produced HS has a cleaning effect of 15.1. The generated HS has low dirt dispersion. It yields forty-six ml of froth from one percent of HS. All of these characteristics show that the herbal HS is of good quality and may be used in daily life (Gadge & Mahavidyalaya, 2017).

Herbal extracts, such as fenugreek, can be used in hair tonics and conditioners to prevent hair loss and maintain hair conditioning. First, the proven fenugreek seeds were extracted with 50 percent ethanol using the maceration method, then freeze-dried and stored in the refrigerator.

Some physicochemical parameters, including pH, foam formation, viscosity, conditioning, and wettability, were assessed once the formulation was prepared. The designed shampoo's pH was found to be 6.6. Formulated shampoo can be an appealing and appropriate product based on wettability and conditioning results. The designed shampoo's pH was in the normal range of six to eight. The shampoo's wetting effect took five minutes, indicating that good quality in contrast to other shampoos on the market. The wettability and conditioning statistics indicate that the designed shampoo has a good chance of being introduced to the market (Noudeh et al., 2011).

Herbal anti-dandruff shampoos were created with herbal-based ingredients such as Lemon Grass Oil, Neem Oil, Henna, Aloe Vera Gel, and other natural substances. Visual inspection, pH, viscosity, percentage of solids contents, dirt dispersion, surface tension, foaming ability, and foam stability, and anti-fungal activity test employing Pityrosporum Ovale strain were all used to evaluate the prepared shampoos. The antifungal activity of Formulation (F8) was good, with a maximal zone of inhibition. The (F8) was extremely safe, with no discomfort or sensitivity. The F8 formulation was subjected to three-month stability tests, which revealed no significant changes in its physicochemical parameters (Potluri et al., 2013).

R. Arora et al., (2019) ware develop and test herbal shampoos made with natural components to replace potentially dangerous synthetic components in shampoo formulations with safe natural alternatives. The shampoo was made with extracts of Aloe vera, Hibiscus rosa-Sinensis, Phyllanthus Emblica, Acacia concinna, Azadirachta indica, Sapindus mukorossi, Glycyrrhiza glabra, Eclipta prostrate, and it was evaluated for organoleptic qualities, physicochemical qualities, and performance tests. That findings show that all of the ingredients used to make shampoo are safe, and the physicochemical test results were excellent. The study suggests that herbal shampoos could be utilized as a natural and effective alternative to synthetic sources for cleaning and controlling hair. Most of the finding using either chemical solvent or traditional method of extraction to extract saponin from the plant or using more synthetic chemical during its shampoo formulation. There is no research conducted concerning the production of shampoo from this plant in Ethiopia and there are only a few research outputs in the open literature related to the production of shampoo from Ziziphus Spina Christi and used synthetic chemical in its formulation

3. Materials and methods

Experiments on the production and characterization of herbal shampoo from the leaf Ziziphus plant were carried out in the laboratories of the Addis Ababa Institute of Technology's School of Chemical and Bioengineering and the Chemistry department of Addis Ababa University. The first portion of the investigations covers everything from sample collection through characterization and optimization of extracted saponin response variables. The experiment's second phase focused on the formulation and characterization of the Ziziphus herbal shampoo with Aloe Vera and Organza commercial herbal shampoo. The experimental procedures followed in this work are shown in Figure 2.

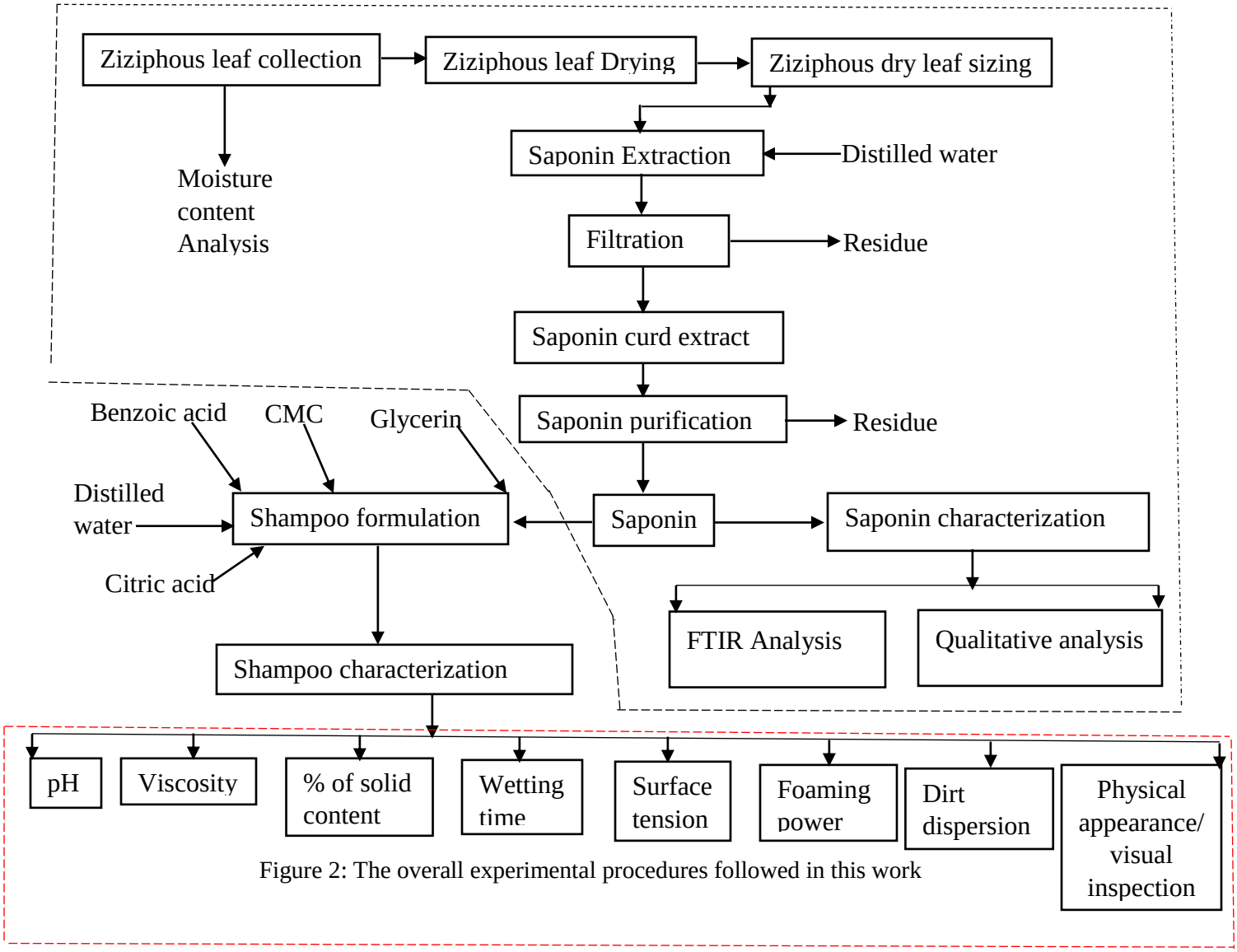


Figure 2: The overall experimental procedures followed in this work

3.1. Materials

The materials, chemicals, and regents like a knife, icebox, different size flask, JANWAY 3505 pH meter, mixer, aluminum foil, Test tubes, Balance, Thermometer, water bath, Bottle, Teaspoon, ruler, Bowl, Stirrer, moisture analyzer, 100 ml capacity plastic containers to store the shampoo, Notebook, and pen, Measuring cylinders, Funnel, oven, refrigerator, cutter mill, sieves, cotton wool, distilled water, carboxyl methylcellulose, Benzoic acid, citric acid, glycerin, sodium hydroxide, n-butanol, sodium chloride, diethyl ether, and ferric chloride, design expert software version 11.1.2 , orginlab 2018 software, standard deviation calculator software, and Microsoft excel 2013 ware used in the experiments.

3.2. Methods

3.2.1. Sample collection area

The fresh plant Ziziphus Spina Christi L. Desf material leaf parts were randomly selected from the two point's latitude 14°05'40.8"N, longitude 38°56'34.1"E, and latitude 14°05'39.4"N, longitude 38°56'34.9"E at Debregenet- Adwa-Tigray state, Ethiopia in October 2020. After collection, the plant material was processed for cleaning by tap water to detach soil, sand, and unnecessary fiber in the field. The cleaned plants' samples were consigned in a plastic container and transported to home and dry on drying frames.

3.2.2. Sample preparation

The air-dried leaf sample was washed by hand two times with tap water and one time with distilled water in the laboratory and dried on drying frames for five days. The dried leaf sample was homogenized into one-millimeter particle sizes using cuter mill. The material was kept refrigerated at room temperature in closed plastic containers until it was needed for further investigation.

3.2.3. Moisture content analysis

Moisture content is often checked by loss on drying, which involves heating the sample and recording the weight loss owing to moisture evaporation. The five hundred gram sample of one mm particle size was oven-dried at 105°C until it had a moisture content of 1 %. Every hour, the weight was measured. The method was repeated until the weight remained constant. The

percentage moisture content of the leaf was determined using the formula described by Eq. (1) (A.A.C.C, 2000).

$$Mositure\ content = [(\frac{W1-W2}{W1})] * 100 \quad \text{.....(1)}$$

Where

- W_1= Original weight of the sample before drying
- W_2= Weight of the sample after drying

3.2.4. Experimental design for saponin extract

The percentage of saponin extraction is functionally related to the interaction effect of parameters such as time and extraction temperature. Appropriate design of experiments was used in this work. The trials were carried out using the statistical experimental design program design expert V 11.1.2. Using response surface methodology (RSM) called central composite design (CCD). Two factors and two levels were employed for the leaf extraction samples.

3.2.4.1 Extraction of saponin

The specified dried powder, a twenty-five gram sample weighted with an Expert pro electrical balance, was put to Pyrex England flasks with a capacity of one liter and 500 ml of distilled water. The combination solutions were shaken for 30 seconds before being placed in a laboratory Sano clave model autoclave, which was set to a pressure of 1.034214 bar, a temperature range of 100°C to 121°C, and a time range of 15 minutes to 40 minutes. The experimental design for extraction of sapoinn was adjusted using design expert version 11.1.2 software (Table 2).

Table 2: The design layout set using design expert version 11.1.2 software

		Factor 1	Factor 2	Response 1
Std	Run	A:Temprature	B:Time	Yield
		℃	Min	%
9	1	110.5	27.5	
5	2	95.6508	27.5	
4	3	121	40	

13	4	110.5	27.5	
8	5	110.5	45.1777	
3	6	100	40	
11	7	110.5	27.5	
10	8	110.5	27.5	
1	9	100	15	
6	10	125.349	27.5	
7	11	110.5	9.82233	
2	12	121	15	
12	13	110.5	27.5	

3.2.4.2. Filtration, and concentrated of saponin extract

The heated mixture solutions of each run were filtered two times using nylon wool and qualitative filter paper 90 mm diameters (model of 101) and (fast, maximum aperture of 20-25μm filter speed < 35s, ash content ≤0.15% and ration of 80±4 g/m^2) by the helping of Kif labo port vacuum filter machine. The Pyrex England flask that contained filtered sample was placed into Grant water bath which was set at temperature of 70 ℃ for eight hours to obtain semisolid mixture.

3.2.5. Quantitative and qualitative analysis of saponin

3.2.5.1. Qualitative analysis of saponin

One ml of extract taken in a cylinder, nine ml of distilled water was added and shaken vigorously for 15s and extract were allowed to stand for ten min. Formation of stable foam one cm indicates the presence of saponins (Thilagavathi et al., 2015).

3.2.5.2. Quantitative analysis of saponins

Saponin can be verified quantitatively by considering the dry powder of the plant sample weighing about 25g and poured into 500 ml of distilled water solution and extracted using Sanoclave. The mixture was sifted and the solid residue of the plant powder was re-extracted with another five hundred ml of distilled solution. The two joint solutions were evaporated above

a water bath at about eighty-five ℃ to form a reduced solution. The concentrated solution was moved into a two hundred fifty mL separating funnel and twenty mL of diethyl ether was added and shaken vigorously to eliminate impurities from the initial solution. The aqueous layer was recuperated for another extraction whereas the ether layer with impurities was castoff. The purification procedure was performed twice, after which sixty ml of n-butanol was poured twice and the mixed n-butanol solutions of hundred twenty ml were purified twice with twenty ml of five percent aqueous NaCl. The staying aqueous solution was moved to a dried pre-weighed porcelain crucible and dried in a drying oven at 60 ℃ to a constant weight, and remaining residue is calculated by using Eq.(2) (Akinseye, 2017).

$$\%\ of\ saponin = \left[\frac{weight\ of\ saponin}{weight\ of\ sample}\right] * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Sources: (Ezeonu & Ejikeme, 2016)

3.2.5.3. FTIR analysis of saponin

A gram of KBr salt and 1.2 mg of sample were carefully weighed and were put together in a mortar, then crushed into a thin pellet. The pellet was placed in the sample container, and the FTIR spectrum was recorded for identification purposes (Kim, 2007).

3.2.5.4. Optimization of process variables for saponin extraction

Using the developed regression model, the process factors were optimized to produce the best percentage of saponin yield. Based on the foregoing analysis, the temperature and times were set in ranges, and the yield objective was set to maximal, the weight was set to 1, and the importance was set to 3 (Table 3).

Table 3: Temperature, time, and yield setting to maximize the extraction yield

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:Temprator	is in range	100	121	1	1	3
B:Time	is in range	15	40	1	1	3
Yield	maximize	3.25	6.25	1	1	3

3.2.6. Formulation of herbal shampoo

Step 1: prepared the mixing formula as showing in the table.

Table 4: The mixing formula used in the formulation of herbal shampoo

No	Ingredient	Function	F_1	F_2	F_3
1	Ziziphus extracts	Biosurfactant (detergents, less extent as emulsifier, foaming properties)	10%	15%	20%
2	Glycerin	Stabilizers, viscosity adjuster, moisturizer	1.5%	1.5%	1.5%
3	Citric acid	Cheating agent, neutralizer, pH adjuster	0.25%	0.25%	0.25%
4	Benzoic acid	Preservative	0.08%	0.08%	0.08%
5	CMC	Thickener	1.25%	1.25%	1.25%
6	Water	Vehicles	86.92%	81.92%	76.92

Step 2: prepare three separate bowl containers with identical volume (500ml) and label each of the container with B_1, B_2, and B_3.

NB=B_1=Bowl one (container one), B_2=Bowl two (container two), and B_3= Bowl three (container three)

B_1 B_2 B_3

Step 3:

- Add F_1 to B_1
- Add F_2 to B_2
- Add F_3 to B_3 and it was homogenized separately using mixer adjusting at 1000rpm for 30 min.

Step 4: determine (measured) the pH value of each formulation shampoo and recorded.

3.2.7. Characterization of the produced herbal shampoos

The produced shampoo were characterized for different criteria for its foam producing ability, viscosity, physical appearance, pH, wetting time, percentage of solid content, surface tension, and dirt desorption.

3.2.7.1. Physical appearance/visual inspection

The recipes prepared were evaluated in terms of their clarity, foam producing ability, and fluidity visually (Revansiddappa et al., 2018).

3.2.7.2. Determination of pH

The pH of a ten percent shampoo solution in distilled water was tested initially, after one month, and after two months at room temperature, 37 °C, and 45 °C. The pH of shampoo should be between 5.5 and 6.0, as this corresponds to the isoelectric point of hair. A shampoo with a pH value of more than 6 will make the hair dry and drab (Dubey et al., 2004).

3.2.7.3. Determine percent of solids contents

A clean dry evaporating dish becomes weighed and 4 grams of shampoo become added. The weights for the dish and shampoo were measured. Most effective the precise weight of the shampoo turned into expected, and the evaporating dish containing the shampoo turned into positioned on a warm plate until the liquid component become absolutely evaporated. After drying, the burden of the shampoo (solids) was calculated by the usage of Eq. (3) (Series, 2019).

$$\%\ of\ solid\ content = \left(\frac{mass\ of\ solid\ left\ after\ boiling}{mass\ of\ shampoo}\right) * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

3.2.7.4. Foaming ability and foam stability

The ability to foam was tested using a cylinder shake method. A 250 ml graduated cylinder was filled with fifty ml of 1 % shampoo solution, which was covered with a hand and shaken ten times. After one minute of shaking, the total amount of the foam contents was recorded. After shaking the flasks, the foam volume was calculated immediately, and then recorded at oneminute intervals for five minutes (R. Arora et al., 2019).

$$\text{Foaming power} = [\text{F1} - \text{F2}] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where: F1 is total foam volume, and F2 is volume of water

3.2.7.5. Dirt dispersion

In a large test-tube containing ten ml of distilled water, two drops of shampoo were introduced. One drop of ink was dropped into the test tube, which was then Stoppard and smashed ten times. The amount of ink in the foam was estimated to be either none, light, medium, or heavy (Series, 2019).

3.2.7.6. Wetting time

A filter paper was cut into one-inch diameter discs having an average weight of 0.58g. The stopwatch was started when the smooth surface of the disc was placed on the surface of a one percent v/v shampoo solution. The wetting time was calculated as the time it took for the disc to start sinking (Badi & Khan, 2014; Dash et al., 2017).

3.2.7.7. Viscosity

Product viscosity plays a significant role in defining and controlling numerous qualities such as shelf life stability, product aesthetics, spreading ability of shampoo on the hair, and product consistency in the package. The viscosities of the produced formulations were measured using a vibrio viscometer at room temperature. In a beaker, forty-one mL of validated shampoo was poured, and an appropriate spindle was immersed in it. At the specified temperature, the interpretations were recorded (Jaraiseh & Hanania, 2018).

3.2.7.8. Surfaces tension

Surface tension measurements were performed with a dropper and a ten percent shampoo solution diluted in distilled water at room temperature (Sharma et al., 2011). Surface tension is greatly impacted by grease or other lubricants, the dropper was thoroughly cleaned with chronic acid and filtered water. Surface tension was calculated by using Eq. (5).

$$R2 = \frac{(W3-W1)n1}{(W2-W1)n2} * R1 \quad \text{.....(5)}$$

Where, W_1 is the weight of the empty beaker and W_2 is the weight of the beaker with distilled water; W_3 is the weight of the beaker with the shampoo solution; n_1 is the number of drops of distilled water and n_2 is the number of drops of the shampoo solution. R_1 is the surface tension of distilled water at room temperature while R_2 is the surface tension of the shampoo solution (Gadge & Mahavidyalaya, 2017).

3.2.7.9. Stability studies

The shampoos' stability was tested by putting them in glass tubes at 37°C and 45°C and comparing them to comparable shampoos kept at room temperature at 25°C. The stabilities were observed initially, one-month, and two-month storage durations (AlQuadeib et al., 2018).

3.3. Statistical data analysis

Results were examined using design expert software version 11.1.2, Microsoft Excel v 2013 and Origin lab 2018 software. The quantitative data obtained were statistically analyzed by calculating the mean of three replicates followed by calculation of the Sum of Square of variance, and Standard error. The results were obtained as mean ± standard error, ANOVA, regressions, tables and graphs. Regression and Pearson correlation coefficient results were used and significance levels < 5% were taken as statistically significant.

4. Result and discussion

4.1. Physical characteristic of the extracted of saponin

The leave of ziziphous Spina Christi plant and the dried and ground of ziziphous plant sample used in the analysis are shown in Fig. 3a and 3b respectively.

a) b)

Figure 3: a. leaves of ziziphous plant, b) dried and ground (1 mm) leaved of Ziziphous plant

The moisture contents of the fresh leaf of ziziphus that were used in the extraction of saponin were 1 %. The parameters indicating the physical characteristics of the extracted saponin are shown in Table 5. It is observed from Table 8 that the extracted ziziphus saponin has opaque, light borwn color with density close to water and pH slightly acidic. The extracted ziziphus saponin samples are shown in Figure 4.

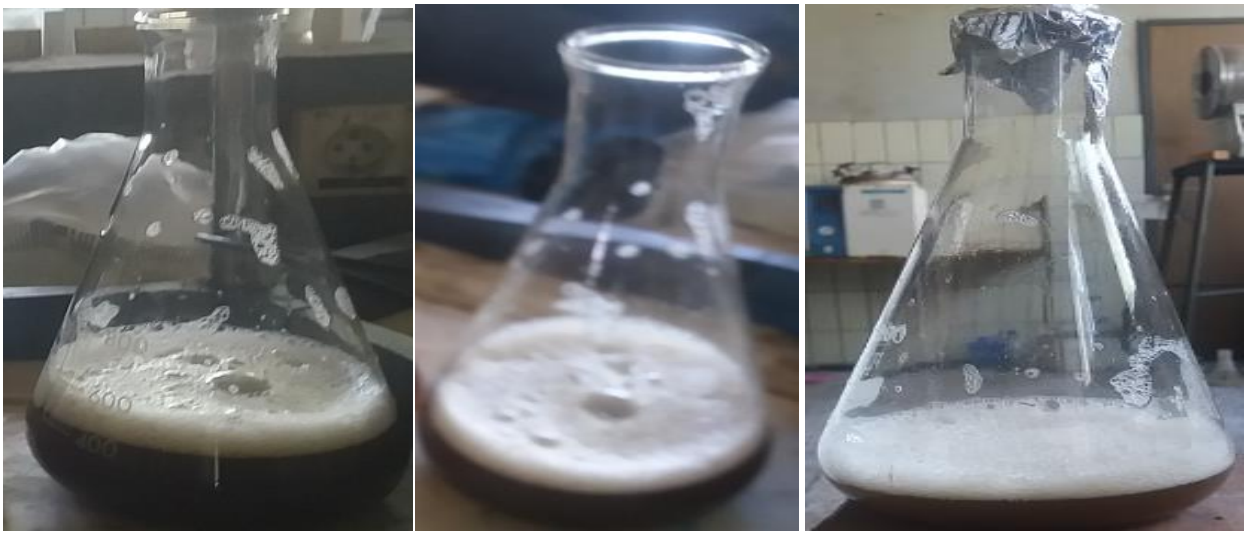


Figure 4: Qualitative analysis of saponin result

Table 5: Physical parameters characterization for the cured extracted of saponin

No	Physical parameters for the cured extracted of ziziphus spnina(saponin)	
1	Color	Light brown
2	Appearance	Opaque
3	Viscosity	2.71 m pas.s
4	Density	1.01912 g/ml
5	pH	6.23

It is to be noted that all the experiments in this study were done in triplicate and the results were average values. It is observed from Table 6 that the maximum saponin yield obtained was 6.25 % that was achieved at run three and the lowest yield was 3.25 % obtained at run nine. This result was two-time compared with the result of saponin isolated from lentils using Soxhlet extraction (3.15%) (Meshram et al., 2021).

Table 6: CCD matrix and purified experimental result of extracted saponin.

		Factor 1	Factor 2	Response
Std	Run	A:Temprature	B:Time	Yield
		℃	Min	%
9	1	110.5	27.5	4.75
5	2	95.6508	27.5	4
4	3	121	40	6.25
13	4	110.5	27.5	5
8	5	110.5	45.1777	5.75
3	6	100	40	4.85
11	7	110.5	27.5	4.85
10	8	110.5	27.5	5
1	9	100	15	3.25

6	10	125.349	27.5	5.75
7	11	110.5	9.82233	3.5
2	12	121	15	4.5
12	13	110.5	27.5	4.75

4.2. Model relating the yield to extraction time and temperature

The model developed for representing the relation between the dependent variable, yield, and the independent variables time and temperature were presented in this section..

4.2.1. Selecting the appropriate model structure

The model structures linear, 2FI, quadratic and quibic were selected for comparison. However, the data for the quality of the various models are given in Table 8. It is observed from Table 7 that the quadratic model has the highest adjusted and predicted R^2 values and the cubic model is excluded because it is aliased. The difference between adjusted and predicted values is 0.0052, which is less than 0.2 in the case of the quadratic model. The P-value for lack of fit (Table 8) is 0.8812, which indicates that the lack of fit P-value was significant. The quadratic model finally was selected based on the design expert software's recommendations.

Table 7: Model summary statistics

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	0.1416	0.9773	0.9727	0.9615	0.3398	
2FI	0.1472	0.9779	0.9705	0.9535	0.4102	
Quadratic	0.1022	0.9917	0.9858	0.9806	0.1708	Suggested
Cubic	0.1147	0.9925	0.9821	0.9684	0.2784	Aliased

Table 8: Lack of fit tests

Source	Sum of Squares		Df	Mean Square	F-value	p-value	
Linear	0.1375		6	0.0229	1.46	0.3733	
2FI	0.1319		5	0.0264	1.68	0.3187	
Quadratic	0.0102		3	0.0034	0.2153	0.8812	Suggested
Cubic	0.0028		1	0.0028	0.1786	0.6943	Aliased
Pure Error	0.0630		4	0.0158			

The statistical significance and the goodness of fit of the developed quadratic model, effect of individual variables and their interactions were analyzed by ANOVA. To check whether the quadratic model is the right model or not, it was vital to perform analysis of variance (ANOVA) at a 95% confidence level for F – value which compares the model variance with residual (error) variance and the probability, P-values were used to check the significance of each coefficient of regression model equation. The F value and P-value of the obtained quadratic model presented (Table 9) were found to be 331.27 and <0.0001, respectively.

4.2.2. Development of reduced model

In developing models it is a common occurrence that not all parameters are significant. In such cases, it is advantageous to get model with reduced number of parameters. Such models are called reduced models. This section presents the reduced model development for the developed quadratic model. The p-value of the main two effects Temperature, and time represent by A, and B respectively were significant based on their estimated p values (<0.05) which indicated that the model was appropriate for use in this experiment and proposing that B^2,B, and A are influenced the yield of saponin. The quadratic terms A^2, and the interactive effects of AB have value of 0.8643, and 0.4871 which are values greater than 0.1000 indicate the model terms are not significant and it could be reduced from the model. The process variables were found to have significant interaction effects on yield. The ANOVA for the reduced model is given in Table 9.

Table 9: ANOVA for reduced quadratic model

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	8.74	3	2.91	331.27	< 0.0001	Significant
A-Temperature	3.28	1	3.28	373.41	< 0.0001	
B-Time	5.33	1	5.33	606.61	< 0.0001	
B^2	0.1214	1	0.1214	13.81	0.0048	
Residual	0.0791	9	0.0088			
Lack of Fit	0.0161	5	0.0032	0.2048	0.9439	not significant

Pure Error	0.0630	4	0.0158			
Cor Total	8.82	12				

The *F* value and the corresponding *p* value for Lack of fit for the reduced quadratic model are 0.2048 and 0.9439, respectively, as shown in Table 9. This shows that there is not enough evidence at the level of 0.05 that there is a lack of fit in the reduced quadratic regression model. Therefore, the reduced quadric model with coefficients shown in Table 10 is taken to be the model relating the yield to the independent variable time and temperature.

Table 10: Coefficient table information

	Intercept	A	B	AB	A^2	B^2
Yield	4.86522	0.640609	0.816498			-0.130978
p-values		< 0.0001	< 0.0001			0.0048

4.2.3. Development of regression model equation in terms of coded and actual factors

When all other factors are maintained constant, the coefficient estimate is the expected change in response per unit change in factor value. In an orthogonal design, the intercept is the overall average response of all the runs. The coefficients are modifications based on the factor settings around that average. The VIFs value (Table 11) are one when the factors are orthogonal; VIFs more than one imply multi-colinearity; the higher the VIF, the more severe the factor correlation. VIFs of fewer than ten are considered tolerable.

Table 11: Coefficients in terms of coded factors

Factor	Coefficient Estimate	Df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	4.87	1	0.0339	4.79	4.94	
A-Temperature	0.6406	1	0.0332	0.5656	0.7156	1.0000
B-Time	0.8165	1	0.0332	0.7415	0.8915	1.0000
B^2	-0.1310	1	0.0352	-0.2107	-0.0512	1.0000

The application of response surface methodology provides an empirical association between the response function and the independent variables. The Design-Expert 11.1.2 software package was used to analysis the statistical significance of each experimental factor and to generate the resultant mathematical prediction models. The equation in terms of coded factors can be used to create predictions about the reaction for certain levels of each ingredient. By default, the components with high levels are coded as positive one; while those with low levels are written as negative one Comparing the factor coefficients, the coded equation may be used to associate the relative influence of the elements. For given values of each element, the equation in terms of actual factors can be used to create predictions about the response. The differences between actual value and predicted value tell weather models are acceptable or not. If the difference are more model are invalid whereas if the differences are very small model are significant. From (Table 12) observed that the different between actual value and predicted value in each runs are small means that model are significant. Eq. (6) shows the final equation in terms of coded factors.

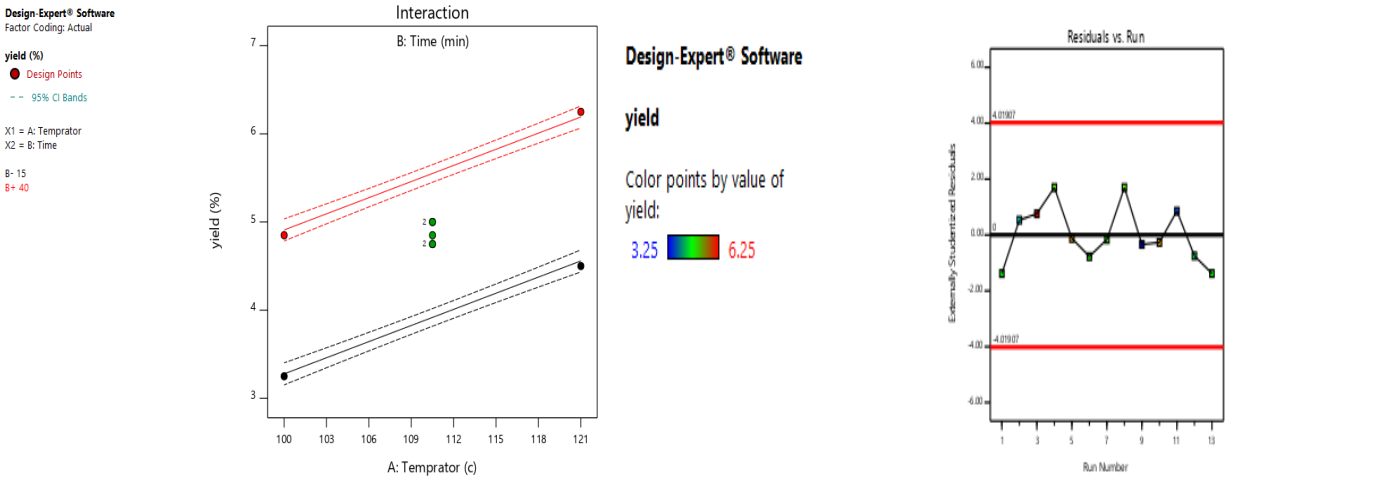
$$Yield = -0.1310\,B^2 + 0.8165B + 0.6406A + 4.87 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

Table 12: Over all report table of actual versus model predicted of total yield

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residuals	Externally Studentized Residuals	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
1	4.75	4.87	-0.1152	0.130	-1.318	-1.383	0.065	-0.536	9
2	4.00	3.96	0.0407	0.380	0.552	0.529	0.047	0.415	5
3	6.25	6.19	0.0587	0.348	0.775	0.756	0.080	0.552	4
4	5.00	4.87	0.1348	0.130	1.541	1.694	0.089	0.656	13
5	5.75	5.76	-0.0080	0.598	-0.134	-0.126	0.007	-0.154	8
6	4.85	4.91	-0.0601	0.348	-0.794	-0.776	0.084	-0.567	3
7	4.85	4.87	-0.0152	0.130	-0.174	-0.164	0.001	-0.064	11
8	5.00	4.87	0.1348	0.130	1.541	1.694	0.089	0.656	10
9	3.25	3.28	-0.0271	0.348	-0.358	-0.340	0.017	-0.248	1
10	5.75	5.77	-0.0212	0.380	-0.287	-0.272	0.013	-0.213	6
11	3.50	3.45	0.0514	0.598	0.865	0.852	0.278	1.039	7

12	4.50	4.56	-0.0584	0.348	-0.771	-0.752	0.079	-0.549	2
13	4.75	4.87	-0.1152	0.130	-1.318	-1.383	0.065	-0.536	12

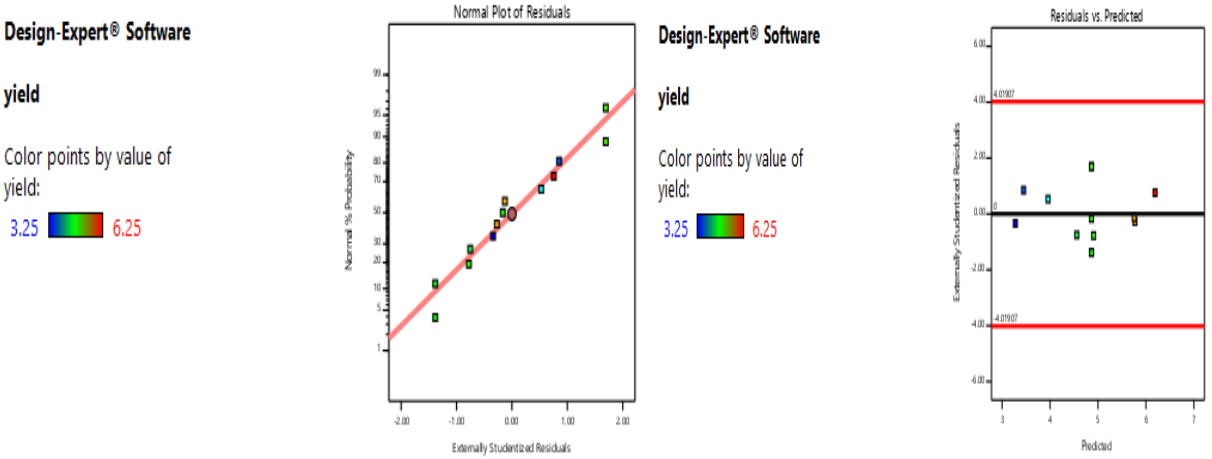
Based on the analysis of variance, the extraction of saponin from ziziphus leaf was significantly influenced by temperature, and time process variables not their interactions. The process variables are time and temperature. This result showed that the advantage of using design of experiments in taking the interaction between variables that affects the saponin extraction process. The individual process variables were concerned was no interaction effect. As a result, the effects of process variable on extraction of saponin were plotted (Figure 5a). Usually, rise in extraction temperature, and extraction time are obtained to increase the yield. The residuals versus experimental run order Figure 5b) tests for lurking factors that may have affected the response throughout the experimental run. The plot should show random scatter, and it has been remarked that all the data points remain within the limits (±4).



a. b.

Figure 5: A. interaction of time and temperature on yield, and B. residuals versus experimental run order

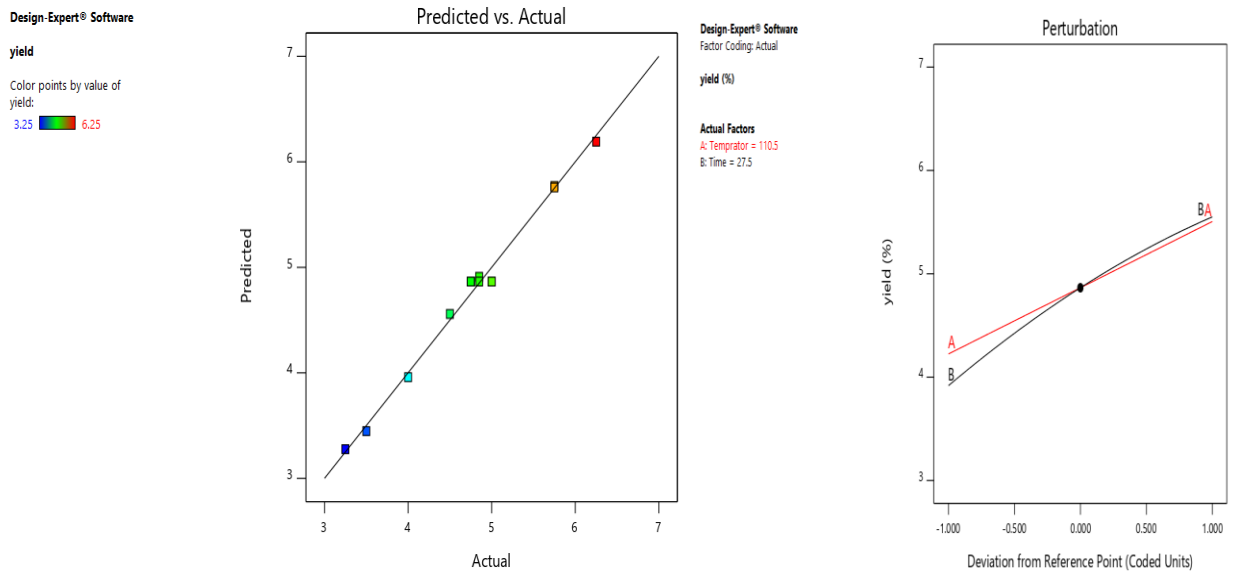
Normal probability plot of the raw data used to test the assumption of normality when using t-test. In the ANOVA, it is typically more effective and straight forward to do this with the residuals. In visualizing the straight line, place more stress on the central values of the plot than on the extremes. The normal probability plot (Figure 6a) designates the residuals result a normal distribution, in which case the points follow a straight line. This indicates the model fulfills the assumption of ANOVA, i.e. the error distribution are approximately normal.



a. b.

Figure 6: a. normal % probability and internally studentzed residuals versus normal plots of residuals, and b. residual versus predicted values

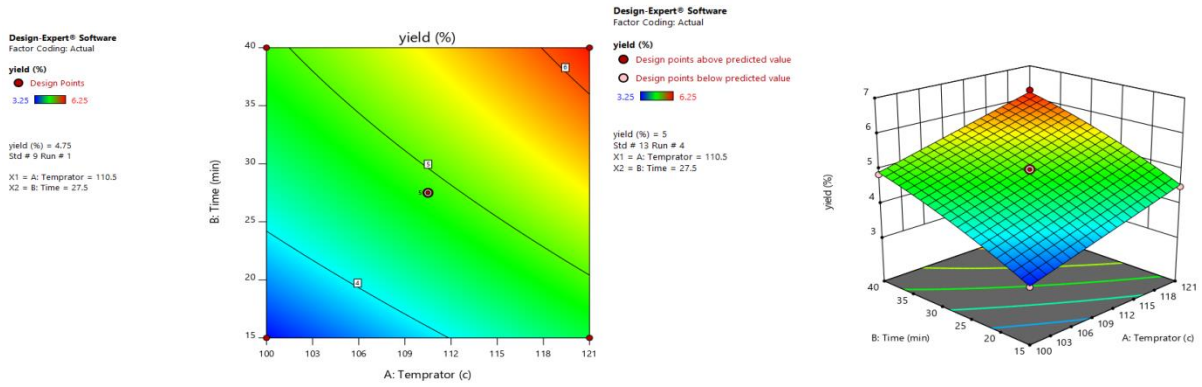
The graph of the predicted values obtained using the developed correlation versus actual values of the two reaction process variables to the percentage of yield. (Figure 7a) showed that the regression model equation gave a very precise description of the experimental data, in which all the points are very similar to the line of perfect fit. The perturbation plot depicts the relationship between all elements' impacts at a certain location in the design space. Only one element is changed across the response's range, while all other factors are held constant. The yield of saponin was drawn by altering only one factor over its range while the other factors were held constant. Perturbation plot can show the relative effects of all the examined independent variables on the yield of saponin. It can be seen from the perturbation plot that temperature and time had significant curving effect. A rise in reaction time would perhaps increase yield up to 6.191% while increase in reaction temperature decrease yield. From (Figure 7b) conclude that reaction time has greatest effects on extraction yield.



a) b)

Figure 7: a) experimental yield vs. predicted yield and b) dvaton from references pointverus yield

The 2D and 3D (Figure 8) response surface interactions between the time and temperature on the yield of saponin extraction. As the temperature and time varies either increasing or decreasing, the yield of saponin changes. The effect of temperature on saponin production was investigated using saponin yield as the response. The range of temperature considered and designed were between 95.6508 ℃ to 125.349 ℃. The time effect was varied from 15 min to 40 min. Generally, an increase in extraction temperature with times found to increase the yield up to some optimal value.



2 D 3D

Figure 8: The 2D and 3D response surface interactions between the time and temperature on the yield of saponin extraction

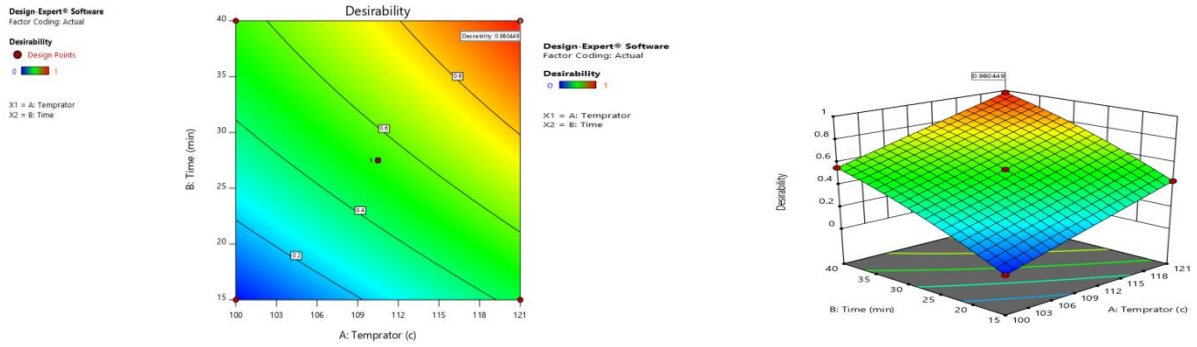
4.3. Optimization of process variables on extraction of saponin

Optimization of the process variables was used to obtain the highest percentage of saponin yield using developed model regression Therefore, optimizing process variables aimed to determine optimum conditions within the range of the values of the process variables by considering the constraints associated with variations of these process parameters. Based on the above analysis in order to obtain the best maximum percentage of yield, the predicted combination of parameters were as follows (Table 13): temperature of 121 °C, and reaction time of 40 min. And all these variables are in range. Under these conditions, the model predicted of yield is 6.191 % with a desirability value of 0.980. The more closely the response approaches the ideal intervals or ideal values, the closer the desirability is to one. In order to verify this prediction, an experiment was conducted in the selected optimum process variables and the result was compared with the statistical experimental design prediction. After the experiment was conducted, the percentage extraction of saponin found to be 98.08% .This result shows that the experimental result was agreed with the predicted value.

Table 13: Optimization of process variables on extraction of saponin

Number	Temperature	Time	Yield	Desirability	
1	121.000	40.000	6.191	0.980	Selected
2	121.000	39.864	6.185	0.978	
3	120.719	40.000	6.174	0.975	
4	121.000	39.542	6.171	0.974	
5	119.444	40.000	6.096	0.949	

The model validations have been determined as optimum levels of the process parameters to achieve yield 6.191. Optimum conditions predicted by the model using desirability ramp and triplicate experiments were conducted using the optimized extraction process conditions and the results are closely related with the data obtained from optimization analysis using desirability functions. Therefore, the numerical optimization can be taken as optimal value because the predicted value is close enough with actual value since desirability is 0.980 which are closed to one (Figure 9).



2D 3D

Figure 9: The 2D and 3D Response surface plot of the interaction effect of time and temperature versus desirability

4.4. FTIR analysis of extracted saponin

FTIR analysis with a wavenumber range of 4000 to 400 cm^{-1} was performed to detect information on the nature of the bonds and to identify different functional groups available on the sapoinn compound. The crystals saponin obtained from aqueous extracts were subjected to FTIR and the spectrum of the compound (Fig.11) showed a characteristic absorption band for the N-H stretch group at 3450 cm^{-1}, 1411cm^{-1} for the C=C group, and sharp absorption at 1634.5 cm^{-1} indicates Organic nitrates. Absorption at 712.23 cm^{-1} is inductive for cis = C–H out-of-plane bending. The FTIR graph result (Fig.10) of the extracted saponin shows similar with the standard FTIRgraph of saponin (appendix A).

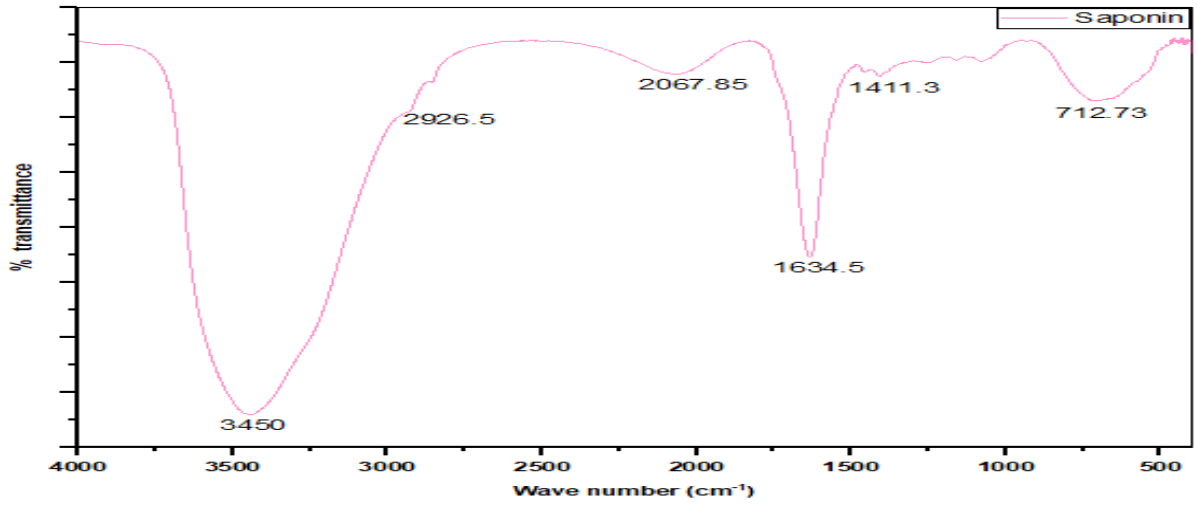


Figure 10: FTIR analysis of a product containing saponin

4.5. Formulation of herbal shampoo

A light yellow herbal shampoo (Figure 11) was formulated by mixing aqueous extracts of ziziphus and other organic additives (Table 4) in define amount. The ziziphus extracts contains saponin which is naturally bio surfactants possessing good detergency and foaming properties. CMC, citric acid, benzoic acid was added as thickener agent, pH adjuster, and preservative respectively. A good shampoo must have adequate viscosity, to facilitate removal from the bottle but must not drip down from the hair during use.

Figure 11: Formulated of herbal shampoo

4.6. Characterization of the formulated shampoo

This section discusses the essential characteristics of the formulated shampoo in comparison to commercial shampoos.

4.6.1. Determination of physical appearance visual inspection

Relative qualities of the herbal and commercial shampoo were assessed by using physical and physicochemical tests. The visual inspection of the physical parameter such as color, appearances, spread ability, and odor of the formulated shampoo is comparable to the marketed herbal shampoos was judged to be satisfactory by all volunteers. Table 14 presents the physical appearance of three formulated shampoos and two marketed shampoos.

Table 14: Physical appearance/ visual inspection result of the three formulated and two marketed shampoo

No	Physical appearance	F_1	F_2	F_3	Aloe Vera shampoo (marketed)	Organza shampoo (marketed)
1	Color	Heavy yellow	Light yellow	Light yellow	Light green	Light green
2	Spread ability	Good	Good	Very good	Very good	Very good
3	Odor	Good	Good	Good	Very good	Very good
4	Appearance	Opaque	Opaque	Opaque	Opaque	Opaque

The results of visual inspection of series of formulations (Table 14) seen are good, and very good characteristics with respect to odor and spread ability. From the three formulated shampoos formulation F_3 has very similar property to both Aloe and organza marketed shampoo. F_3 was found to have the best result, and it was selected as the best formulation by the volunteers.

4.6.2. Investigation of the stability characteristics of the shampoos

The three formulated, and two marketed shampoo were studied for stability at 25 ℃, 37 ℃ and 45 ℃ at the initial, after a month, and after two month time period. The shampoos were found to be stable at all the three temperatures, and time periods with respect to colour, odor, spread ability and appearance. This suggests good stability of the formulated shampoos throughout their shelf life.

4.6.3. Determination of pH

The pH of shampoos are found to significant for beating and improving the qualities of hair, reducing irritation to the eyes and steadying the ecological balance of the scalp. The pH of shampoo should be between 5.5 and 6.0, as this corresponds to the isoelectric point of hair. A shampoo with a pH value of more than 6 make the hair dry and drab (Dubey et al., 2004). Table 15 shows the pH for the three formulations and two marketed shampoo at temperatures 25℃, 37℃ and 45℃ and at the beginning, after a month and after two months. The two formulated (F_2 and F_3), and organza marketed shampoo were acid balanced and were ranged 5.5 to 5.9 which is ranged from 5.8067 ±0.0107 to 5.9333±0.00533. The two marketed shampoo whose pH range

between 5.8022 ±0.00356 to 6.3322 ±0.00356. From Table 18 it is observed that the pH value of F_2 and F_3 were 5.8644 ±0.00711 and 5.8067 ±0.0107 which are closely related to Organza and pH of the skin and were designated as the best formulations.

Table 15: Evaluation for pH (mean± SD, n=3) for the three formulation and two marketed shampoo at (initial, after a month and after two month), and at (25℃, 37℃ and 45℃).

Time for sample	Temperature in℃	Formulation I	Formulation II	Formulation III	Aloe Vera Shampoo	Organza shampoo
At initial time	25	5.9333 ±0.00533	5.8644 ±0.00711	5.8067 ±0.0107	6.3322 ±0.00356	5.8022 ±0.00356
	37	5.9344 ±0.00711	5.8544 ±0.00889	5.8044 ±0.0551	6.3322 ±0.00356	5.8033 ±0.00533
	45	5.9344 ±0.00711	5.8622 ±0.00356	5.8067 ±0.0107	6.3322 ±0.00356	5.8067 ±0.0107
After a month	25	5.9344 ±0.00711	5.8667 ±0.0107	5.8089 ±0.00775	6.3333 ±0.00533	5.8022 ±0.00356
	37	5.9367 ±0.0107	5.8667 ±0.0107	5.8011 ±0.0498	6.3356 ±0.00889	5.8022 ±0.00356
	45	5.9356 ±0.00889	5.8644 ±0.0047	5.8021 ±0.0739	6.3322 ±0.00356	5.8033 ±0.00533
After two month	25	5.9289 ±0.0142	5.8544 ±0.0047	5.8067 ±0.0107	6.3344 ±0.0124	5.8078 ±0.0099
	37	5.9267 ±0.00815	5.8667 ±0.0107	5.8067 ±0.0107	6.3333 ±0.00533	5.8044 ±0.00711
	45	5.9244 ±0.00356	5.8633 ±0.00533	5.8078 ±0.0099	6.3344 ±0.0047	5.8056 ±0.00889

The influence of the three formulated, and two marketed shampoo were studied for stability of pH value at storage conditions of 25°C, 37 °C, and 45 °C throughout the storage periods of initial, after a month and after two month obtainable no significant differences contrasted to the results obtained at zero time at 25 °C. We concluded that all the formulated and marketed shampoos are physically stable.

4.6.4. Determination of viscosity

Table 16 presents the viscosity of the three formulated and two marketed shampoo brands at 25℃, 37℃ and 45℃, initially, after a month and after two months. From Table 19 it is observed that the viscosity of the tested shampoos change slowly with change in temperature. The shampoo formulations were temperature-dependent. The shampoo formulations were pseudo-plastic in nature. Pseudo-plastic behavior is attractive quality in shampoos. At low temperature the shampoos showed high viscosity and increase temperature causes viscosity drop. Shampoo F_3 showed pseudo plastic behavior the same as the commercial (reference), which is a desirable quality in a shampoo formulation. F_3 was 381 ±0.924 at 25℃ and initial time which are closely related to Aloe Vera marketed shampoo compared with two formulation, and it was selected as best formulation.

Table 16: Viscosity value (mean ± SD, n=3) for the three formulated and two marketed shampoo brands at (initial, after a month and after two month), and at (25℃, 37℃ and 45℃).

Time for sample	Temperature in℃	Formulation I	Formulation II	Formulation III	Aloe Vera Shampoo	Organza shampoo
At initial time	25	280.3333 ±0.533	315.6667 ±1.067	381 ±0.924	478.6667 ±0.533	773.3333 ±0.533
	37	268.3333 ±1.067	305.6667 ±1.067	358.3333 ±0.533	477.3333 ±1.067	685 ±0.924
	45	264.3333 ±1.067	302.6667 ±1.067	354.3333 ±0.533	414.3333 ±0.533	577 ±0.924
After a month	25	280.6667 ±1.067	315.6667 ±1.067	381 ±1.6	478.6667 ±0.533	773.6667 ±1.067
	37	273.3333 ±0.533	312.6667 ±1.067	361.3333 ±0.533	481.6667 ±1.067	686.6667 ±0.533
	45	265.3333 ±1.411	303.3333 ±1.411	353.6667 ±0.533	414.6667 ±0.533	577.3333 ±0.533
After two	25	281.3333 ±1.067	317.6667 ±1.067	381.6667 ±1.067	479.3333 ±0.533	773.6667 ±0.533

month	37	273.6667 ±0.533	312.3333 ±1.067	361.6667 ±1.411	479.6667 ±1.067	686.3333 ±1.067
	45	265.6667 ±1.411	303 ±1.6	353.6667 ±0.533	414.3333 ±0.533	577.3333 ±0.533

When compared to the values obtained at zero time, the viscosity did not change appreciably between the different storage time periods. Viscosity, on the other hand, fluctuates with storage temperature (as storage temperature rises, viscosity value falls). This shows that all shampoos designed and marketed are physically stable during their shelf life.

4.6.5. Foaming ability and foam stability analysis

The cylinder shaking method is used to determine the foam volume of the marketed and designed shampoo (Table 17). One percent of 50 mL of shampoo solution was poured into a 250-mL graduated cylinder, which was then encased by hand and shaken ten times at room temperature, 37 °C, and 45 °C for time periods of initial, after a month, and after two months. The ideal foam volume must remain constant during the study period (Bouranen, 2017). The total volume of the foam content after one minute of shaking was recorded. It was found to be 125.35 ±0, 187.1667 ±2.87, and 155.5 ±1.617 for F_3, Aloe Vera, and Organza respectively. Foaming ability was observed to be good for F_3 comparison with the F_1 (73.3333 ±0.533ml), and F_3 (95 ±1.848 ml).

Table 17: Evaluation of foaming ability and foam stability (mean foam volume (cm^3) ± SD, n=3) for the three formulated and two marketed shampoo brands at (initial, after a month and after two month), and at (25℃, 37℃ and 45℃).

Time for sample	Temperature in℃	Formulation I	Formulation II	Formulation III	Aloe Vera Shampoo	Organza shampoo
At initial time	25	73.3333 ±0.533	95 ±1.848	125.35 ±0	187.1667 ±2.87	155.5 ±1.617
	37	76.3333 ±2.325	92 ±1.6	129.2 ±2.459	189.1667 ±3.713	160.1667 ±2.096
	45	70.6667 ±2.371	96.6667 ±3.734	130.6167 ±3.074	189.45 ±2.08	170.5 ±4.509
After a	25	73.6667 ±1.411	101.3333 ±1.923	128.8 ±1.077	190.6167 ±2.15	160.05 ±3.441

month	37	70.25 ±2.275	95 ±1.848	129.6167 ±2.15	189.0833 ±2.934	159.5 ±6.602
	45	72.4167 ±2.314	93.8333 ±5.193	134.9167 ±2.677	187.1667 ±2.87	167.1667 ±2.146
After two month	25	73.6667 ±1.067	96.6667 ±2.134	133.5 ±3.296	191.8333 ±2.314	163.1667 ±2.627
	37	73.3333 ±1.411	95 ±1.848	127.6167 ±1.228	188.0833 ±2.934	161.3333 ±3.698
	45	71 ±2.772	95 ±1.848	125.4 ±0.08	187.1667 ±2.87	155.5 ±1.617

The effect of the storage conditions at 25°C, 37 °C, and 45 °C on the foam profile of the formulated and marketed shampoos throughout storage periods of initial, after a month and after two month showed no significant differences compared to the results obtained at zero time at 25 °C. The foam made by formulated shampoo continued unchanged within the five minute time period. This verifies that all the formulated shampoos are physically stable.

4.6.6. Evaluation of wetting ability

The ability of a surfactant to wet varies depending on its concentration, which is commonly used to assess its effectiveness. Diffusion, surface tension, concentration, and the type of the wetted surface are just a few of the processes and components that go into making a wetting prodigy. Surface tension must be reduced by each wetting cause. The findings of the three formulation shampoos and two marketed shampoos (Table 18) demonstrate that F3 has the highest detergent concentration because it has the shortest wetting time (52±4.234min) compared to F1, which has the longest wetting time (92.6667±2.325min)..

Table 18: Evaluation of wetting ability (mean second ± SD, n=3) for the three formulated and two marketed shampoo brands at (initial, after a month and after two month), and at (25℃, 37℃ and 45℃).

Time for sample	Temperature in℃	Formulation I	Formulation II	Formulation III	Aloe Vera Shampoo	Organza shampoo
At initial	25	92.6667 ±2.325	85 ±4.62	52 ±4.234	20.3333 ±2.97	32.3333 ±2.325
	37	92.3333	81.6667	53.6667	23.6667	32.6667

time		±5.254	±7.693	±1.067	±1.923	±2.325
	45	100 ±1.848	85.6667 ±3.734	52 ±4.234	22 ±0.924	32.6667 ±1.923
After a month	25	101 ±1.848	75.6667 ±8.585	51 ±1.848	23.6667 ±2.823	34 ±1.848
	37	90.6667 ±1.923	88.6667 ±1.411	55 ±1.848	20.6667 ±1.411	39 ±1.848
	45	102.3333 ±1.067	89.6667 ±1.067	56 ±1.848	24 ±1.848	32 ±1.6
After two month	25	91 ±6.976	71.6667 ±18.348	51.6667 ±1.923	20.3333 ±2.97	34.6667 ±3.245
	37	93 ±12.906	84.6667 ±7.749	52 ±4.234	19.3333 ±2.97	35 ±2.445
	45	94.6667 ±3.847	83 ±6.663	51.6667 ±2.97	25.6667 ±1.923	33.3333 ±3.734

The effect of storage temperatures of 25°C, 37°C, and 45°C on the wetting ability profile of F_3 developed and both marketed shampoos during the initial, one-month, and two-month storage periods yielded no significant differences when compared to the data obtained at zero time at 25 °C. However, the wetting ability of F_1 and F_2 differed significantly across all storage conditions and timeframes. The lone F_3 designed shampoo, as well as both commercialized shampoos, was found to be physically stable.

4.6.7. Determination of percentage of solid content

Effective shampoos often have a solid content of 22–29 percent, making them simple to apply and rinse out of the hair. It was too liquid and washed away quickly if there weren't enough particles in the shampoo (Sharma et al., 2011). The solid material percentages (Table 19) reveal that F1 and F2 were determined to be below the required range (13.0833 ± 0.833 to 18.2967 ± 0.0533%), and to wash away easily. F_3, Aloe Vera, and organza shampoo were 23.0767 ±0.123, 24.5167 ±0.0267, and 27.0833 ±0.133 respectively. We conclude that F_3 was acceptable at 25℃ and initial time which is closely related to Aloe Vera marketed shampoo compared form the other two formulations.

Table 19: Solid content percentage (mean ± SD, n=3) for the three formulation and two marketed shampoo brands at (initial, after a month and after two month), and at (25℃, 37℃ and 45℃).

Time for sample	Temperature in℃	Formulation I	Formulation II	Formulation III	Aloe Vera Shampoo	Organza shampoo
At initial time	25	14.0833 ±0.133	18.0833 ±0.112	23.0767 ±0.123	24.5167 ±0.0267	27.0833 ±0.133
	37	14.0833 ±0.133	18.0933 ±0.149	23.0833 ±0.133	24.4333 ±0.107	27.0833 ±0.133
	45	14.1567 ±0.00533	18.2967 ±0.0533	22.7833 ±0.0533	24.5333 ±0.0533	27.0167 ±0.0267
After a month	25	14.12 ±0.192	18.1167 ±0.187	23.1333 ±0.213	24.4 ±0.16	27.1167 ±0.187
	37	14.15 ±0.24	18.09 ±0.144	23.0733 ±0.117	24.5 ±0.185	24.5 ±0.185
	45	14.0933 ±0.149	17.6667 ±0.133	23.1333 ±0.213	24.39 ±0.118	27.25 ±0.231
After two month	25	14.0833 ±0.133	18.1167 ±0.187	23.0167 ±0.0267	24.4667 ±0.0448	27.0867 ±0.139
	37	13.8333 ±0.133	18.2533 ±0.0053	23.0167 ±0.0267	24.4333 ±0.107	27.0067 ±0.0107
	45	14.1567 ±0.0107	18.26 ±0.016	23.0533 ±0.0427	24.4 ±0.16	26.9667 ±0.0533

Shampoo is physically stable for the duration of the stability test at a specific temperature (Vijayalakshmi et al., 2018). The effect of storage temperatures (25 °C, 37 °C, and 45 °C) on the percentage of solid content of designed and marketed shampoos during the initial, one-month, and two-month storage periods revealed no significant differences when comparing data taken at 25 °C at zero time to data taken at 25 °C at zero time. In this method, the shampoos' physical stability is proved.

4.6.8. Dirt dispersion analysis

Dirt dispersion is an important criterion for determining how well shampoos clean. To achieve better washing activity, dirt should remain in the water component (P. Arora et al., 2011). None means that good results in the dirt dispersion test because there was no ink distribution in their foam in the formulation F_3, Aloe Vera, and Organza tested shampoos recorded (Table 20) as none means that good results in the dirt dispersion test because there was no ink distribution in their foam.

Table 20: Dirt dispersion for the three formulation and two marketed shampoo brands at (initial, after a month and after two month), and at (25℃, 37℃ and 45℃).

Time for sample	Temperature in℃	Formulation I	Formulation II	Formulation III	Aloe Vera Shampoo	Organza shampoo
At initial time	25	Light	None	None	None	None
	37	Light	Light	None	None	None
	45	Light	Light	None	None	None
After a month	25	Light	Light	None	None	None
	37	Light	Light	None	None	None
	45	Light	Light	None	None	None
After two month	25	Light	Light	None	None	None
	37	Light	Light	None	None	None
	45	Light	Light	None	None	None

The influence of storage temperatures of 25°C, 37°C, and 45°C on the dirt dispersion profile of marketed and formulated shampoos throughout initial, one-month, and two-month storage periods revealed no significant differences when compared to the data obtained at 25°C at zero time, and it was physical stability.

4.6.9. Determination of surface tension

Surface tension reduction is one of the indications for good detergency. If a shampoo reduces the surface tension of pure water from 72.28 dyne/cm to around 40 dyne/cm, it is considered to have good cleansing properties (R. Arora et al., 2019). The surface tensions of the

shampoos examined varied from 30.9067 ±0.126 to 35.0333 ±0.187 dyne/cm (Table 21), which are satisfactory with the lowest surface tension suggesting the strongest cleaning performance.

Table 21: Surface tension (mean ± SD, n=3) for the three formulation and two marketed shampoo brands at (initial, after a month and after two month), and at (25℃, 37℃ and 45℃).

Time for sample	Temperature in℃	Formulation I	Formulation II	Formulation III	Aloe Vera Shampoo	Organza shampoo
At initial time	25	31.5 ±0.231	32.5 ±0.231	32.51 ±0.199	34.5267 ±0.223	35.0333 ±0.187
	37	31.4333 ±0.162	31.7033 ±0.205	33.51 ±0.232	34.4867 ±0.213	34.4967 ±0.236
	45	31.24 ±0.617	32.3667 ±0.233	33 ±0.231	34.4133 ±0.349	34.38 ±0.116
After a month	25	33.2567 ±0.222	32.21 ±0.24	32.6433 ±0.119	34.5133 ±0.25	34.4633 ±0.238
	37	31.5167 ±0.208	31.6167 ±0.116	33.26 ±0.245	34.5 ±0.231	34.2967 ±0.243
	45	30.9067 ±0.126	31.7367 ±0.121	32.4933 ±0.222	34.4967 ±0.226	34.25 ±0.231
After two month	25	31.52 ±0.233	32.4267 ±0.0594	33.4833 ±0.179	34.58 ±0.233	34.3267 ±0.355
	37	31.2933 ±0.129	31.57 ±0.242	33.27 ±0.204	34.4967 ±0.226	34.38 ±0.347
	45	30.9067 ±0.126	31.5 ±0.231	32.66 ±0.171	34.3633 ±0.342	34.38 ±0.116

Moreover, the influence of the storage states at 37℃, and 45℃ on the measurement of the surface tension of F_2, F_3, Aloe Vera, and Organza shampoos throughout at initial, after a month and after two month of the storage periods are obtainable no significant change compared to the results obtained at zero time at 25 °C. But the impact of the storage conditions at 37℃, and 45℃ on the measurement of the surface tension of F_1 during storage periods at initial, after a month and after two month are presented has slightly difference compared to the results obtained

at zero time at 25 °C. This confirms that only F_2 and F_3, Aloe Vera, and Organza are physically stable.

5. Conclusion and recommendation

5.1. Conclusion

The goal of this study was to create and characterize a herbal shampoo that minimizes hair loss when combing and is stable and functionally effective without the use of any synthetic chemicals. Herbal shampoo was created using the aqueous extract of ziziphus leaf plant extracts rather than synthetics, which are traditionally used in Tigray, Ethiopia, for hair washing. The saponin extraction parameters from ziziphus leaf were studied and optimized using the CCD RSM method. The yield of saponin increases as the temperature and residence time increase; however, a temperature of 140 °C and a reaction period of 40 minutes were discovered to be the optimum values, which are about 6.19%.

In a series of tests to assess the quality of these shampoos, the physicochemical properties of both formulated and commercialized shampoos were evaluated and compared. Three developed and two marketed shampoo brands were examined in terms of their physical appearance/visual inspiration, pH levels, foam formation and foam stability, viscosity, wetting time, surface tension, and dirt dispersion. All of the prepared shampoos' pH and surface tension values meet the standards' requirements, indicating that they are chemically sound. Many of the created shampoos' features were found to be within the usual range, however some were out of range for some shampoo. The results demonstrated that there were no significant changes in the formulated shampoo's physicochemical attributes stability at different temperatures and times when compared to the reference, and they are all physically sable. Formulation (F_3) shampoo exhibited all of the ideal shampoo qualities and exhibited similar properties to the two reference shampoos, hence it was chosen as the best formulation. Finally, the research is critical for ensuring the creation of an alternative type of herbal shampoo with a wide range of cosmetic applications, as well as meeting customer needs and eliminating the need for foreign currency.

5.2. Recommendation

Further research is required on F_3 to determine cleaning effectiveness, conditioning performance, skin sensitization, eye irritation, and toxicity test.

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Appendix A

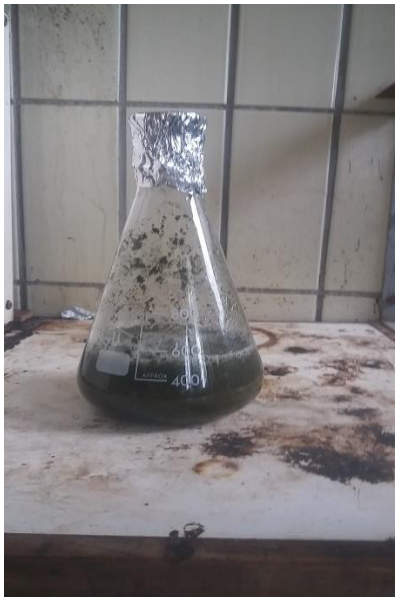
Dry sample

Moisture analyzer

Curer mill machine

1 mm particle size

Electronic balance



1:20 g/ml mixture solution

Sano Clave model of Autoclave

Hot cured Extracted saponin

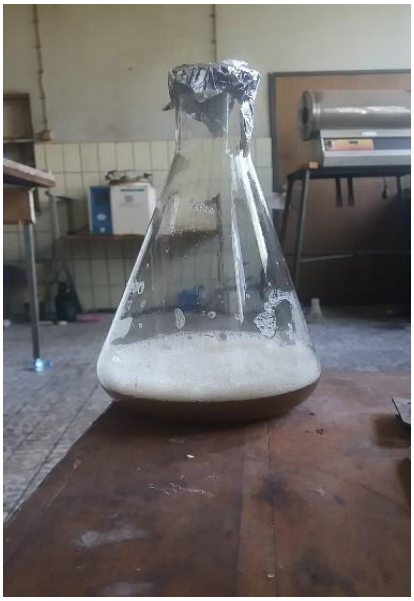
Nylon wool Filtered saponin

Crude filtered saponin using qualitative filter paper with the aid of vacuumed pressurized machine

Water bath

Concentrated saponin

Quantitative saponin test result



Qualitative saponin test result

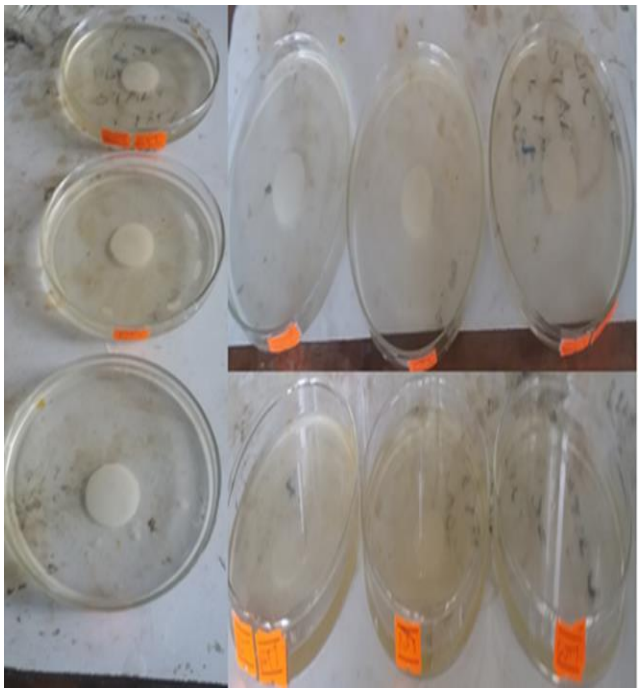
Homogenizers

Final formulated product

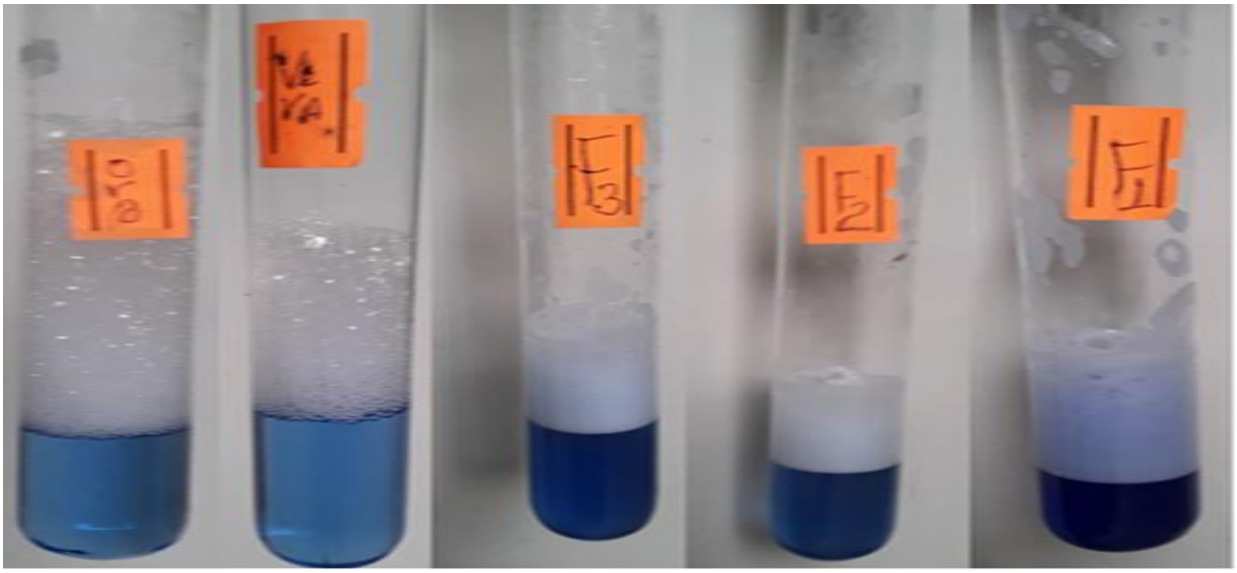
Viscosity measurement

Percentage of Solid content determination

Foam volume ability and stability



Wetting time measurement



Dirt dispersion analysis

Figure 5: Specific locations of the two sampling collected area

Sources: Google map-satellite

The two brand marketed shampoo

A) Latitude 14°05'40.8"N, longitude 38°56'34.1"E,

B) Latitude 14°05'39.4"N, longitude 38°56'34.9"E

Appendix B

The column information of experimental factors and levels for extraction of saponin from leave sample of ziziphus plant in design expert version 11 software.

Name	Units	Type	Changes	Std. Dev.	Low	High

Temperature	℃	Factor	Easy	0	100	121
Time	Min	Factor	Easy	0	15	40
Yield	%	Response		0		

The total Factors used set in the design expert version 11 soft ware

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding
A	Temperature	103.89	100.00	121.00	0.0000	Actual
B	Time	25.00	15.00	40.00	0.0000	Actual

Factors table information

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Temperature	C	Numeric	95.65	125.35	-1 ↔ 100.00	+1 ↔ 121.00	110.50	8.57
B	Time	Min	Numeric	9.82	45.18	-1 ↔ 15.00	+1 ↔ 40.00	27.50	10.21

Moisture content of dry ziziphus leaf sample.

		Drying Time (hour)						Moisture Content (%)
	Particle Size (mm)	0	2	4	6	8	10	
Sample Weight (gm)	1	500	498.25	496.75	495.75	495.15	495.00	1

Build Information

File Version	11.1.2.0		
Study Type	Response Surface	Subtype	Randomized
Design Type	Central Composite	Runs	13
Design Model	Quadratic	Blocks	No Blocks

Build Time (ms)	39.00		

. Sequential model sum of squares

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs Total	297.60	1	297.60			
Linear vs Mean	8.62	2	4.31	214.83	< 0.0001	
2FI vs Linear	0.0056	1	0.0056	0.2597	0.6226	
Quadratic vs 2FI	**0.1217**	**2**	**0.0609**	**5.82**	**0.0324**	**Suggested**
Cubic vs Quadratic	0.0074	2	0.0037	0.2797	0.7671	Aliased
Residual	0.0658	5	0.0132			
Total	306.42	13	23.57			

Fit summery

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	< 0.0001	0.3733	0.9727	0.9615	
2FI	0.6226	0.3187	0.9705	0.9535	
Quadratic	**0.0324**	**0.8812**	**0.9858**	**0.9806**	**Suggested**
Cubic	0.7671	0.6943	0.9821	0.9684	Aliased

1. PH value

a) The measured PH value at 25℃ in initial time periods.

Measured Parameter PH value		Trial one	Trial two	Trial three	Average	Average mean ±SD n=3
Aloe Vera marketed shampoo						
Required time 5 minutes	1 min	6.8	6.85	6.9	6.85	
	2min	6.65	6.75	6.8	6.73333	
	3 min	6.33	6.35	6.33	6.3367	

	4 min	6.33	6.33	6.33	6.33	
	5min	6.33	6.33	6.33	6.33	6.3322 ±0.00356
Organza marketed shampoo						
Required time 5 minutes	1 min	6.83	6.79	6.9	6.84	
	2 min	6.65	6.45	6.5	6.533333333	
	3 min	5.8	5.82	5.8	5.806666667	
	4 min	5.8	5.8	5.8	5.8	
	5 min	5.8	5.8	5.8	5.8	5.8022 ±0.00356
Formulation III						
Required time 5 minutes	1 min	6.6	6.45	6.7	6.583333333	
	2 min	6.2	6.23	6.35	6.26	
	3 min	5.81	5.8	5.84	5.82	
	4 min	5.8	5.8	5.8	5.8	
	5 min	5.8	5.8	5.8	5.8	5.8067 ±0.0107
· Formulation II						
Required time 5 minutes	1 min	6.89	6.85	6.95	6.896666667	
	2 min	6.62	6.4	6.5	6.506666667	
	3 min	5.9	5.86	5.86	5.873333333	
	4 min	5.86	5.86	5.86	5.86	
	5 min	5.86	5.86	5.86	5.86	5.8644 ±0.0071
· Formulation I						
Required time 5 minutes	1 min	6.9	6.95	6.93	6.926666667	
	2 min	6.45	6.05	6.15	6.216666667	
	3 min	5.95	5.93	5.94	5.94	
	4 min	5.93	5.93	5.93	5.93	
	5 min	5.93	5.93	5.93	5.93	5.9333 ±0.0053

2. Wetting time

b) The measured wetting value at 25℃ in initial time periods.

Measured Parameter wetting	Trial one	Trial	Trial	Average	means ±SD, N=3

time		two	three		
Organza marketed shampoo	0:00:30	0:00:35	0:00:32	0:00:32	32.3333 ±2.325
Aloe Vera marketed shampoo	0:00:24	0:00:18	0:00:19	0:00:20	20.3333 ±2.97
Formulation III	0:00:51	0:00:48	0:00:57	0:00:52	52 ±4.234
Formulation II	0:01:30	0:01:20	0:01:25	0:01:25	85 ±4.62
Formulation I	0:01:30	0:01:35	0:01:33	0:01:33	92.6667 ±2.325

3. Viscosity Value

c) The measured Viscosity value at 25℃ in initial time periods.

Measured Parameter Viscosity	Trial one	Trial two	Trial three	Average	Means± SD n=3
Organza marketed shampoo	774	773	773	773.3333333	773.3333 ±0.533
Aloe Vera marketed shampoo	479	478	479	478.6666667	478.6667 ±0.533
Formulation III	382	380	381	381	381 ±0.924
Formulation II	317	315	315	315.6666667	315.6667 ±1.067
Formulation I	281	280	280	280.3333333	280.3333 ±0.533

4. Foaming power and foaming stability

d) The measured foaming power and foaming stability value at 25℃ in initial time periods.

For Aloe Vera marketed shampoo	Trial one	Trial two	Trial three	Average	Mean ± SD ,n=3
Foam recorded (F1)	230	235	230	231.6666667	
Water recorded (F2)	44.75	44.25	44.5	44.5	
foaming power=F1-F2	185.25	190.75	185.5	187.1666667	187.1667 ±2.87
For Aloe Organza marketed shampoo					
Foam recorded (F1)	202	200	198	200	
Water recorded (F2)	44.75	44.5	44.25	44.5	
foaming power=F1-F2	157.25	155.5	153.75	155.5	155.5 ±1.617
For Formulation III					

Foam recorded (F1)	170	170	170	170	
Water recorded (F2)	44.65	44.65	44.65	44.65	
foaming power=F1-F2	125.35	125.35	125.35	125.35	125.35 ±0
For Formulation II					
Foam recorded (F1)	131	130	129	130	
Water recorded (F2)	34	35	36	35	
foaming power=F1-F2	97	95	93	95	95 ±1.848
For Formulation I					
Foam recorded (F1)	110	110	110	110	
Water recorded (F2)	36	37	37	36.66666667	
foaming power=F1-F2	74	73	73	73.33333333	73.3333 ±0.533

5. % of solid content

$$\%\ of\ solid\ content = \left(\frac{mass\ of\ solid\ left\ after\ boiling}{mass\ of\ shampoo}\right) * 100 \qquad (3)$$

- Where, M0 is the mass of clean evaporating dish
- M1 is mass of evaporating dish plus mass of shampoo before drying
- M2 is mass of shampoo only
- M3 is mass of evaporating dish plus shampoo left after drying
- M4 is mass of solid left after boiling[M3 –M0]
- M4a is average mass of solid left after boiling
- M2a is average mass of shampoo only

e) The % of solid content calculated value at 25℃ initial time periods.

% of Solid content determination for organza marketed shampoo

$$\%\ of\ solid\ content = \left(\frac{mass\ of\ solid\ left\ after\ boiling}{mass\ of\ shampoo}\right) * 100 \qquad (3)$$

Measured Parameter	Trial one	Trial two	Trial three	Average
M0	42.85	42.5	42.35	42.57
M1	46.85	46.5	46.35	46.57
M2	4	4	4	4
M3	43.89	43.58	43.47	43.65
M4	1.04	1.08	1.12	1.08

$$\%\ of\ solid\ content = \left(\frac{M4a}{M2a}\right) * 100$$

$$\%\ of\ solid\ content = \left(\frac{1.08g}{4g}\right) * 100\ = 27\%$$

- **% of Solid content determination for Aloe Vera marketed shampoo**

$$\%\ of\ solid\ content = \left(\frac{mass\ of\ solid\ left\ after\ boiling(M4a)}{mass\ of\ shampoo(M2a)}\right) * 100$$

Measured Parameter	Trial one	Trial two	Trial three	Average
M0	79.3	79.5	79.02	79.27
M1	83.3	83.5	83.02	83.27
M2	4	4	4	4
M3	80.28	80.47	80.01	80.25
M4	0.98	0.97	0.99	0.98

$$\%\ of\ solid\ content = \left(\frac{M4a}{M2a}\right) * 100$$

$$\%\ of\ solid\ content = \left(\frac{0.98g}{4g}\right) * 100\ = 24.5\%$$

- **% of Solid content determination for Formulation III shampoo**

$$\%\ of\ solid\ content = \left(\frac{mass\ of\ solid\ left\ after\ boiling}{mass\ of\ shampoo}\right) * 100 \qquad (3)$$

Measured Parameter	Trial one	Trial two	Trial three	Average
M0	45.17	45.20	44.97	45.11
M1	49.17	49.20	48.97	49.11
M2	4	4	4	4
M3	46.05	46.12	45.93	46.03
M4	0.88	0.92	0.96	0.92

$$\%\ of\ solid\ content = \left(\frac{M4a}{M2a}\right) * 100$$

$$\%\ of\ solid\ content = \left(\frac{0.92g}{4g}\right) * 100\ = 23\%$$

- **% of Solid content determination for Formulation II shampoo**

$$\%\ of\ solid\ content = \left(\frac{mass\ of\ solid\ left\ after\ boiling}{mass\ of\ shampoo}\right) * 100 \qquad (3)$$

Measured Parameter	Trial one	Trial two	Trial three	Average
M0	32.65	32.68	32.62	32.65
M1	36.65	36.68	36.62	36.65
M2	4	4	4	4
M3	33.35	33.4	33.36	33.37
M4	0.70	0.72	0.74	0.72

$$\% \; of \; solid \; content = \left(\frac{M4a}{M2a}\right) * 100$$

$$\% \; of \; solid \; content = \left(\frac{0.72g}{4g}\right) * 100 \; = 18\%$$

- **% of Solid content determination for Formulation I shampoo**

$$\% \; of \; solid \; content = \left(\frac{mass \; of \; solid \; left \; after \; boiling}{mass \; of \; shampoo}\right) * 100 \qquad (3)$$

Measured Parameter	Trial one	Trial two	Trial three	Average
M0	45.27	45.25	45.29	45.27
M1	49.27	49.25	49.29	49.27
M2	4	4	4	4
M3	45.83	45.79	45.87	45.83
M4	0.56	0.54	0.58	0.56

$$\% \; of \; solid \; content = \left(\frac{M4a}{M2a}\right) * 100$$

$$\% \; of \; solid \; content = \left(\frac{0.56g}{4g}\right) * 100 \; = 14\%$$

NB: Triplicate experiment was performed and mean ± SD, n=3 are listed in the final result report of percent of solid content.

6. **Surface tension measurement**

$$R2 = \left[\frac{w3 - w1}{w2 - w1}\right] * \left(\frac{N1}{N2}\right) * R1 \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots (5)$$

f) The calculated surface tension value at 25℃ initial time periods.

- **The surface tension for formulation I shampoo**

Measured Parameter	Trial one	Trial two	Trial three	Average
W1	79.48	79.50	79.46	79.48
W2	123.20	123.22	123.18	123.20
W3	127.73	127.77	127.75	127.75
N1	24.80	25.20	25.00	25.00
N2	63.58	63.60	63.62	63.60
R1	72	72	72	72

- W1a=79.48g W2a= 123.20g
- W3a=127.75g R1= 72 dyne/cm
- N1a=25 N2a=63.60

$$R2 = \left[\frac{w3a - w1a}{w2a - w1a}\right] * \left(\frac{N1a}{N2a}\right) * R1 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

$$R2 = \left[\frac{127.75g - 79.48g}{123.20g - 79.48g}\right] * \left(\frac{25}{63.60}\right) * 72\ dyne/cm$$

$$R2 = \left[\frac{48.27g}{43.72g}\right] * \left(\frac{25}{63.60}\right) * 72\ dyne/cm = 31.25\ \text{dyne/cm}$$

- **The surface tension for formulation II shampoo**

Measured Parameter	Trial one	Trial two	Trial three	Average
W1	79.48	79.54	79.42	79.48
W2	123.20	123.26	123.14	123.20
W3	127.70	127.77	127.78	127.75
N1	24.40	25.60	25.00	25.00
N2	61.00	61.30	61.60	61.30
R1	72	72	72	72

- W1a=79.48g W2a= 123.20g
- W3a=127.85g R1a= 72 dyne/cm
- N1a=25 N2a=61.30

$$R2 = \left[\frac{w3a - w1a}{w2a - w1a}\right] * \left(\frac{N1a}{N2a}\right) * R1 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

$$R2 = \left[\frac{127.85g - 79.48g}{123.20g - 79.48g}\right] * \left(\frac{25}{61.30}\right) * 72\ dyne/cm$$

$$R2 = \left[\frac{48.37g}{43.72g}\right] * \left(\frac{25}{61.30}\right) * 72\ dyne/cm = \text{ 32.5 dyne/cm}$$

- **The surface tension for formulation III shampoo**

Measured Parameter	Trial one	Trial two	Trial three	Average
W1	79.18	79.48	79.78	79.48
W2	123.18	123.25	123.18	123.20
W3	127.50	128.00	127.75	127.75
N1	24.50	25.50	25.00	25.00
N2	61.00	61.15	61.30	61.15
R1	72	72	72	72

- W1a=79.48g W2a= 123.20g
- W3a=128.5g R1= 72 dyne/cm
- N1a=25 N2a=61.15

$$R2 = \left[\frac{w3a - w1a}{w2a - w1a}\right] * \left(\frac{N1a}{N2a}\right) * R1 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

$$R2 = \left[\frac{128.5g - 79.48g}{123.20g - 79.48g}\right] * \left(\frac{25}{61.15}\right) * 72\ dyne/cm$$

$$R2 = \left[\frac{49.02g}{43.72g}\right] * \left(\frac{25}{61.15}\right) * 72\ dyne/cm = \text{ 33 dyne/cm}$$

- **Surface tension of marketed Aloe Vera shampoo**

Measured Parameter	Trial one	Trial two	Trial three	Average
W1	79.20	79.48	79.76	79.48
W2	123.00	123.35	123.26	123.20
W3	127.48	128.12	127.65	127.75

N1	24.40	25.59	25.00	25.00
N2	57.56	57.54	57.58	57.56
R1	72	72	72	72

- W1a=79.48g W2a= 123.20g
- W3a=128.00g R1= 72 dyne/cm
- N1a=25 N2a=57.56

$$R2 = \left[\frac{w3a - w1a}{w2a - w1a}\right] * \left(\frac{N1a}{N2a}\right) * R1 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

$$R2 = \left[\frac{128.00g - 79.48g}{123.20g - 79.48g}\right] * \left(\frac{25}{57.56}\right) * 72\ dyne/cm$$

$$R2 = \left[\frac{48.52g}{43.72g}\right] * \left(\frac{25}{57.56}\right) * 72\ dyne/cm = \ 34.71\ \text{dyne/cm}$$

- **The surface tension for marketed Organza shampoo**

Measured Parameter	Trial one	Trial two	Trial three	Average
W1	79.00	79.68	79.76	79.48
W2	123.10	123.35	123.16	123.20
W3	127.08	128.52	127.65	127.75
N1	24.40	25.29	25.30	25.00
N2	58.60	58.56	58.58	58.58
R1	72	72	72	72

- W1a=79.48g W2a= 123.20g
- W3a=128.20g R1= 72 dyne/cm
- N1a=25 N2a=58.58

$$R2 = \left[\frac{w3a - w1a}{w2a - w1a}\right] * \left(\frac{N1a}{N2a}\right) * R1 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

$$R2 = \left[\frac{128.20g - 79.48g}{123.20g - 79.48g}\right] * \left(\frac{25}{58.58}\right) * 72\ dyne/cm$$

$$R2 = \left[\frac{48.72g}{43.72g}\right] * \left(\frac{25}{58.58}\right) * 72\ dyne/cm = \ 34.24\ \text{dyne/cm}$$

NB: Triplicate experiment was performed and mean ± SD, n=3 are listed in the final result report of percent of solid content.

7. **Dirt dispersion observe**

The dirt dispersion observe at 25℃ in initial time periods.

A1					
Dirt dispersion observe	None	Light	Moderate	Heavy	Average
trial one	6	0	0	0	
trial two	6	0	0	0	
trial three	6	0	0	0	
Total	18	0	0	0	
A3					
Dirt dispersion observe	None	Light	Moderate	Heavy	Average
trial one	5	1	0	0	
trial two	4	2	0	0	
trial three	6	0	0	0	
Total	15	3	0	0	
A2					
Dirt dispersion observe	None	Light	Moderate	Heavy	Average
trial one	6	0	0	0	
trial two	6	0	0	0	
trial three	6	0	0	0	
	18	0	0	0	
A4					
Dirt dispersion observe	None	Light	Moderate	Heavy	Average
trial one	5	1	0	0	
trial two	4	1	1	0	
trial three	0	5	1	0	
Total	9	7	2	0	
A5					

Dirt dispersion observe	None	Light	Moderate	Heavy	Average
trial one	0	5	1	0	
trial two	0	5	1	0	
trial three	0	5	1	0	
Total	0	15	3	0	

- Where A1 is represented to organza marketed shampoo
- A2 is represented to aloe Vera marketed shampoo
- A3 is represented to formulation III
- A4 is represented to for formulation II
- A5 is represented to for formulation I

N.B: The experiment was similarly done at 25 ℃, 37 ℃, and 45℃ temperature on initial, after a month, and after two month time periods to investigate its stability.