Investigation of emulsion prepared from Endod-based-saponin extract and paraffin oil

Haymanot Ewunetie Wale

Thesis Submitted to the Department of Chemical Engineering

School of Mechanical, Chemical and Material Engineering

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Chemical Engineering (Specialization in Process Engineering)

Office of Graduate Studies

Adama Science and Technology University

Adama, Ethiopia

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Declaration

I declare that this Master Thesis entitled "**Investigation of emulsion prepared from Endod-based-saponin extract and paraffin oil**" has not been submitted for the award of any academic degree, diploma, or certificate in any other university or any other institution. All sources of materials that are used for this thesis have been duly acknowledged through citation.

Haymanot Ewunetie Wale

Student name	Signature	Date

Recommendation

I, the advisors of this thesis, hereby certify that I have read the revised version of the thesis entitled "***Investigation of emulsion prepared from Endod-based-saponin extract and paraffin oil"*** prepared under my guidance by Haymanot Ewunetie Wale submitted in partial fulfillment of the requirements for the degree of Master's of Science in Chemical Engineering. Therefore, I recommend the submission of the revised version of the thesis to the department following the applicable procedures.

Dr. Mulugeta Yilima

Advisor	Signature	Date

Approval Page of M.Sc. Thesis

I, the advisors of the thesis entitled "***Investigation of emulsion prepared from Endod-based-saponin extract and paraffin oil***" and developed by Haymanot Ewunetie Wale certify that the recommendation and suggestions made by me and appropriately incorporated into the final version of the thesis.

Dr. Mulugeta Yilima _____ _____

Advisor Signature Date

We, the undersigned, members of the Board of Examiners of the thesis by Haymanot Ewunetie Wale have read and evaluated the thesis entitled "***Investigation of emulsion prepared from Endod-based-saponin extract and paraffin oil***" and examined the candidate during the open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Chemical Engineering.

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Finally, approval and acceptance of the thesis is contingent upon submission of its final Copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and School Graduate Committee (SGC).

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LIST OF ABBREVIATIONS

P. dodecandra	Phytolacca dodecandra
CMC	Critical micelle concentration
E_{24}	Emulsification index
FT-IR	Fourier Transform Infrared spectroscopy
NMR	Nuclear Magnetic Resonance
FS	Foam stability
DEE	Diethyl ether
S. mukorossi	Sapindus mukorossi
OFAT	one factor at a time
B. aegyptiaca	Balanites aegyptiaca
C. quinoa	Chenopodium quinoa
V. amygdalina	Vernonia amygdalina

LIST OF NOTATIONS

hr	hour
g	gram
g/mL	gram per milliliters
L	liter
mL	milliliters
M	molar
%	percentage
v/v	volume per volume
min	minute
g/L	gram per liters
mN/m	micro Newton per meter
cm	centimeter

ABSTRACT

Endod (phytolacca dodecandra) berries have traditionally been used as soap for washing cotton clothes in Ethiopia for centuries. P. dodecandra, locally called "Endod", produces a series of triterpenoid saponins that possess very potent and useful biological properties. In this research, we used P. dodecandra berries for the extraction of saponin as a biosurfactant. Distilled water/ethanol mixture was used for the extraction process. The effect of operational parameters such as extraction time (1-5hr), extraction temperature (40-80 °C) and ratio of sample-to-solvent (1:5-1:20 g/mL) were evaluated in terms of obtaining high extraction yield, total saponin. Extracted saponin was mixed with paraffin oil for preparation of emulsion. Extracted saponin was characterized using properties such as foam stability, surface tension, critical micelle concentration (CMC). It also characterized using FT-IR and NMR analysis. Emulsification index of the extract was also evaluated. The optimum extraction conditions were of sample to solvent ratio of 1:10g/mL, extraction time of 3h and extraction temperature of 70 °C. CMC value of 1.2g/L with 40.04 mN/m surface tension was observed. The most stable foam was obtained in presence of 0.4 wt% saponin solution at room temperature. The presence of low pH did not show significant effect on foam stability at room temperature with CMC concentration. Moreover foam stability was observed as the temperature was raised from room temperature to 60°C at CMC concentration. Further increases in temperature had negative effect on the stability of foam. The emulsification index of the extracts was evaluated at different concentrations with paraffin oil and emulsification activity was directly increased with concentration of extract. The stability of prepared emulsion was checked by long-time storage for 30days. FT-IR analysis of extract from the berries of P. dodecandra revealed that the existence of saponin functional group.

Keywords

P. dodecandra, biosurfactant, saponin, surfactant properties of saponin and emulsion

CHAPTER ONE

1. INTRODUCTION

1.1 Background of the study

Surfactants are a class of chemical compounds possessing both hydrophobic and hydrophilic moieties that distribute themselves between two immiscible fluids, with the effect of reducing the surface/interfacial tensions and causing the solubility of polar compounds in non-polar solvents. They display properties, such as detergency, solubilization, and lubrication; have stabilizing and foaming capacity; and form phase dispersion (Káren G.O. Bezerra et al., (2021); Barradas et al., (2015)). Surfactant is an abbreviation for surface active agent, which literally means active at a surface (Yangxin et al., 2008). They are multifunctional compounds made up of polar and nonpolar components and produce emulsions at the interface of liquids with different polarity (Sanches et al., 2021). Surfactants are the components responsible for forming and stabilizing emulsion-based products. These molecules adsorb into oil–water interfaces during homogenization, reducing the interfacial tension and enhancing further droplet disruption. Additionally, the surfactants provide a protective layer around the droplets, which improves the long-term stability and inhibits their aggregation (Schreiner et al., 2020)

Nowadays surfactants are one of the most produced compounds in the world and used in different industries like cosmetics, pharmaceuticals, food, textile, paper, polymer, plastic and machinery manufacture (Mnif et al., 2018). But now various studies are emphasizing towards biosurfactants directly produced from renewable natural material (Wojciechowski, 2013). Natural surfactants are bio-degradable, biocompatible and less toxic and hence pose less threat to the environment (Pradhan & Bhattacharyya, 2017). Biosurfactants are produced by a range of living organisms. Natural or green surfactants (biosurfactants) are classified as plant based biosurfactant, microbial based biosurfactant and animal based biosurfactants based on their origin (Bronzo et al., 2021).

Plant-based surfactants, such as saponins, have gained increasing attention as they are environmentally safe, less toxic, biodegradable, and renewable (Wisetkomolmat et al., 2020). Those Plant-based surfactants are widely distributed throughout the planet, being present in different parts of plants, such as the roots, stems, seeds, fruit, and leaves (Bronzo et al., 2021).

Surfactants from plants have received special attention because, in addition to the history of using these surfactants in ancient communities like detergents and shampoos, the yield in extraction processes may be higher than those obtained for microbial biosurfactants (Du et al., 2020); (Káren G.O. Bezerra et al., 2021). Saponins were first discovered and extracted from the root of Saponaria officinalis in the early 1800's. Since then, closely related substances have been found in roots of other plants such as Gypsophila arrostii, or in the bark of Quillaja saponaria (Jurado Gonzalez & Sörensen, 2020). Saponins are secondary metabolites produced by numerous plants (Góral & Wojciechowski, 2020).

Saponins, a class of nonionic biosurfactants derived from plants. They are triterpenic or steroidal glycosides largely distributed in plant materials. Their structural diversity is reflected in their physicochemical (foaming, emulsification, solubilization, sweetness, bitterness) and biological properties (haemolytic, antimicrobial, moluscacide, insecticide, ichthyocide), which are exploited in many applications in food, cosmetic and pharmaceutical industries (Tmáková et al., 2015); (Tiwari et al., 2020). Saponins can reduce the surface tension and dissolve in water easily due to their highly hydrophilic properties (Fathordoobady et al., 2020).

The essential components that stabilize, modify, and control the texture and rheological properties of products are surface-active molecules whose properties, in combination with the processing conditions, determine the final quality of the products (Jurado Gonzalez & Sörensen, 2020). Nowadays, a significant challenge to be overcome in the field of emulsions is the introduction of natural products to act as surfactants (Schreiner et al., 2020). Emulsions are thermodynamically unstable, and therefore tend to breakdown over time due to various physicochemical mechanisms, including gravitational separation, flocculation, coalescence, particle coalescence, Ostwald ripening, and phase separation. For this reason, stabilizers are included in emulsion formulations to improve their long-term stability, such as emulsifiers, texture modifiers, ripening inhibitors, and weighting agents. Emulsifiers are one of the most important stabilizers used in any emulsion formulation (Mcclements & Jafari, 2017). Recently, there has been considerable interest in using saponins as natural emulsifiers due to their ability to efficiently form and stabilize emulsions (Zhu et al., 2019).

1.2 Statement of problem

Surfactants are among the most used ingredients in beauty and hygiene products. These molecules are responsible for the formation of emulsions, foams, wetting capacity, cleaning dispersant, and even antioxidant and antimicrobial activity, and play an essential role in formulations. However, most of the surfactants in these products are derived from petroleum and are toxic and non-renewable Examples of such surfactants are sodium dodecyl sulfate alkylphenol ethoxylates, linear alkylbenzene sulfonates, dialkyl quats (Atta et al., 2021) and ammonium lauryl sulfate (Sanches et al., 2021). The residues of synthetic surfactants in soils and groundwater are harmful to the environment and also have toxic effects on human health. Therefore, there is a need to replaced synthetic surfactants by biodegradable surfactants, with low toxicity or non-toxic and sustainable. Natural surfactants, derived from microorganisms and plants, are an alternative that has been extensively studied and even commercially applied by some companies around the world (Káren G.O. Bezerra et al., 2021).

Many biosurfactant compounds are derived from renewable plant resources and have surface active. Saponins are a structurally diverse class of compounds widely distributed across the plant kingdom. The aim of this study was important to provide and to establish biosurfactant/saponin by extracting from Endod berries this can be minimize the use of synthetic surfactants and decreases the negative impact of synthetic surfactants on environment and human health.

1.3 Research Objectives

1.3.1 General objective

The general objective of this research is to investigate emulsion prepared from Endod derived saponin and paraffin oil.

1.3.2 Specific objective

Specific objectives are to:

- Extract and characterize of saponin from Endod (*phytolacca dodecandra*)
- Evaluate the effect of distilled water/ethanol ratio on saponin yield
- Optimize the effect of time, temperature and sample to solvent ratio on the extraction of saponin from Endod
- Investigate the impact of (pH, temperature) on the activity of saponin
- Prepare and characterize emulsion

1.4 Significance of the study

The synthesis of saponin from Endod can be advantages, because Endod can be used as soap in ancient time for washing close in Ethiopia for a long period, this promotes to protect and the usage of this plant in Ethiopia for today also. After Aklilu Lemma, an Ethiopian scientist discovered a natural treatment against Bilharzia also known as Schistosomiasis from Endod, the useful biological properties including antifungal, anti-protozoan, spermicidal and insecticidal properties was also studied. These biological properties can be due to the presence of the bioactive compounds (saponin tannin, protein and flavonoids). The presence of saponin in this plant was determined previously but this study determines the surfactant properties and emulsification activity of saponin from Endod berry, this information gives to our country investor or foreign investor about production of saponin from this plant.

Saponin extracted from this plant is also environmentally friendly, sustainable and biodegradable. The physicochemical properties (foaming, surface active, emulsifier, wetting) play effective to form stable emulsion and foam and solubility of (polar and non- polar compounds) to form phase dispersion. The study is significant because it adds to previous information Endod saponin as an emulsion ingredient and berry of Endod plant that can be used for the production of saponin is a non-edible feedstock.

1.5 Scope of the Study

This thesis work mainly focused on:-

- Extracting saponin from berry of P. dodecandera.
- Examining the effect of extracting (time, temperature and sample to solvent ratio) on the extraction of saponin from the berry of P.dodecandera is clearly investigating and determining their optimum.
- Characterizations of the extracting saponin using FTIR and NMR analysis and their physiochemical properties (foaming property, surface tension activity and CMC value) and also to test performance are investigating.
- The emulsification activity of saponin and characterization of emulsion are investigating.

CHAPTER TWO

2. LITRATURE REVIW

2.1 Surfactant

Surfactants are chemical compounds found between phases with different degrees of polarity and hydrogen bonds (Varjani & Upasani, 2017). Surfactants are tensio active agents responsible for the cleaning property of detergents and can be synthetic or natural origin the latter of which are denominated biosurfactants. Surfactants are amphipathic compounds with hydrophilic and hydrophobic portions that preferably partition at the interface between liquid phases with different degrees of polarity, such as oil/water or air/water interfaces. This characteristic reduces the surface tension of liquids through specific, preferential interactions at surfaces and interfaces due to the presence of hydrophilic and hydrophobic portions in the same molecule. The non-polar (hydrophobic tail group) portion of a surfactant is often a hydrocarbon chain and with one or more saturated, unsaturated, hydroxylated or branched fatty acids, whereas the polar portion (hydrophilic head group) may be ionic (cationic or anionic), non-ionic, or amphoteric may be an ester group, hydroxyl, phosphate, carboxyl, or sugar (Bronzo et al., 2021); (Káren Gercyane O. Bezerra et al., 2018). Based on the solubility properties, the surfactants can either be ahead or a tail. The head is often associated with the solubilizing group (lypophilic or hydrophilic) and the tail non-solubilizing group (hydrophobic or lipophobic) Figure 2.1 show the hydrophobic tail and hydrophilic head group (Saputra et al., n.d.); (Atta et al., 2021).

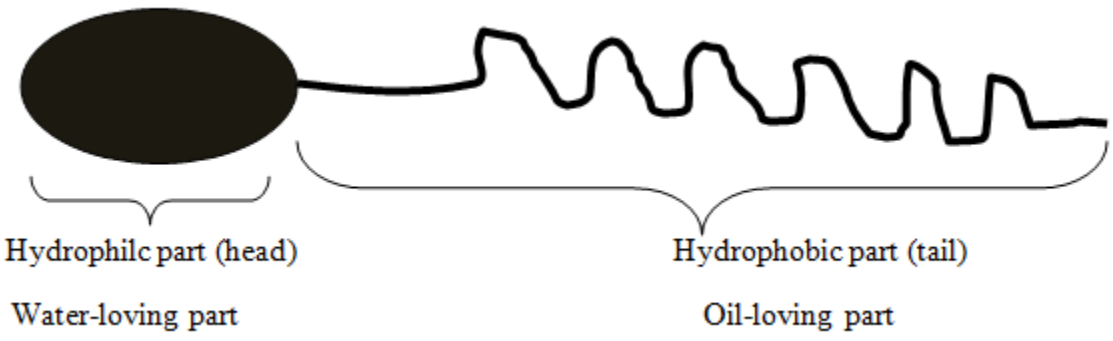

Figure 2.1 A typical structure of surfactant showing the head and tail (Atta et al., 2021).

Surfactants increase the aqueous solubility of hydrophobic molecules, reducing the surface/ interfacial tension of air/water and oil/water surfaces/interfaces. Surface tension decreases with the increase in surfactant concentration. In the aqueous medium up to the formation of micelles, which are aggregated structures with the hydrophilic portion positioned towards outside of the molecule and the hydrophobic portion positioned towards the inside (Bronzo et al., 2021).Scientists classify surfactants generally per their charge as nonionic, zwitterionic cationic, and anionic. Anionic surfactants possess negatively charged heads, and cationic surfactants are positively charged and hence are preferably applied in sandstone and carbonate reservoirs, respectively to prevent excessive adsorption. Amphiphiles with no net charge (nonionic) are used in conjunction with cationic or sometimes anionic surfactants to give better performance. Zwitterionic surfactants are the less explored type of surfactants in chemical EOR applications due to cost, although they are highly tolerant to temperature and salinity (Atta et al., 2021).

2.2 Biosurfactants

Natural, green, or renewable surfactants or simply biosurfactant are terms used to describe a diversity of amphiphilic molecules synthesized/derived from plants, animals (including humans), and microorganisms (Vieira et al., 2021); (Kumar & Das, 2018b). Biosurfactants are structurally diverse surface-active compounds obtained from renewable resources, whose amphiphilic structure modifies the properties of surfaces or interfaces of complex systems (Moutinho et al., 2021). Natural surfactants can be obtained either from sources of either plant or animal origin (Tmáková et al., 2015). Currently, biosurfactants are becoming an attractive alternative in the market as the manufacturers are producing eco-friendly biosurfactants from different natural and sustainable sources (Akbari et al., 2018). Biosurfactants are compounds that are produced by plants and animals, but are largely produced by microorganisms, such as bacteria, yeasts, and filamentous fungi and have combination of several properties including biomolecules (proteins, carbohydrates, and lipids) reduces surface tension to act as an emulsifier. All biosurfactants are amphiphilic and consist of polar and nonpolar parts (Sanches et al., 2021). Biosurfactants are classified as plant based biosurfactant, microbial based biosurfactant and animal based biosurfactants based on their origin (Bronzo et al., 2021). They are used various segments, partially or complete replacing chemical surfactants in mostly consumed products, such as detergents for washing clothes, household cleaning and personal hygiene products and

cosmetics. In the medical field, biosurfactants can provide as antimicrobial, anti-tumour and anti-inflammatory agents because of their bioactivity (Ribeiro & Sarubbo, n.d.).

The efficiency and effectiveness of a biosurfactant and surfactants in general are determined based on the ability to reduce surface/interfacial tension, emulsifying capacity and the critical micelle concentration (CMC). A lower surface/ interfacial tension, lower CMC and higher emulsification index indicate a more efficient and effective surfactant (Góral & Wojciechowski, 2020). The effectiveness of a biosurfactant is estimated by its ability to reduce ST, where a good biosurfactant is able to reduce the ST of water from 72 to 35 mN/m and the interfacial tension (IT) (Domínguez Rivera et al., 2019). Efficiency is determined through the critical micelle concentration (CMC) – the minimal concentration for surfactant action, defined as the solubility of a surfactant in an aqueous solution or the minimal concentration required to reach the lowest surface or interface tension.

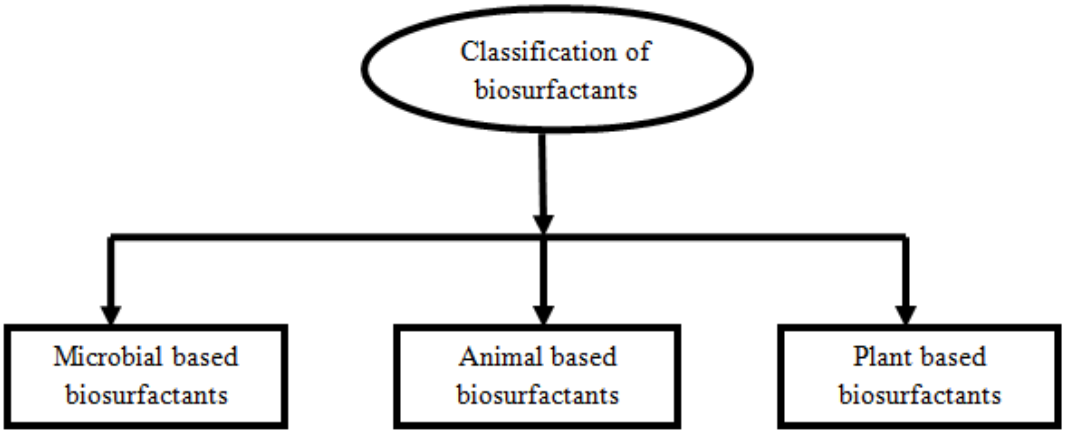

Figure 2.2 Classification of biosurfactant

2.2.1 Microbial based biosurfactant

The term microbial surfactant only refers to biosurfactants produced from microbial cultures (Henkel & Hausmann, 2019). Microbial surfactants or biosurfactants are a natural class of surface-active molecules with diverse structures that are produced by different microorganisms, including bacteria, yeasts and filamentous fungi. They are amphiphilic compounds, comprising a hydrophilic head and a hydrophobic tail (Souza et al., 2017). These microorganisms can produce biosurfactants from oleaginous substrates, including agro-industrial waste products, such as glycerol, corn steep liquor and waste frying oil, which makes the industrial application of biosurfactants viable by lowering the production costs (Ribeiro & Sarubbo, n.d.). Microbial biosurfactants are classified based on the type of producing microorganism and chemical composition. The main classes of microbial biosurfactants are Glycolipids, polymeric surfactants, lipopetides, fatty acids, particulate surfactants, and phospholipids. Glycolipids, phospholipids, and lipopetides have low molecular mass, whereas polysaccharides, proteins, lipoproteins, and lipopolysaccharides have high molecular mass (Káren Gercyane O. Bezerra et al., 2018).

Table 2.1 Microbial based biosurfactants and their surfactant properties

Substrate	**Types of microorganisms**	**Biosurfactant**	**CMC**	**Surface tension**	**Reference**
Diesel contaminated sites	*Pseudomonas aeruginosa* DS10-129	Rhamnolipid	22 mg/l	27 mN/m	(Rahman et al., 2010)
soy molasses	Candida bombicola	Sophorolipids	6 mg/ l at pH 6	37 mN/ m at pH 6	(Solaiman et al., 2004)
vegetable fat waste	Candida glabrata	Crud biosurfactants cotain protein, sugar and	1g/ml or 1%	24 mN m-1.	(de Gusmão et al., 2010)

		fatty acid			
different sugar alcohols with the presence of vegetable oil	Pseudozyma parantarctica JCM 11752	mannosylribitol lipid(glycolipid)	1.6×10−6 M	23.7 mN/m,	(Morita et al., 2012)
different sugar alcohols with the presence of vegetable oil	Pseudozyma parantarctica JCM 11752	mannosylarabitol lipid (glycolipid)	1.5× 10−6 M	24.2 mN/m,	(Morita et al., 2012)
Marine wastes	A novel marine bacterium *Rhodococcus* sp.,strain PML026	Purified Trehalolipid	250 mg/L	29 mN/m	(Mnif et al., 2018)

2.2.2 Animal based biosurfactants

Like the microbial sources, animal sources have also been used to obtain biosurfactants. Biosurfactants extracted from animal include casein, cholesterol, wax, gelatin, lecithin, and wool fat. The biosurfactants are widely applicable in various industries based on its chemical structure. Gelatin is extracted by partial hydrolysis from collagen and has wide application in the food industry. Lecithin derived from egg yolk is being employed in pharmaceutical preparation (Kumar & Das, 2018a). Casein is a milk protein that accounts for ~80% of milk's total protein content (Xu et al., 2011).

2.2.3 Plant based biosurfactant

Plants have an amazing ability to produce a wide variety of secondary metabolites, like alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones, and coumarins (Khan et al., 2018). Saponins belong to a various group of naturally occurring surface-active compounds. It is known as a plant-derived natural surfactant (Nafiunisa et al., 2019). Saponins are secondary metabolites naturally occurring in plant material and belong to glycosides group. These compounds are intensively used nowadays in pharmaceutical industry. In the ancient times saponins were widely used as household detergents, because of their amphiphilic nature due to the presence in their structure of a lipid-soluble aglycone and water-soluble chains. Saponins are natural surfactants with lipophilic aglycone and hydrophilic glycosyl groups (Fathordoobady et al., 2020); (Ligor et al., 2018).

The name "saponin" is derived from the Latin word sapo, meaning soap-like foam-generating ability, and the amphiphilic properties derived from the structure containing an isoprenoid-derived aglycone (sapogenin) attached to one or more sugar chains by either an ether or ester linkage. Structural classification of saponins is primarily based on their sapogenin skeletons, which can be divided into two main groups—Triterpenoid saponins and steroid saponins. Saponins are natural compounds of a diverse group generally consists of sugar (glycon) and non-sugar part (aglycon) connected by a glyosidic linkage (Yu Pu Juang, 2020); (Mohlakoana & Moteetee, 2021).

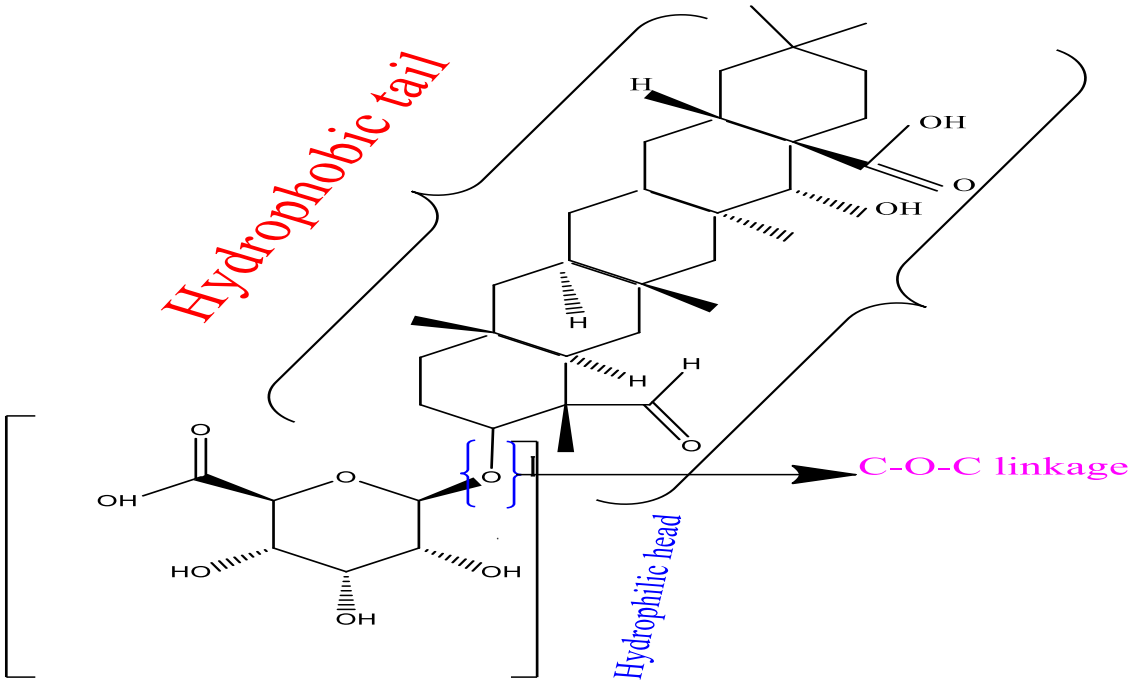

Figure 2.3 Saponin structure (Nguyen et al., 2020)

Saponins on hydrolysis yielded glycon (sugar) and aglycone (sapogenin). According to the structure of the aglycone or sapogenin, saponins are classified as neutral and acid type (Desai et al., 2009)

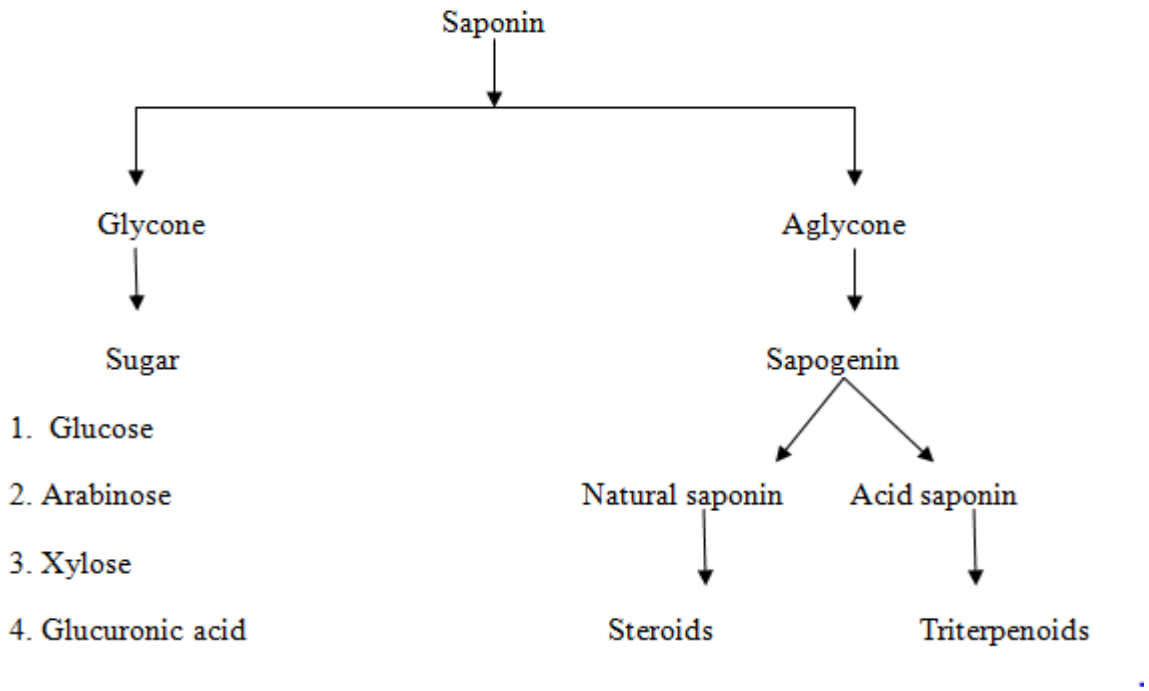

Figure 2.4 Saponin classification (Desai et al., 2009).

Table 2.2 Saponin from different plant source and their surfactant properties

Saponin	**Surface tension**	**CMC**	**Foam property**	**Reference**
Saponin extract from Saponaria ocinalis	32.6mN/m	0.5 g/L		(Fathordoobady et al., 2020)
Saponin extracted from lentils	37.39 mN/m	0.6g/L	80% at CMC value of saponin	(Meshram et al., 2021)
Saponin extracted from *Camellia oleifera seed*	50.0 mN/m	0.5g/L	86.0% at 0.5% of saponin solution	(Chen et al., 2010)
Saponin extracted from Pu'er Tea seeds	30.01 mN/m under CMC	0.05% /0.05g/L		(Yang & Li, 2020)

Saponin extracted from Soapwort (*Saponaria officinalis*)	34.5 mN/m at the CMC value	0.64-1.06 g/L	85% of foam after 1hr	(Jurado Gonzalez & Sörensen, 2020)
Saponin extracted from S. mukorossi	41.8 mN/m	0.243g/L	80% after 5 min f with 0.75g/L solution	(Tmáková et al., 2015)

2.2.3.1 Saponin extraction method

The first step in the processing of saponin involves their extraction from the plant matrix. As in any extraction process, the extraction solvent, extraction conditions (such as temperature, time, pH, solvent to feed ratio), and the properties of the feed material (such as composition and particle size) are the main factors that determine process efficiency and the properties of the end product (Guclu-Ustundag & Mazza, 2007).

In general the extraction techniques employed in saponin extraction can be classified into two categories, the conventional and the green technologies. The conventional extraction techniques are maceration, Soxhlet, and reflux extraction, where the modern extraction techniques are ultrasound-assisted, microwave-assisted, and accelerated solvent extraction. The conventional extraction is relied on the solubility of solute from plant materials into solvent. Therefore, it often utilizes a large quantity of solvent to extract the desired solute, even though sometimes is aided with elevated temperature by heating, and mechanical stirring or shaking. On the other hand, the green extraction techniques involved less hazardous chemical synthesis, safer chemicals used, energy efficiency, use of renewable feedstock, and pollution prevention (Cheok et al., 2014)

The saponin-rich fraction could be purified from the crude extract by a number of strategies including acetone or diethyl ether precipitation and then buthanol used as capture of saponin from the crud extract, recrystallization from 95% ethanol solvent, separatory-funnel partition, and column chromatography with macroporous resin (Diaion HP-20, AB-8 and D4020) or reversed phase silica gel. The purification or isolation of saponin from crud extract is important for the use and study of their properties (Guo et al., 2018).

2.3 Endod (phytolacca. dodecandra)

Phytolacca dodecandra is a sprawling woody climber with stems reaching 5–10m in length, with erect, racemic, dioecious flowering stalks, and pinkish or red berries. The species is widely distributed over parts of Sub-Saharan Africa, South America, and Asia. In Ethiopia, the plant can be found at elevations between 1600 and 3000m above sea level. The active ingredient, a saponin labeled 'lemmatoxin', Toxicological, chemical, and agrobotanical studies on *endod* were done during the period 1970 to 1990. Berries from *P. dodecandra* have traditionally been used as soap for washing cotton clothes in Ethiopia for centuries. Berries are ground in either fresh or dried form and used directly as soap. Ground leaves can also be used, but are less potent compared to berries. The plant is also used for various medicinal purposes (Esser et al., 2003).

Phytolacca dodecandra (L'Herit) is native to sub-Saharan Africa and Madagascar. It is a member of the Phytolaccaceae family, and commonly known in Ethiopia as "endod." Other local names include soapberry, African soapberry (English), Phytolaque (French), and Fitolaca (Spanish), and in Tanzania it is called chihakahaka (Beressa et al., 2020). The African soapberry plant, P. dodecandra, locally called "Endod", produces a series of triterpenoid saponins that possess very potent and useful biological properties including antifungal, anti-protozoan, spermicidal and insecticidal properties (Zeleke et al., 2017).

Figure 2.5 Fresh P. dodecandra plants

2.4 Emulsion

Emulsion is a mixture of two immiscible liquids, which generally forms during various chemical processes/ equipment such as water flooding of heavy oil reservoirs, water treatment membranes, and packed bed separators. For instance, emulsions can be categorized as water-in-oil emulsions (with water droplets as a dispersed phase in the flow of oil as the continuous phase), oil-in-water emulsions (with oil droplets in the flow of water), and more complex configurations of emulsions such as water-in-oil-in-water emulsions (Goodarzi & Zendehboudi, 2019). Emulsions are thermodynamically unstable, and therefore tend to breakdown over time due to various physicochemical mechanisms, including gravitational separation, flocculation, coalescence, particle coalescence, Ostwald ripening, and phase separation. For this reason, stabilizers are included in emulsion formulations to improve their long-term stability, such as emulsifiers, texture modifiers, ripening inhibitors, and weighting agents (Mcclements & Jafari, 2017).

Emulsifiers are one of the most important stabilizers used in any emulsion formulation. In addition to their ability to stabilize emulsions, the nature of the emulsifier used also determines the ease of emulsion formation and the functional attributes of the final product. Consequently, selection of an appropriate emulsifier is one of the most important decisions when formulating emulsion-based products. Emulsifiers are typically amphiphilic molecules that have both

hydrophilic and hydrophobic groups on the same molecule, such as small molecule surfactants, phospholipids, proteins, polysaccharides, and other surface-active polymers. Emulsifiers play a fundamental role in facilitating the initial formation of lipid droplets during homogenization and minimize the separation of the phases due to the capacity to control the clustering of globules, which favours the stability of heterogeneous systems (Mcclements & Jafari, 2017); (Ribeiro & Sarubbo, n.d.).

CHAPTER THREE

3. MATERIAL AND METHODS

Experiments on the production and characterization of emulsion from Endod plant were carried out in the laboratories of Adama science and Technology University School of mechanical chemical and material engineering chemical engineering department. The first portion of the investigations covers everything from sample collection through characterization. The experiment's second phase focused on the preparation and characterization of an emulsion using saponin from Endod berries and with paraffin oil.

3.1 Materials and Chemicals

Materials/Equipment

Phytolacca dodecandra berries were collected from Ameya. For the experiment pH meter, mixer, aluminum foil, Test tubes, mass balance, water bath, grinder, measuring cylinders, Funnel, sieves, Petri Dishes, Samples Holder, Ultra-Sonicator, Magnetic Stirrer with Hot Plate, Hot-Air Oven, Cotton and Whatman No. Filter Paper, beaker, Conical Flasks were used.

Chemicals

Distilled water, citric acid, n-butanol, sodium chloride, diethyl ether, ethanol, Methanol and liquid paraffin were used for extraction of saponin and for the preparation of emulsion.

3.2 Methods

3.2.1 Moisture content analysis

Moisture content was often checked by loss on drying, which involves heating the sample and recording the weight loss owing to moisture evaporation. 25 g of Endod berries was oven-dried at 105℃ until moisture was removed completely from the berries. Every hour, the weight was measured. The method was repeated until the weight remained constant. The percentage moisture content of the berry was determined using equation 3.1 described by (Lambert et al., 1991).

$$\text{Moisture content (\%)} = \frac{W1-W2}{W1} \times 100 \quad \text{..... (3.1)}$$

Where

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

3.2.2 Preparation of Endod berry powder

Fresh Berries of Endod (*P. dodecandera*) were collected from Ameya. The fresh berries were washed twice with distilled water to remove dust particles. Then the cleaned berries were dried at room temperature until completely dried to reach constant weight. The dried berries were crushed into powder using POLYMIX® PX-MFC 90D laboratory miller and sieved with 0.2mm mesh. The prepared powder was stored under dry conditions for further use. The overall procedure of powder preparation from Endod berries is illustrated in Figure3.1

Figure 3.1 Diagrammatic representation of Endod berry powder preparation

3.3 Extraction of saponin from Endod (Phytolacca dodecandera)

3.3.1 Distilled water/ethanol ratio selection for extraction

The first things for extraction of active compounds from plant have been to determining solvent type. Aqueous ethanol was mostly used solvent for extraction of saponin from plant (Sarvin et al., 2018). In this study, distilled water/ethanol mixture (100/0, 90/10, 80/20, 70/30 and 60/40v/v) was used to determine the best combination for extraction of saponin from Endod berry based on yield of saponin. For experimental determination 20g of dried powder was taken in a 250mL screw-capped Erlenmeyer flask containing 200mL of solvent to conduct the extraction. The sample flask was kept at 60℃ for 3hr under constant stirring. Then, the obtained mixture was filtered using Whitman No.1 filter paper and the obtained filtrate (extract) from each solvent was separately stored for analysis purpose. The extracts was evaporated over a water bath at 80-90℃ to reduce the volume of the concentrate to 40mL.The concentrated solution was transferred into a 250mL separating funnel and 50mL of diethyl ether and 60mL n-buthanol step wise were added and shaken vigorously to remove impurities. The solution was evaporated at 60℃ to dryness in a hot air-oven until complete dried and saponin yield was measured and determine the best solvent mixture for extraction (Nizioł-Łukaszewska & Bujak, 2018).

3.3.2 Effect of diethyl ether and n-buthanol on saponin yield

On the basis of literature reports the amount of diethyl ether used 20 mL or 50 mL and n-buthanol used 40 mL, 50 mL and 60 mL for 20 g of sample (Pn et al., 2019). In this work the amount of diethyl ether and n-buthanol used in the extraction of saponin from P.dodecandera on the extraction yield were investigated and the effect of diethyl ether and n-buthanol were examined using OFAT experimental method.

3.3.2.1 Effect of diethyl ether

Saponin compounds were partially purified by different solvent diethyl ether was one of them. Its amount was varied depending on the extract partitioning and it can affect the yield of saponin extraction (Kim & Park, 2001). In this study; the effect of diethyl ether was studied at 20mL, 30mL, 40mL, 50mL and 60mL and extraction yield was investigated with a constant n-buthanol amount i.e. 60mL (Nizioł-Łukaszewska & Bujak, 2018).

3.3.2.2 Effect of n-buthanol

Buthanol is one of the most important solvent for the extraction of saponin for purification steps for the capture of saponin from the extracts. The amount of buthanol used for extraction varies depending on the structure and the type of saponin as well as plant extracted (Rai et al., 2021). In this study the, extraction yield at 30 mL, 40 mL, 50 mL, 60 mL and 70 mL of n-buthanol were investigated under a constant diethyl ether amount obtained in the above steps (Yang & Li, 2020).

3.4 Extraction processing description

The extraction of saponin was performed using berry extract of p. dodecandra with the help of a magnetic stirrer on a hot plate. Extracts were prepared from 20g of ground Endod berries with 200mL of solvent. The extraction solvent were distilled water and ethanol mixed at a volume ratio of 80/20v/v. The process of extraction was conducted on hot plate temperatures at 40℃, 50℃, 60℃, 70℃ and 80℃ with continuous stirring for 1 hr, 2hr, 3 hr, 4 hr and 5 hr with sample to solvent ration 1: 20g/mL, 1:15g/mL, 1:10g/mL and 1:5g/mL. The extracts were filtered and the resulting filtrate was collected and the remaining plant residue was subjected to another initial volume of solvent and extract and the extracts were filtered again. The filtrates were combined and evaporated over a water bath at about 80-90℃ to reduce the volume of concentrate to 40mL. The concentrated solution was transferred into a 250mL separating funnel and 50mL of diethyl ether was added and shaken vigorously to remove impurities from the original solution. The processes repeated twice. The resulting aqueous phases were combined and purified twice with 50mL of n-butanol while the ether layers with impurities were discarded. The combined n-butanol solution was evaporated at 60℃ to dryness in a hot air-oven until complete drying of n-buthanol solution to powder extracted (Yang & Li, 2020); (Ashour et al., 2019). The yield of saponin calculated from equation 3.2. The overall procedure of extraction of saponin from Endod berries is illustrated in Fig. 3.2.

$$\text{Total saponin content (\%)} = \frac{\textbf{weight of saponin extracted}}{\textbf{weight of the plant material used}} \times 100 \quad \ldots\ldots\ldots\ldots \quad (3.2)$$

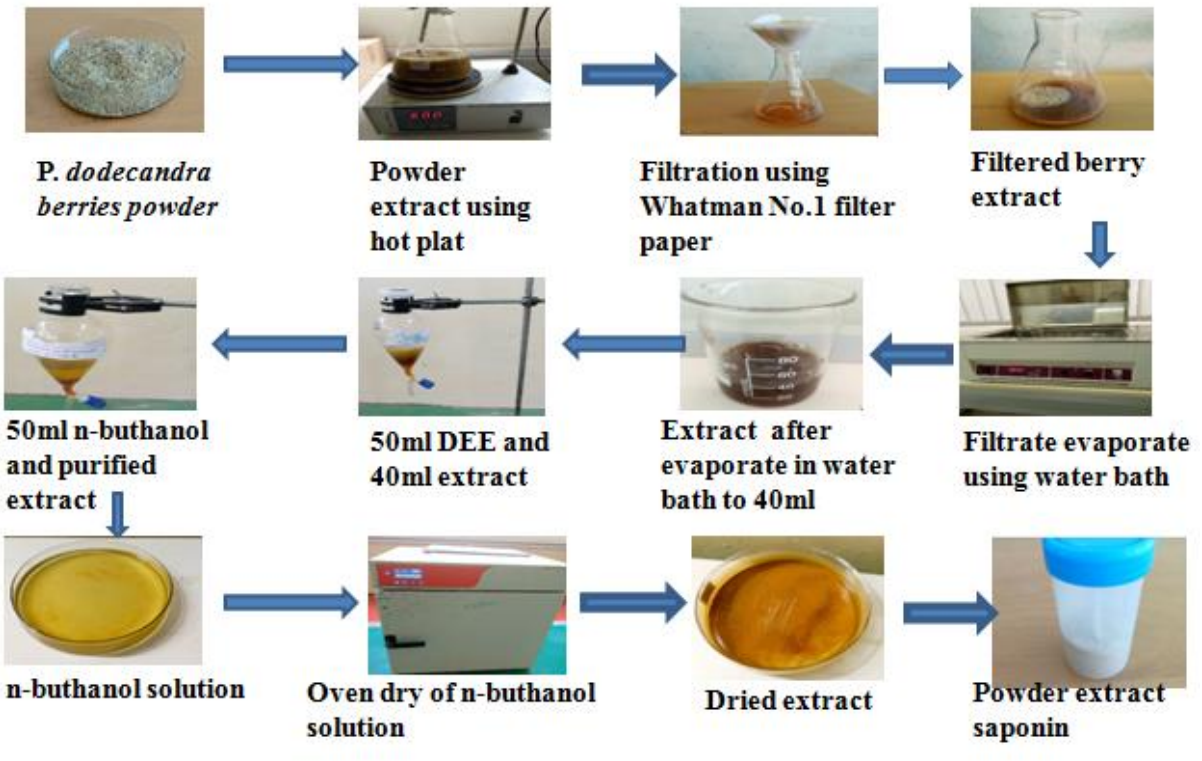

Figure 3.2 Steps for extraction of saponin from P. dodecandra

3.4.1 The effect of operational parameters on saponin extraction yield

In this study, the effect of various operational parameters such temperature, time and sample to solvent ratio were analyzed for different ranges on the extraction yield. The optimal extraction conditions were investigated for further use in the extraction of saponin from Endod berry. The experimental method used for in this study was one factor at a time (OFAT). To perform OFAT, only one factor was varied, and others were kept constant (Akbari, Abdurahman, Yunus, et al., 2019).

3.4.1.1 The effect of time

The extraction time is one of the most important factors that affect the extraction yield of saponin in the extraction process (He et al., 2022). In this work the extraction time was varied as 1hr, 2hr, 3hr, 4hr and 5hr. The experiment was conducted separately at different times by adding 20g of Endod powder in 250mL Erlenmeyer flask containing 200mL of Ethanol-distilled water solvent (sample to solvent ratio 1:10g/mL) and kept it at 60℃ under constant stirring. Diethyl ether and n-buthanol were used in purification process also kept constant (50mL) for each on all process

separately based on subsequent procedures. Based on the results observed the yield of saponin extracted had been investigated.

3.4.1.2 The effect of temperature

Temperature affects the mass transfer rate and the solubility of some compounds in the extraction process (Nafiunisa et al., 2019). Effect of extraction temperature was studied at 40℃, 50℃, 60℃, 70℃ and 80℃ the experiment was conducted separately by adding 20 g berries powder in 250 mL Erlenmeyer flask containing 1:10 g/mL sample to solvent ratio and under optimum time obtained with a constant stirring. Diethyl ether and n-buthanol were used in purification process also kept constant for all subsequent procedures. The extraction yield was investigated under optimal extraction time obtained in the above procedure.

3.4.1.3 The effect of sample to solvent ratio

Sample to solvent ratio significantly affect the solubility of bioactive compound in extraction solvent under extraction process. Generally, the volume of extraction solvent mainly depend on the amount of the sample (He et al., 2022).The extraction yield at 1:20 g/mL, 1:15 g/mL, 1:10 g/mL and 1:5 g/mL were explored under the above obtained optimal extraction temperature (70℃) and optimal time (3hr) and thus the optimal extraction sample to solvent ratio was determined.

3.5 Characterization of extracted saponin

3.5.1 FTIR (Fourier transform infrared) spectroscopy

FT-IR analysis was performed to further confirmation on the functional groups of the surfactant. The analysis was done by mixing a small portion of the surfactant with KBr salt and compressing it into the small tablet. Bruker FT-IR spectrometer was implemented to record the infrared spectrum under the resolution of 4$cm^{-1}$and the wave number range of 4000–400cm^{-1} (Khayati et al., 2020). The functional groups and the absorption/ molecular vibration peaks present in the extracted saponin was identified through the FTIR analysis and compared with that of the FTIR transmittance spectra/absorbance spectra with the previous literature (Yekeen et al., 2020).

3. 5. 2 NMR (Nuclear Magnetic Resonance) spectroscopy

The chemical shift was investigated by ^{1}H NMR and ^{13}C-NMR. ^{1}H NMR and ^{13}C-NMR spectra were recorded on a 500 MHz Bruker spectrometer. Measurements were carried out using dimethyl sulfoxide (DMSO) solvent (Sharma & Paliwal, 2013); (Nowrouzi et al., 2020a). Sample was dissolved in DMSO solvent and about 600µl was poured in NMR tube and observed on the applied magnetic field

3.6 Testing of the properties and performance of extracted saponin

3.6.1 Foaming properties

Saponin can be determined based on foaming properties (Singh & Kaur, 2018). In this study; the foaming properties of the extracts was assessed by measuring foam stability with distilled water. Foam test was measured using 100mL beaker in the laboratory. The extract was dissolved in distilled water at the concentrations of 0.1w%, 0.2w%, 0.3w%, 0.4w% and 0.5w%. The solution was stirred for 1min using magnetic stirrer in the beaker as shown in the figure 3.3 and the foam height was measured. Foam stability was also recorded with equation 3.3 (Góral & Wojciechowski, 2020). Foam stability/height was also studied under different conditions (pH and temperature). 0.1M NaOH and 0.1M HCl were added to the extract to adjust the pH (3, 7 and 9) (Barreiro, et al., 2021). For the effect of temperature, the extract was heated to room temperature, 40℃, 60℃ and 80℃.

$$\text{Foam stability (FS) \%} = \frac{\textbf{Total height of the foamed sample(cm)after time t}}{\textbf{Initial height(cm)}} \times 100 \ldots\ldots\ldots \quad (3.3)$$

The foam height indicates the foam strength of the surfactant solution and foam stability is calculated by parameter (FS), defined as the ratio of the foam height after time t to that measured in the first stage (Meshram et al., 2021).

Figure 3.3 Foam formations using magnetic stirrer for 1min.

3.6.2 Surface tension and critical micelle concentration (CMC) of saponin

In this study, surface tension of (0.1-1.7g/L) extracted saponin were carried out using distilled water as a standard material/solvent at room temperature with drop weight method and the result were compared with in the literatures. In this experiment the sample was first loaded into the syringe and drops of the sample were allowed to fall and collected in a beaker. 20 drops of solution was used and determine surface tension with equation 3.4 (Permprasert & Devahastin, 2005).

$$R_2 = \frac{(W3-W1)\,n1}{(W2-W1)\,n2} \times R_1 \quad \text{..... (3.4)}$$

Where, W_1 is weight of empty beaker, W_2 is the weight of beaker with distilled water, W_3 is Weight of beaker with saponin solution, n_1 is number of drops of distilled water, n_2 is a number of drops of saponin solution, R_1 is the surface tension of distilled water at room temperature (at room temperature the surface tension of distilled water considered72mN/m) and R_2 is the surface tension of the saponin solution (Fazlolahzadeh & Toosi, 2015).

To determine the critical micelle concentration (CMC) of the saponin surfactant, the surface tension values were measured at different surfactant concentrations. A plot of the surface tension versus the surfactant concentration was made and the surfactant CMC was taken as the surfactant concentration with no significant change in surface tensions of saponin solution (Yekeen et al., 2020). The effect of pH on surface tension and CMC also determined based on (Jurado Gonzalez & Sörensen, 2020). pH value (3, 7 and 9) was adjusted using 0.1M NaOH and 0.1M HCl and

determine the effect on surface tension and CMC of saponin extracted from Endod berries compared with the value of surface tension and CMC without pH adjusted (Ralla et al., 2018).

Figure 3.4 Drop-weight methods for surface tension test of saponin from P. dodecandera berry.

3.6.3 Emulsification index (E_{24})

Emulsification index of saponin was measured using concentration of saponin (0.1%, 0.2%, 0.3%, 0.4% and 0.5 %) in emulsified layer and using paraffin oil. 5mL of emulsified layer and 5mL of paraffin oil were stirred for 2min and left to stand for 24hr at room temperature and emulsification index was determined using equation 3.5 (Hajimohammadi et al., 2016)

$$\text{Emulsification index } (E_{24}) = \frac{\textbf{Height of emulsion layer (cm)}}{\textbf{Total height of the soluton (cm)}} \times \mathbf{100} \quad \text{..... (3.5)}$$

3.7 Emulsion preparation

Emulsions were prepared by homogenizing the water phase and the oil phase. The aqueous phase contained emulsifier (extracted saponin) (Z. Li et al., 2018). In this work Emulsion was prepared by mixing (0.5-3w %) of the extract with oil/water ratio of 10/90v/v (Ralla et al., 2018). First saponin extract was mixed with aqueous phase followed by the drop wise addition of the oil under continuous stirring at 200rpm for 3min using a mechanical stirrer. Then emulsion prepared by mechanical stirring method homogenized using Ultrasonication method, the ultrasonic agitation by sound waves with more than 20 kHz frequency breaks coarse droplets into nanoemulsions (Aswathanarayan & Vittal, 2019). The stability of prepared emulsion was examined by exposing in long-term storage though visual observation. To evaluate the storage stability of prepared emulsion at 5, 10, and 30days at room temperature after emulsion was prepared (Schreiner et al., 2020); (Dahlawi et al., 2020).

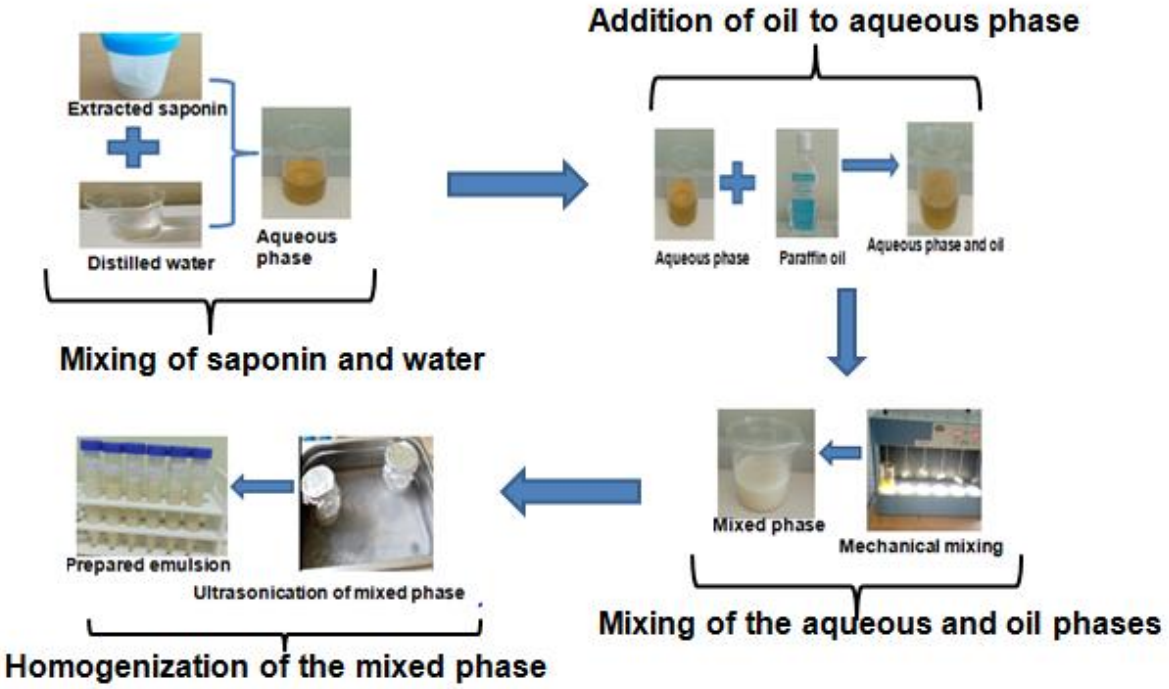

Figure 3.5 Emulsion preparation steps

CHAPTER FOUR

4. RESUALT AND DISCUSSION

4.1Moisture content analysis

The Moisture content was determined using equation 3.1 and is presented in Table 4.1. As illustrated in the table 4.1, the moisture content of Endod berries was conducted in triplicates and the average moisture content was taken as mean . From table 4.1 the result has shown, 71.8 %(w/w) of saponin.

Table 4.1 Moisture content of Endod berries

No	Endod before drying (gram)	Endod after drying (gram)	Loss (gram)	Moisture content (%)	Moisture content average (%)
1	25	7.25	17.75	71	
2	25	6.9	18.1	72.4	71.8
3	25	7	18	72	

4.2 Extraction of saponin from *phytolacca dodecandera*

4.2.1 Distilled water/ethanol ratio

water and ethanol have been extensively utilized in the extraction of saponin from the plant sources (Guo et al., (2018); Mohaddes-Kamranshahi et al., (2019)). The extraction solvent was examined to obtain the suitable solvent for the extraction process. In this study, it was reported that distilled water-ethanol mixture on the yield of saponin extracted from Endod berries were investigated and the effect of solvent ratio on the extraction yield was also studied. Figure 4.1 shows that the effect of solvent ratio in the extraction yield of saponin from Endod berries, it indicates distilled water - ethanol mixture (80/20v/v) was the best solvent ratio for extraction of saponin from Endod berry with 15.3% saponin yield which is the highest from the rest of combinations ratio. The yield was decreased as the amount of ethanol increased from the

maximum yield 14.03% and 13.97% when distilled water/ethanol ratio was70/30 v/v and 60/40 respectively.

Similar results were also reported on the extraction of saponin from *Sapindus Mukorossi* that, distilled water –Ethanol mixture is suitable for the extraction of saponin. The mixture of distilled water to ethanol in saponin extraction was 80/20v/v and the yield of saponin from this plant was higher at this ratio, since ethanol is completely evaporated from the extract during evaporation for the purification step (Köse & Bayraktar, 2016). The yield of saponin extracted from different plant was higher with the mixture of distilled water to ethanol solution of 80/20v/v (Pn et al., 2019). Due to its low toxicity and economic accessibility, aqueous ethanol has been commonly employed to extract saponins from plant (Sarvin et al., 2018). Water is significantly increasing the extraction efficiency due to high polarity of the water. Water is used for the extraction surfactant property compounds (saponin) and adding ethanol to the water is more effective on yield and foaming agent and surfactant properties of saponin (Berry et al., 1991).

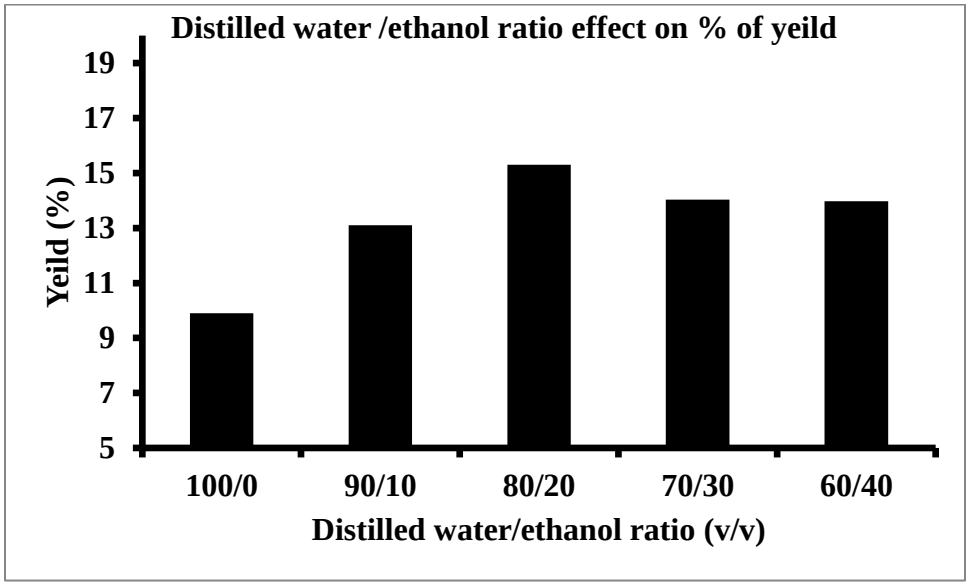

Figure 4.1 Effect of distilled water/ethanol ratio on saponin yield (%)

4.2.2 Effect of diethyl ether and n-buthanol amount on yield

Diethyl ether is used as a constituent agent because; saponins are not soluble in ether and can precipitate saponins. The n-butanol solution separated to obtain more pure compounds, namely saponins (Wardatun & Mahmudah, 2019). The effects of amount of diethyl ether and n-buthanol on saponin yield from Endod berry were investigated based on one factor at a time method for all experiment the amount of used sample was 20g.

4.2.2.1 Effect of diethyl ether amount

Purification of saponin was done by using the addition of solvent diethyl ether (Rijai et al., 2016). Figure 4.2 shows that the effect of diethyl ether amount on yield of saponin. Diethyl ether amount used for this study were 20mL, 30mL, 40mL, 50mL and 60mL whereas n-buthanol amount was kept constant i.e. 60mL and the yield of saponin were 11.28%, 11.51%, 12.09%, 13.78% and 13.01% respectively. The result shows that 50mL diethyl ether is best for extraction of saponin from Endod berries.

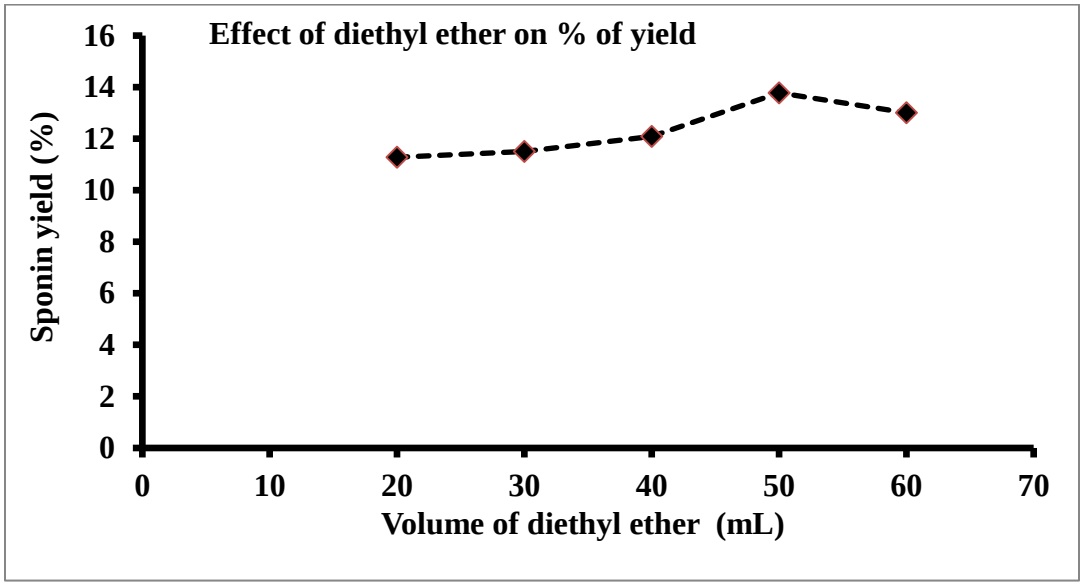

Figure 4.2 Effect of diethyl ether on saponin yield (%)

Nizioł-Łukaszewska & Bujak, (2018), reported that 50mL of Diethyl ether for the extraction of saponin from plant for purification was preferable since the amount of diethyl ether beyond this amount can decrease the yield of saponin extracted due to the removal of saponin in Diethyl ether phase because there may be the formation of more than one phase precipitation in the process and also in most case the uses of high Diethyl ether in this process leads wastage of time in separation stage for the next steps and cost of Diethyl ether use.

4.2.2.2 Effect of n-buthanol amount

Amount of n-buthanol was one of the main factors that determine yield of saponin from plant matrix and n- buthanol can be used for the separate saponin from other active components (Wardatun & Mahmudah, 2019). In this work 30mL, 40mL, 50mL, 60mL and 70mL of n-buthanol was used to investigate the yield of extraction with a constant diethyl ether

concentration i.e. 50mL from the above step. Figure 4.3 shows that varying the amount of n-buthanol were investigated the yield of saponin extracted from Endod berries. As can be seen the figure 4.3 the yield of saponin increases when used 30mL, 40mL, and 50mL. Saponin yield from Endod berry was higher when 50mL of n-buthanol was used with the value of yield was 15.26% and as the amount of n-buthanol increase the yield decreased. The concentration/amount of n-buthanol solution in the extraction of saponin from plant matrix mostly used in literature was 50mL since, increased in amount of n- buthanol increased the temperature for the removal of n- buthanol and also the time to remove n-buthanol from saponin, this leads to decrease in the saponin extraction yield due to the increment of temperature and time for the removal of n-buthanol (Khan et al., 2018). Ashour et al., (2019), reported use of high n- buthanol amount in extraction process leads to increases in environmental disadvantages with remarkable amounts, the need of heating, the need of drying, and time consuming.

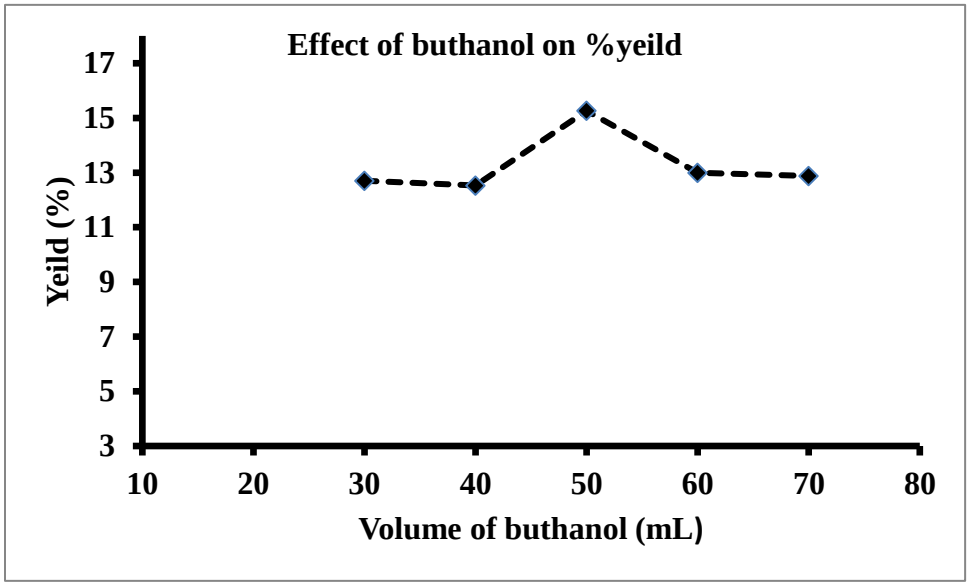

Figure 4.3 Effect of buthanol amount on saponin yield (%)

4.3 Effect of operational parameters on yield of saponin extraction

4.3.1 Effect of time on extraction of saponin

Studies have revealed that the percentage of extraction yield tends to increase as the extraction time increases; however, higher exposure or contact of the plant matrix can cause the degradation of biologically active compounds (saponin). Therefore, the selection of proper time

in extraction methods is necessary to obtain high recovery in terms of quality and quantity and saponin yield (Hadidi et al., 2020). In this study, the yield of total saponin content was examined at different extraction times 1 hr, 2hr, 3hr, 4hr, and 5hr, while the other two factors including, temperature of extraction and sample to solvent ratio were constant 60 ℃ and 1:10g/mL respectively. As we have seen in figure 4.4 at the beginning of extraction time saponin yield show significant increased i.e. 1 hr, 2 hr and 3 hr the yield value was 12.81%, 13.83% and 14.39% respectively. However, once the extraction time reached 3hr, the yields did not show significant change as the time was increased, this might be due to the degradation of bioactive compounds. The result obtained from this study is 3hr as an optimum extraction time for saponin extraction from Endod. Patel & Khare, (2022), reported the length of extraction of saponin from plant matrix was not longer than 3hrs and the same results also reported in the extraction of saponin from Sapindus laurifolia plant. According to report by He et al., (2022), extending the extraction time beyond 3hr may cause negative reactions, the yield decline and wasting too much energy. Therefore, extraction time was fixed at 3hr for further experiments.

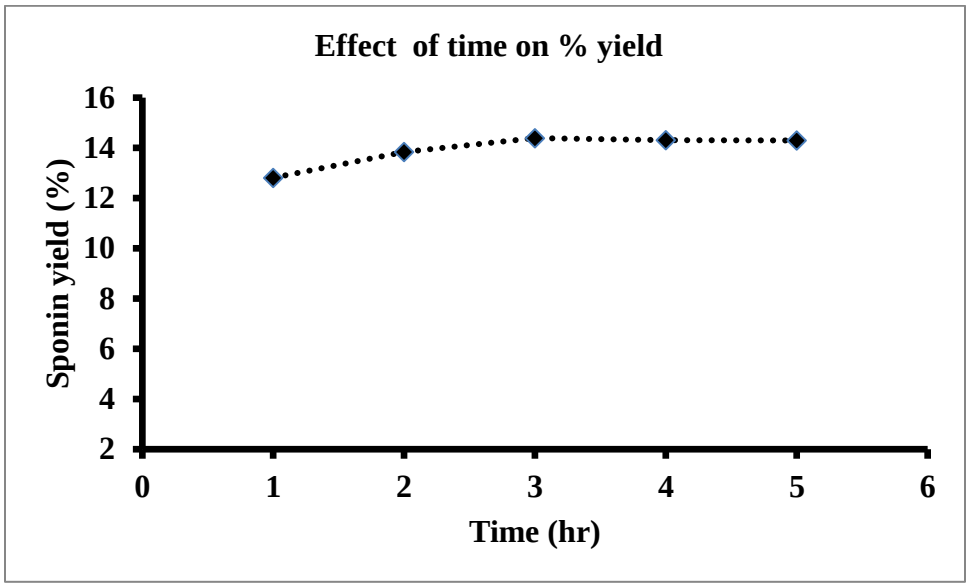

Figure 4.4 Effect of extraction time on saponin yield (%)

4.3.2 Effect of temperature on extraction of saponin

The results of saponin extraction temperature conditions at 40℃, 50℃, 60℃, 70℃ and 80℃ have been investigated. The extraction yield was gradually increased with the temperature rise i.e. the yield of saponin were 15.39%, 18.85%, 19.55% and 20.57% at a temperature 40℃, 50℃, 60℃, 70℃ respectively and reached a peak at 70°c as shown in Figure 4.5. The highest saponin yield

(20.57%) was achieved at 70℃, so the optimal extraction temperature was 70℃. Saponin yield decreased as temperature was increased to 80℃ at this temperature the yield was 18.39%. As the temperature continued to raise; the structure of saponin was damaged, the extraction rate was reduced on the contrary (Yang & Li, 2020). In the process of natural compound extraction, temperature highly affected the result. At higher temperatures, the solubility of the extracted compounds was increased because of its decreased viscosity, improving the mass transfer and accelerating the extraction process (Nafiunisa et al., 2019). The saponin extraction yield decreased as the temperature increased beyond 70℃, this may be, due to the damage of saponin structure from the extract this leads to the reduction of saponin extraction yield. This result was in agreement with saponin extracted from *Camellia oleifera* in which saponin yield increased with temperature from 40℃ to 70℃ and no longer increased the saponin yield over the temperature of 70℃ (Liu et al., 2016) and saponin extracted from Pu'er tea seeds (Yang & Li, 2020). Saponin extracted from F enugreek (*Trigonella-foenum graecum*) with a temperature of (40-80℃) the maximum yields of recovery was achieved at 70℃ (Akbari, Abdurahman, Yunus, et al., 2019).

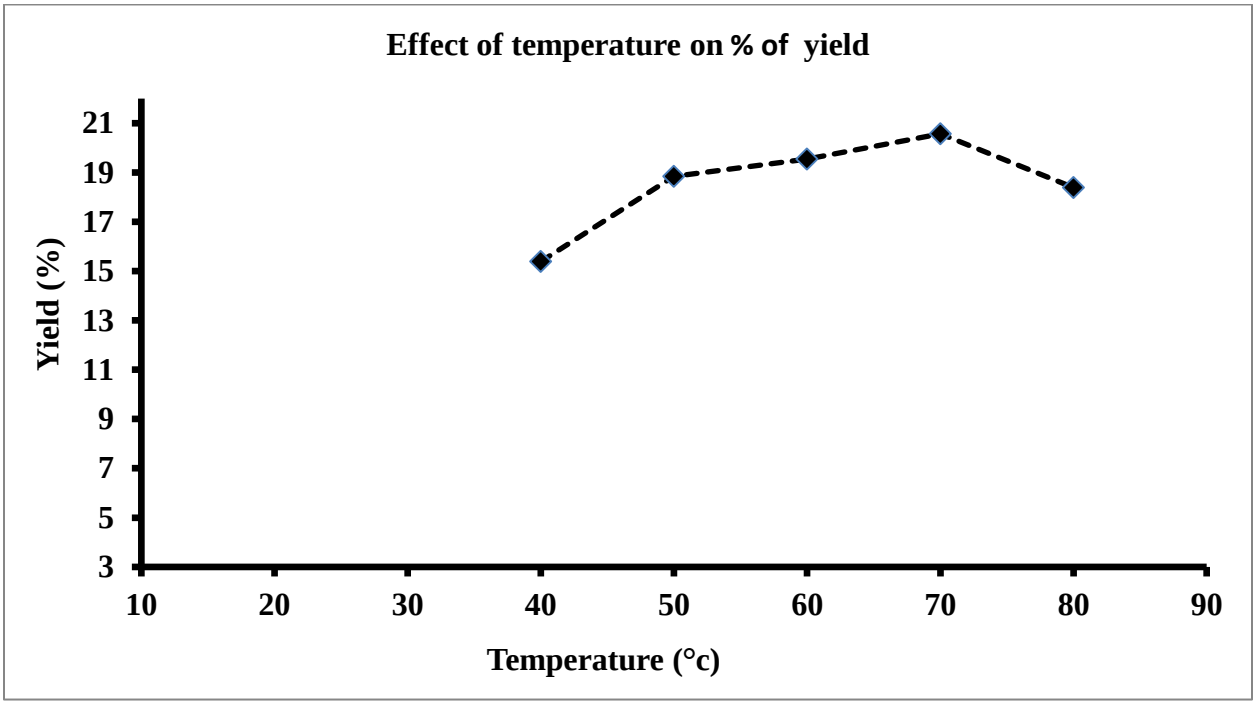

Figure 4.5 Effect of extraction temperature on saponin yield (%)

4.3.3 Effect of sample to solvent ratio on saponin yield

Saponin yield was increased with sample to solvent ratios from 1:5g/mL to 1:10 g/mL as shown in the figure 4.6, the yield was 12.77% for 1:10g/mL and further increase in feed to solvent ratios led to a decline in extraction yield (12.14% for 1:15g/mL) means more ethanol and water content in the extraction medium, which results in higher solvent generates more heat within the system which may also lead to the evaporation of bioactive compounds (saponin). Therefore, the optimal sample to solvent ratio was 1:10g/mL. This result was agreement with saponin from Fenugreek (*Trigonella-foenum graecum*) with ratio of sample to solvent (1:8-1:16 g/mL) maximum yield of recovery was achieved at 1:10g/mL and saponin from Polygonatum kingianum Collett & Hemsl with sample to solvent ratio (1:5-1:30 g/mL) maximum extraction yield was achieved at 1:10g/mL(Yunus, et al., (2019); He et al., (2022)) . Generally, a larger solvent: material ratio means a larger concentration gradient favoring mass transfer. The reason behind the negative effect of feed-to solvent ratio on the yield could be the poor transformation of seed matrix to the solvent. Since more solvent than the equilibrium point would result into the low mass transfer of bioactive compounds. In addition to that higher solvent generates more heat within the system which leads to the evaporation of bioactive compound due to more temperature usage to evaporate solvent from the extracts compounds (Akbari, Abdurahman, & Yunus, 2019). According to Sarvin et al., (2018), report use of a large amount of solvent was not considered cost-effective due to the increased cost of solvents and energy. The relation of sample to solvent ratio on saponin extraction yield was expressed in figure 4.6 below.

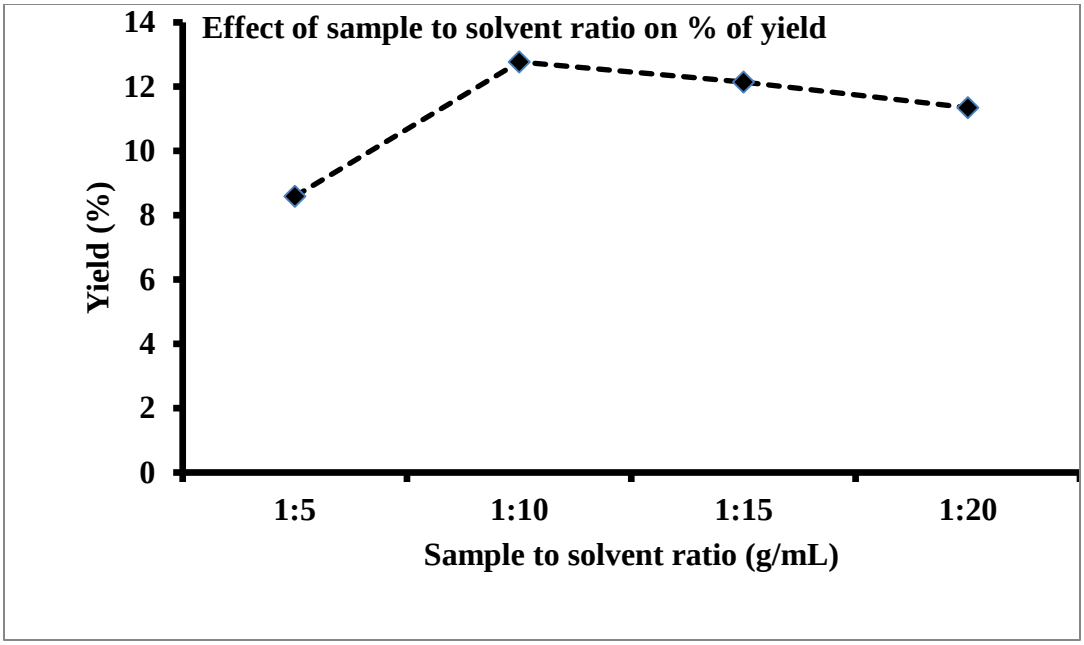

Figure 4.6 Effect of sample to solvent ratio on saponin yield (%)

4.4 Characterization of saponin extract from Endod

4.4.1 FTIR analysis

In order to identify the main functional groups of the extracted saponin, FT-IR spectra of the saponin extracts was determined. To confirm if the extracted substance was actually a saponin or not, the infrared spectra of the extracted saponin were recorded between 4000 − 500 cm^{-1} and were compared with the infrared spectra of the identified infrared spectra of saponin reported in previous literature. Hydroxyl stretching vibration was noted at 3281 cm^{-1}, Absorption of carbon-hydrogen (C-H) was at 2922 cm^{-1}. At 1734 cm^{-1}, a carbonyl (C=O) group stretching vibration peak was observed. At 1612 cm^{-1} the C=C stretching vibration peak was observed. At 1014 cm^{-1}, the spectrum has a C-O-C stretching vibration peak. Figure 4.17 shows all the transmittance spectra of saponin extracted from Endod.

The results showed that the transmittance spectra of the extracted saponin were similar to the spectra reported in previous studies. Imuetinyan et al., (2022) obtained the transmittance spectra of the -OH stretching of extracted saponin from V. amygdalina leaves *at* 3281$cm^{-1}$and carbon-hydrogen (C-H group) stretching peaks at 2926 cm^{-1}. Yekeen et al., (2020) and (Yuan et al., (2018) obtained the transmittance spectra of (carbonyl) C=O stretching of extracted saponin from Camellia oleifera and Sapindus Mukorossi *at* 1717 cm^{-1} ~ 1616 cm^{-1} and C-O-C stretching at 1076 cm^{-1}~1068.99cm^{-1} which is oligosaccharide linked to sapogenins. (Carbonyl) C=O

stretching of extracted saponin from soapwort plant at 1731cm^{-1} and C-O-C stretching at 1046cm-1 (Nowrouzi et al., 2020a)

Generally, the FTIR analysis of the extracted saponin in comparison with the infra-red spectra of saponins reported in various literatures confirmed that the substance isolated from P.dodencandera is indeed a saponin.

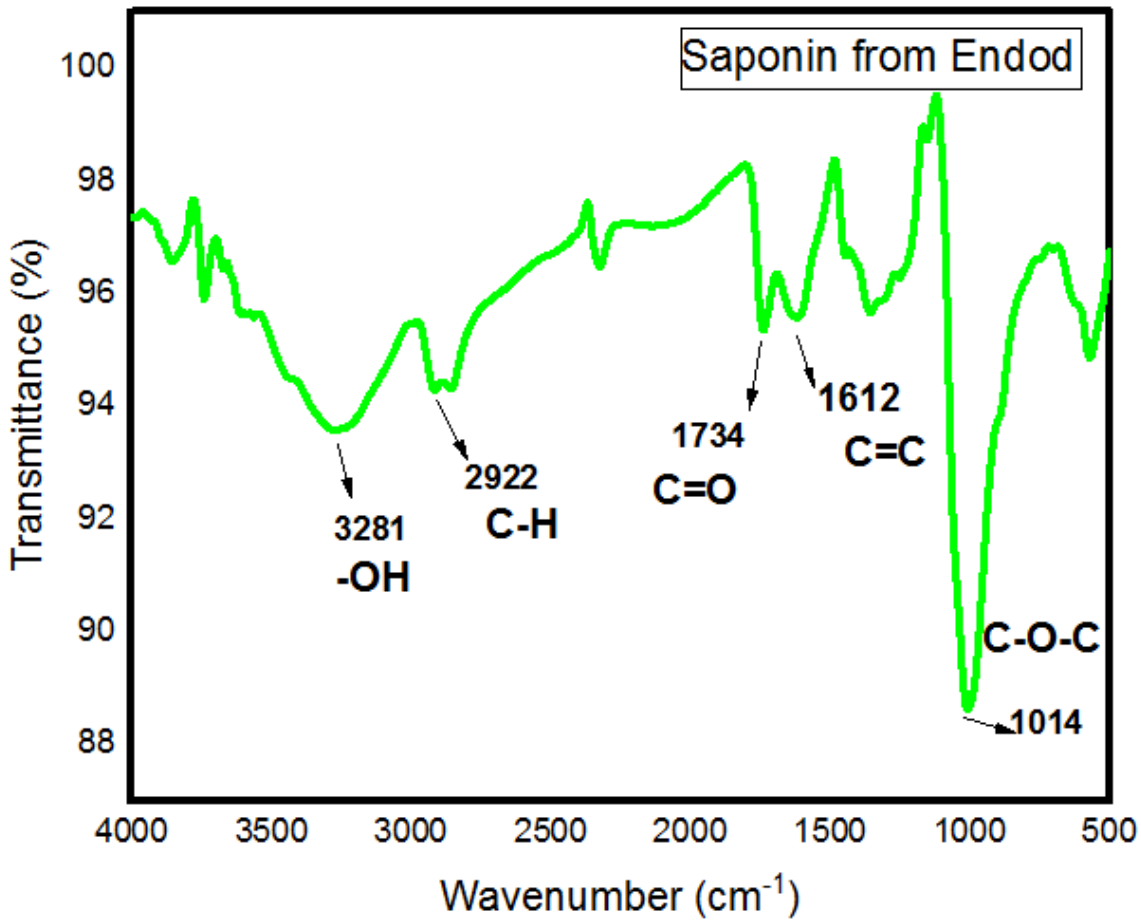

Figure 4.7 FTIR spectrum of saponin extracted from P.dodecandera berries

4.4.2 Nuclear Magnetic Resonance (NMR) analysis

The ^{1}H-NMR ^{13}C-NMR and spectrums of extracted saponin from Endod berry were illustrated in Figure 4.18 and figure 4.19 respectively. The NMR spectrum was characterized by comparing the chemical shifts of some single bond C–H pairs and olefin resonances with literature data. Chemical shifts in the 2.68-5.27 ppm show mainly protons in the oligosaccharide while chemical changes at 0.70-2.34 ppm are mainly related to protons in the aglycone part of saponin (Nowrouzi et al., 2020a). The presence of a larger number of multiplets in the region of 3-4 ppm

was a good indication of the presence of saccharide units (Štujbera et al., 2018). The methyl group in the extracted saponin was shown at chemical shift of 0.7, 0.84, 0.88 and 1.39ppm and olefin resonance at the chemical shift of 5.15ppm in the ^{1}H NMR spectrum. In the ^{13}C-NMR spectra the methyl group was shown at a chemical shift of 14.34, 15.61, 16.56 and 17.1ppm and olefin resonance 103.64ppm. The spectrum of this extracted saponin was given chemical shift peak around 3.67ppm indicates that there is -OH group in the extract. In the ^{1}H-NMR spectra less intensive peak appears around 5.24 ppm which is due to (C=C) group and this result agree with FTIR spectral data of this isolated compound. NMR analysis of the extracted saponin in comparison with the NMR spectra of saponins reported in various literatures based on the chemical shift confirmed that the substance isolated from Endod probably saponin (Sharma & Paliwal, (2013); Huang et al., (2021); Hajimohammadi et al., (2016))

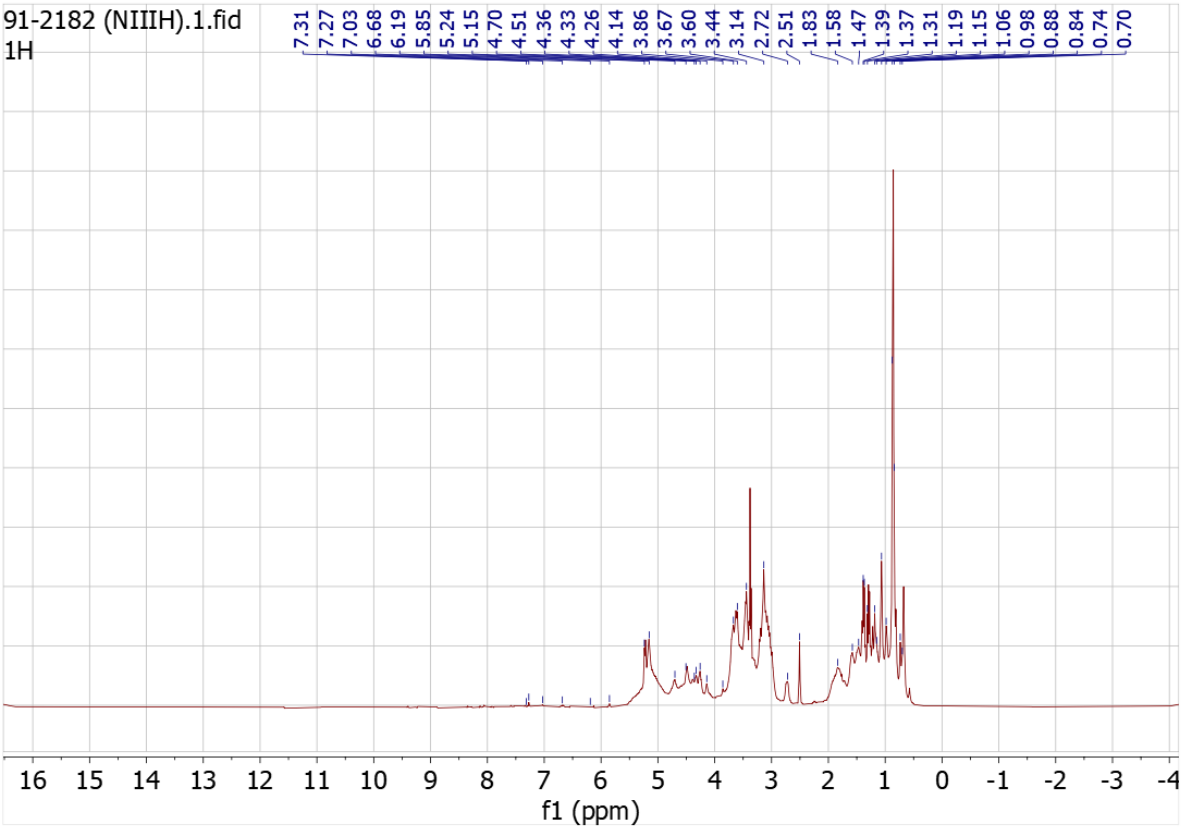

Figure 4.8 ^{1}H NMR spectra of saponin from Endod

Table 4.2 ^{1}H NMR chemical shift in (ppm) of extracted saponin

Chemical shift (ppm)	**Proton description**
~0.7, ~0.84, ~ 0.88 and ~ 1.39	C-H (methyl group)
~5.15 and ~ 5.24	C=C group (olefin resonance)
~3.67	-OH group

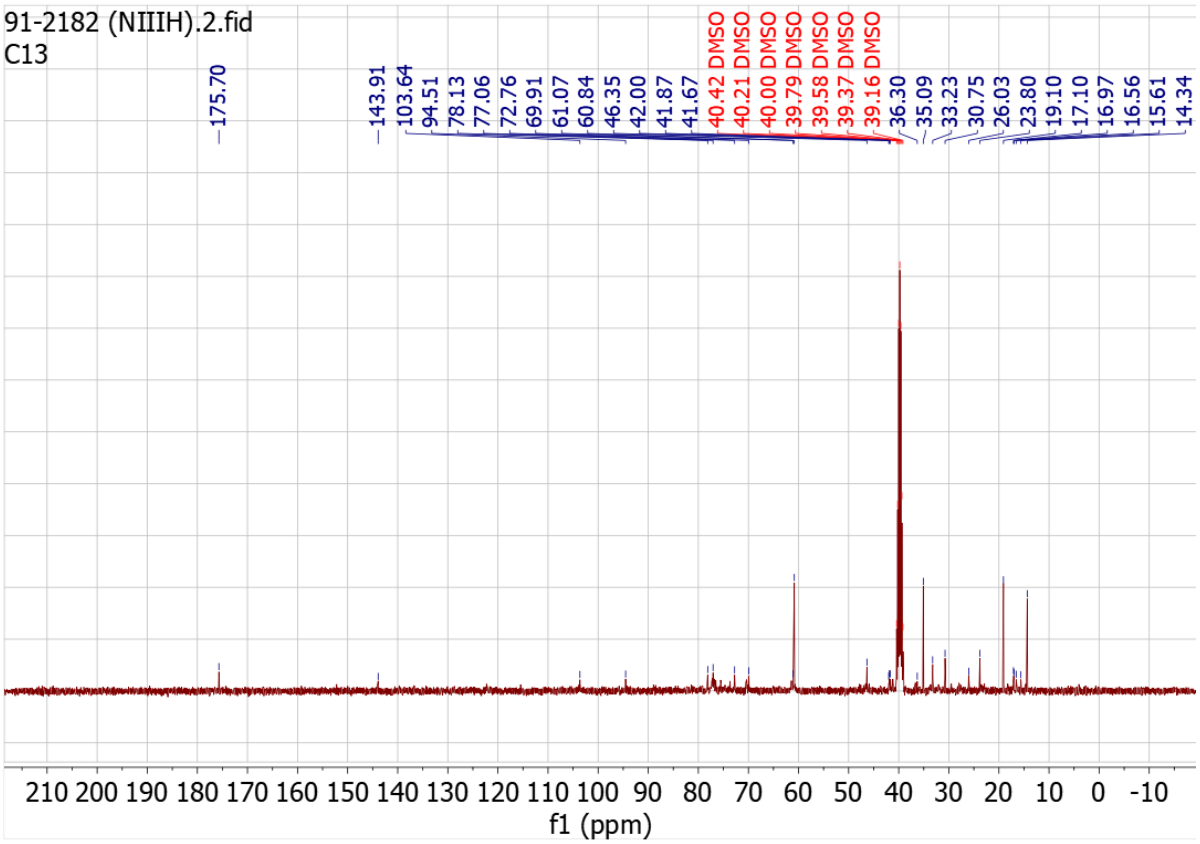

Figure 4.9 ^{13}C-NMR spectra of saponin from Endod

Table 4.3 ^{13}C-NMR chemical shift in (ppm) of extracted saponin

Chemical shift (ppm)	**Proton description**
~14.34, ~15.61, ~16.56 and ~17.1	C-H (methyl group)
~103.64	C=C group

4.5 Testing of properties and performance of saponin from Endod berry

4.5.1 Foaming properties

Foaming properties of saponin extracted from Endod were identified based on foam height and stability. Figure 4.7shows the foam height with respect to time for different concentrations of saponin and thus represents the formability as a function of saponin concentration. The foam height increases with increasing saponin concentration. It is clear from the results that the foam height is directly proportional to the saponin concentration then foam height of the extracted saponin at immediate have been 16cm, 30cm, 33cm, 36cm and 37cm for 0.1wt%, 0.2 wt%, 0.3 wt%, 0.4 wt% and 0.5 wt%, respectively. The height of foam have been decreased as the time increased, the value of the foam height at time 360min was 5cm, 15cm, 13cm, 17cm and 15cm for 0.1 wt%, 0.2 wt%, 0.3 wt%, 0.4 wt% and 0.5 wt% respectively and foam stability also decreased with time. Foaming increases with increasing concentration due to availability of more surfactant in the films to stabilize the foam (Pradhan & Bhattacharyya, 2017) The same trends have been reported previously for different types of surfactants and foam height versus time plot is also used to indicate the stability of the foam (Majeed et al., 2020). The foam stability analysis showed that the height of foam stabilized by higher saponin concentration (0.3 wt%, 0.4 wt% and 0.5 wt %) was quite stable and eventually attains a fixed value at particular time.

The most stable foam was obtained in presence of 0.4 wt% saponin solution. The foam height decay profile was characterized by a slower destruction in foam volume for a longer time. The general aspect of the curves obtained shows little difference, which reflects good foam stability versus time for 0.4%. The stability of foam value 0.4% saponins solution was 61.1% at 300min, representing the good foam stability of the saponins extracted from Endod berries. Foam with foam stability values higher than 50% can be regarded as stable (Nowrouzi et al., 2020b). This result confirmed with saponin extracted from soapnut (*Sapindus Mukorossi*) in which the stability of foam increased as a concentration of saponin and The most stable foam was obtained in presence of 0.4 wt% saponin solution (Yekeen et al., 2020). This was not means that the foam power always increased with the concentration of solution hence, as we have seen in the graph foam height did not show wide significant difference with the concentration of solution.

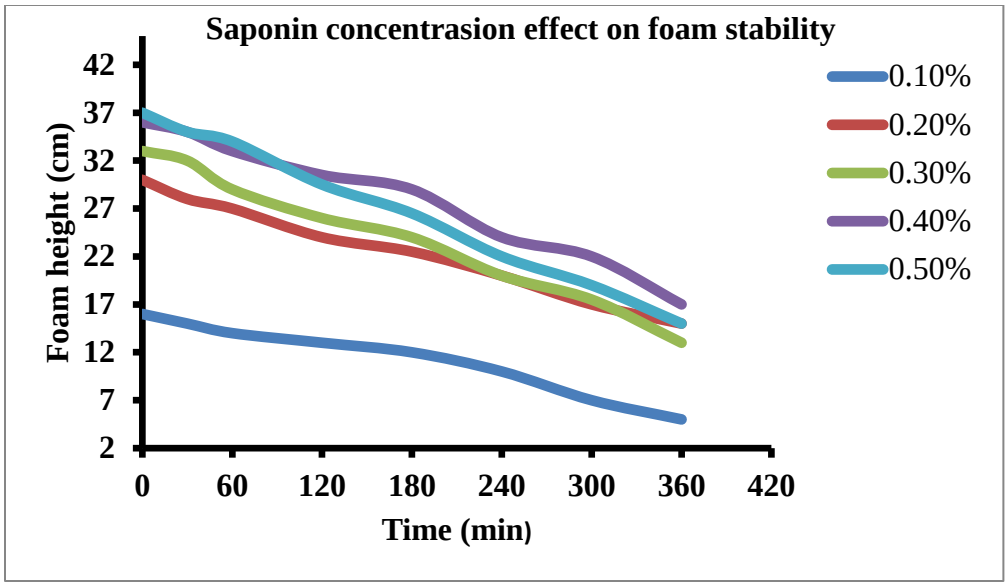

Figure 4.10 Effect of saponin concentration on foam stability

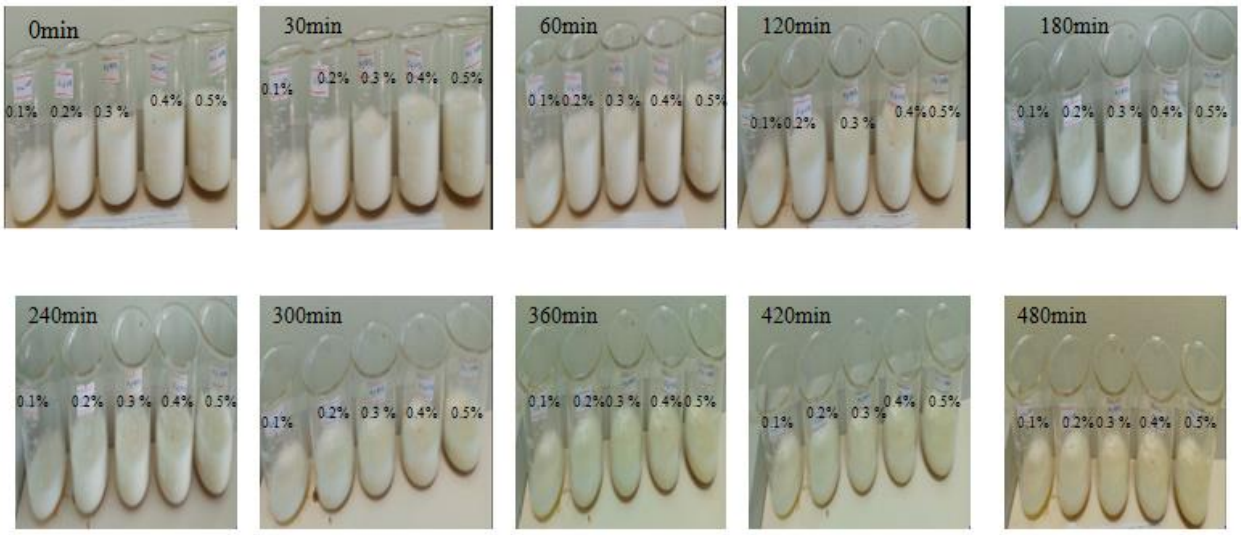

Figure 4.11 Foam decay profile for different concentration of extracted saponin- stabilized foam at room condition

4.5.1.1 Effect of temperature on foam stability

The impact of different conditions to determine the effect on foam stability of saponin extracts. In this study, the effect of temperature on foam stability of saponin extract was investigated at room temperature, 40℃, 60℃ and 80℃ . The effect of temperature on foam stability was determined at CMC value of the extract. Addition of heat considerably increased the capacity to form large height of foam that remained stable for long periods of time. Figure 4.9 shows that foam height increases for 25℃ 40℃ and 60℃ foam height have been 12cm, 16cm and 27cm

respectively at 0min and decreased at 80℃ with foam height 24cm at 0min. The height and stability of foam decreased with time. The foam height decay profile show in figure 4.10 that the foam stability was lower at high temperature. The stability of foam at 60℃ was higher and decreased at 80℃ . Foam stability of 60℃ at 300min was 55.6% but the foam stability of 80℃ was 45.8% at 300min. The foam stability of 60℃ is high as compared from other temperature. The same result also reported foam stability was high at 60℃ and the height of the foam decreased beyond this temperature (Barreiro, 2021). Lower foam stability at high temperature is due to lower solution viscosity which means that solution viscosity decrease as temperature increases similarly the foam stability of saponin was lower at high temperature (Yekeen et al., 2020). From the figure 4.9 it can be observed that the foam height decreased as the time and the foam was decayed.

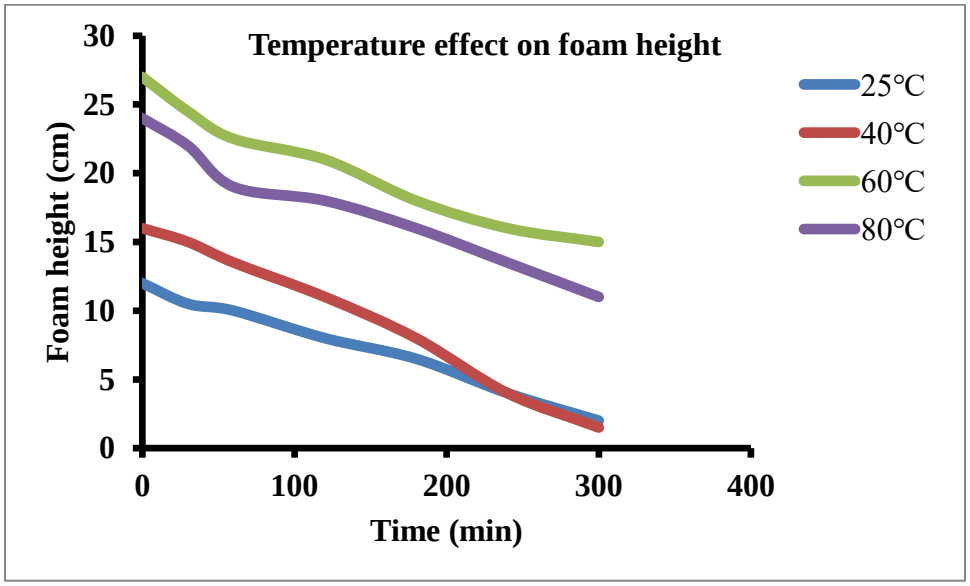

Figure 4.12 Temperature effects on the stability of saponin foam

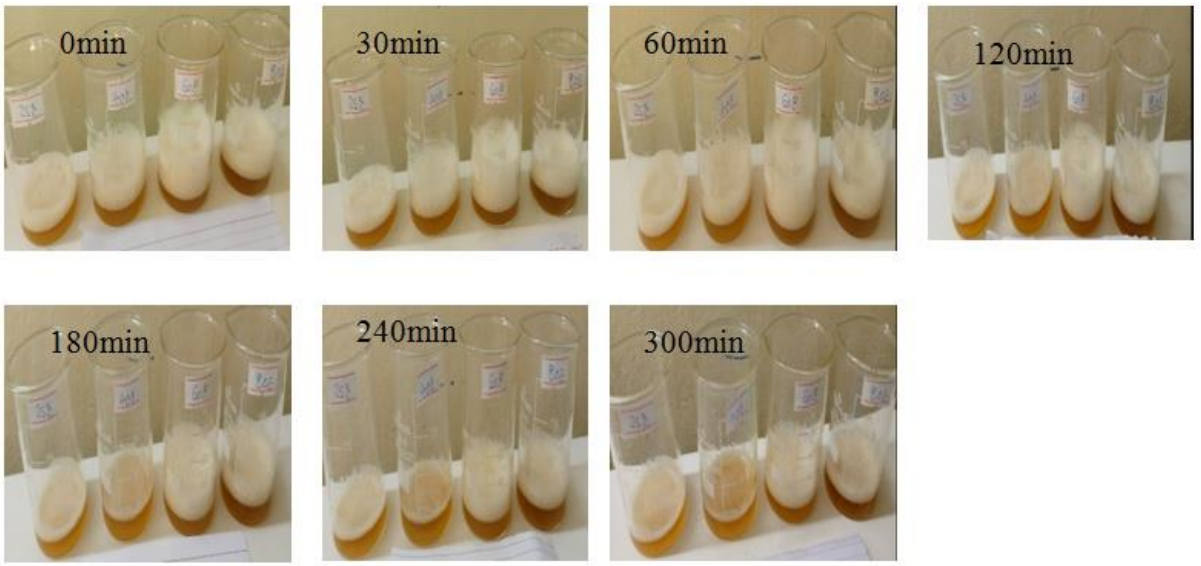

Figure 4.13 Foam height of saponin at CMC concentration with different temperature

4.5.1.2 Effect of pH on foam stability

The effect of pH (3, 7 and 9) on foam stability was studied using 0.1M NaOH and 0.1M HCl in aqueous solution of saponin extract. Foam height of the extract was evaluated at CMC value of the extract. Figure 4.11 shows that foam height decreased as the time was increased and become decayed as show in figure 4.12. The height of the foam at the begging(0min) were 22cm, 19cm and 15cm for pH =3, pH = 7 and pH =9 respectively, and foam height at 240min 15cm, 7cm and 4.5 for pH =3, pH = 7 and pH =9 respectively. The stability of foam at pH =3 after 240min was 68.18% but the foam stability of pH = 7 and pH =9 with this time was 36.84% and 30% respectively, as compared with the foam stability at this time pH =3 has high foam stability. The result revealed that lower pH values have high foam stability since most literatures reported that saponin foam is favorable to be used in acidic system. The electrostatic interaction minorly affects the foaming properties of saponin from plant (Bibi et al., 2019). The same results have been reported from (Jurado Gonzalez & Sörensen, 2020), in which saponin foam height was decreased with high pH value.

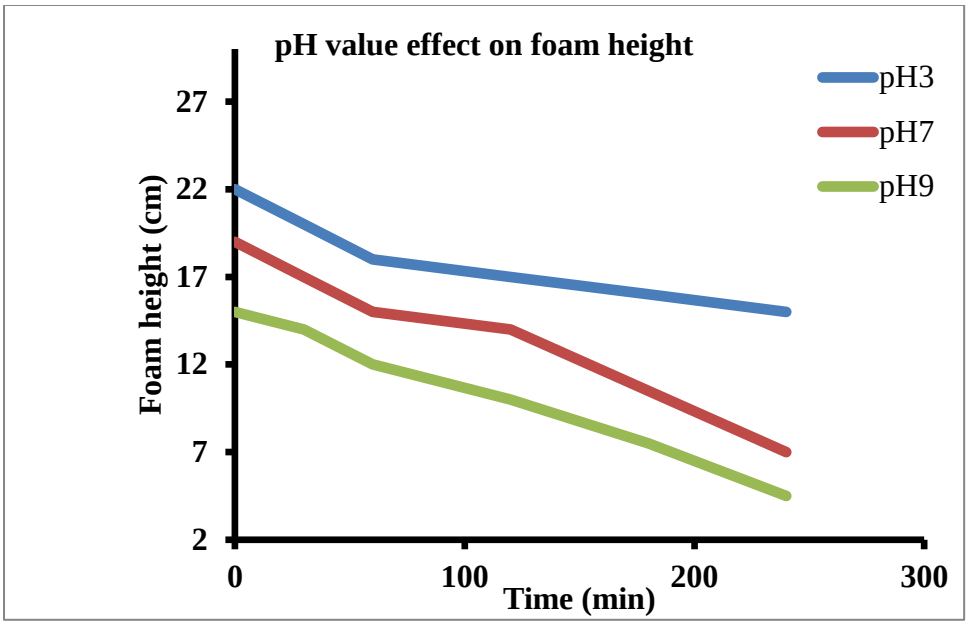

Figure 4.14 the effect of pH on stability of saponin foam

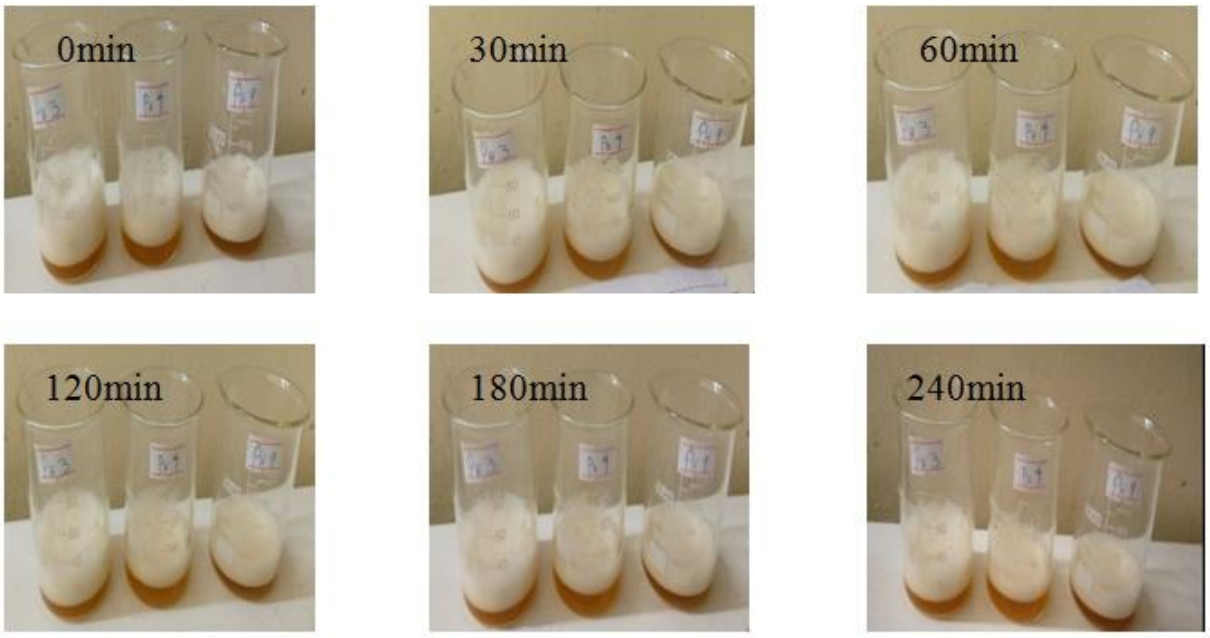

Figure 4.15 foam decay of saponin at CMC concentration with different pH

4.5.2 Surface tension and critical micelle concentration

Surfactants in aqueous solutions reduce the surface tension by minimizing the forces acting on the solution surface (Tmáková et al., 2015). Jurado Gonzalez & Sörensen, (2020) reported that; Saponins tend to be more effective water-soluble surfactants with high CMC values and a surface tension above the CMC of 38-40 mN/m. In this study the surface tensions of saponin from Endod with surfactant concentration (0.1-1.7g/L) has been examined and the critical micelle concentration of the extract have been determined. The relationship between the concentration of surfactant in aqueous solution and surface tension has been shown in the Figures 4.13. The plotted graphs showed that the surface tension decreases with the increasing surfactant concentration. In this work it is very clear that an increase in surfactant concentration lowers the surface tension of the solution from figure 4.13. Once surfactant concentration reaches a certain value, the value of surface tension becomes almost insignificant with the increasing surfactant concentration; this concentration is CMC value of saponin. The result shows that; the CMC values of Endod extract ranged between 1.2-1.7 g/L surface tension of 40.04mN/m at the CMC. Compared with other natural sources of saponins, the CMC values of Endod extract was in moderate range.

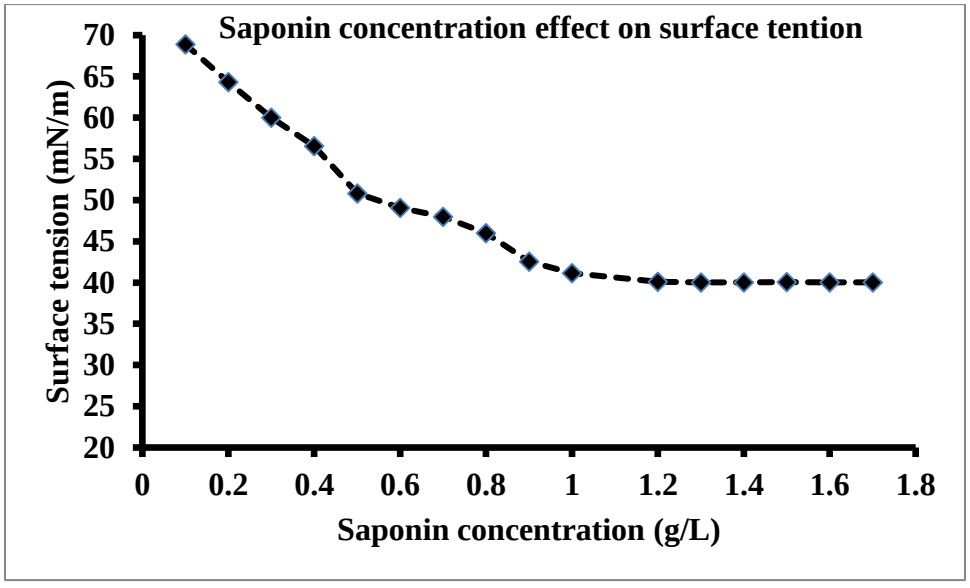

Figure 4.16 Surface tension of saponin in aqueous solution with different concentration

4.5.2.1 Effect of pH on surface tension and CMC (critical micelle concentration)

The effect of PH on surface tension and CMC value of saponin extracted from Endod berry was determined using 0.1M NaOH and 0.1M HCl in aqueous solution to adjust the pH value (3, 7 and 9). Figure 4.14 described different concentration of saponin in distilled water. The result show that as the pH gets lower the surface tension also decreased and also the CMC value. The value of CMC for pH =3, pH =7 and pH =9 were 1.2g/mL, 1.2g/mL and 1.3g/mL respectively with surface tension value of 40.12N/m, 41.11N/m and 41.78mN/m. This means that low pH value was favored for reduction of surface tension of air-water interface. (Jurado Gonzalez & Sörensen, (2020), Bibi et al., (2019)) reported that low pH value of surfactant solution is important in the reduction in surface tension also low value of CMC used for the reduction. The presence of some special groups on saponins, such as carboxyl groups, will greatly impact CMC. Affected by carboxyl groups, the formation of micelles is sensitive to pH, and CMC varies greatly under different pH conditions or for low-pH conditions, there is a carboxyl group (COOH) that may not dissociate into $COOH^-$ at lower pH of saponin. This means that the charge of saponins at low pH is neutral and may not negatively affect the surface active properties of saponins. Due to this reason the surface tension value of saponin varies depending on the pH value of solution.

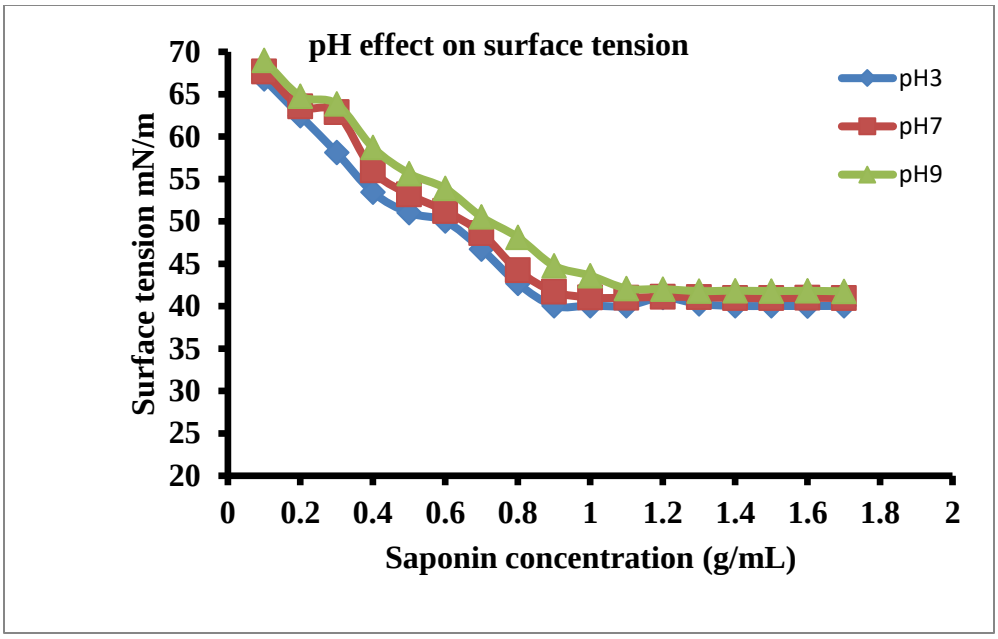

Figure 4.17 the effect of pH on surface tension of saponin in aqueous solution

4.5.3 Emulsification activity of saponin

The emulsification index of the extracts was evaluated at different concentrations. Figure 4.15 describes emulsification index of saponin extracted from Endod berry with different concentration. The emulsification index of saponin increased with saponin concentration. Emulsification index value of saponin concentration 0.1wt%, 0.2 wt%, 0.3 wt%, 0.4 wt% and 0.5 wt% have been 43.86%, 46.43%, 56.99%, 57.9% and 60.93 respectively. The result shows that emulsification index increased with the concentration of saponin increased. This indicates that the activity of saponin as emulsifier increased with concentration which mean, emulsification activity of saponin increased with concentration. This result is confirmed/agreed with (Kime et al., 2015) in which saponin extracted from B. aegyptiaca bark extract having similar result, as the concentration of saponin extract increased from 1g to 4g the emulsification activity also increased with concentration. Káren G.O. Bezerra et al., (2021) also reported that emulsification activity of saponin extract from C. quinoa in coconut oil increased from the saponin concentration within its CMC value to 10CMC. This is why the stability of emulsion was indicated a significant correlation between the concentrations and emulsions formation surfactant. Figure 4.15 indicated that the effect of saponin concentration on emulsification activity with paraffin oil. The emulsification activity of saponin increased with concentration. Therefore, with an increase in E_{24} resulted in an increase in dispersion of aqueous phase in oil

phase. Figure 4.16 shows that the emulsification profile of saponin in different concentration. The result was confirmed with the emulsification activity of saponin extracted from Glycyrrhiza glabra using saponin concentration 0, 2%, 4%, 6%, 8% and 10% w/v were increased due to dispersion of aqueous phase and oil phase (Hajimohammadi et al., 2016).

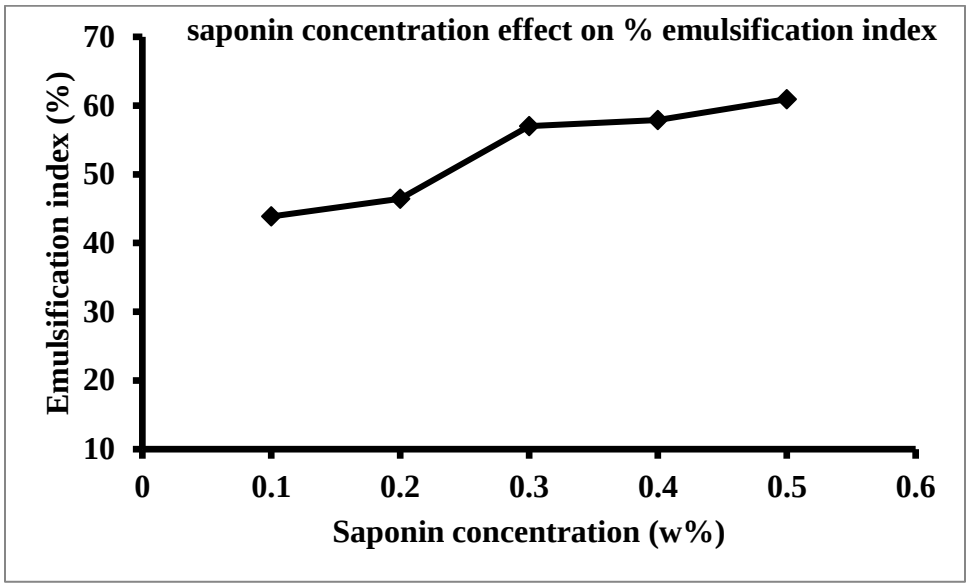

Figure 4.18 Effect of saponin concentration on emulsification index use paraffin oil

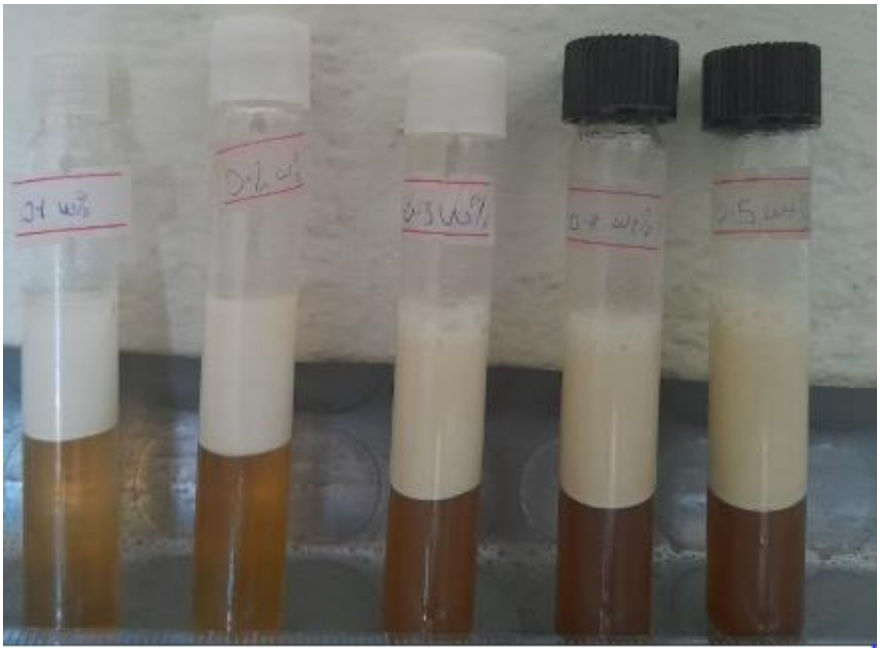

Figure 4.19 Emulsification profile of saponin with different concentration

4.6 Emulsion characterization

4.6.1 Effect of long-term storage on emulsion stability

emulsion stability is an important parameter to be considered, the stability of the emulsions over time was evaluated (Schreiner et al., 2020). The storage stability of emulsion is a particularly important parameter for understanding the shelf life and potential applications of the emulsified products (Dahlawi et al., 2020). The saponin molecules are aligned at the oil/water interface to emulsify the two immiscible phases. Saponins form a viscoelastic interfacial membrane through hydrogen bonds between adjacent sugar residues. The adsorbed saponins provide a self-assembled protective layer around the oil droplets, inhibiting the aggregation of droplets and improving the long-term stability of the emulsion (Liao et al., 2021). The stabilities of emulsion use different saponin concentration were investigated for 5, 10 and 30 days after emulsion prepared. Emulsions were milky white but the color of the emulsions was depending on emulsifier concentration (R. Li et al., 2020). These experiments were performed using emulsions at neutral pH and at room condition. Figure 4.18 shows the emulsion appearances after 5, 10 and 30 days after preparation. Visual observations indicated that there was no change in the appearance of emulsion prepared using 0.5% and 1% of saponin concentration throughout the entire period and little change in the appearance/color of the 1.5%, 2%, 2.5% and 3% saponin concentration after 10days due to the concentration of saponin amount that changes the color of prepared emulsion though storage time. The prepared emulsion was stable for 5days without change for all concentration. No creaming was observed though entire period of storage when the emulsifier concentration was 1 wt%. For economic and sustainability reasons, it is practical to use the lowest amount of emulsifier possible to form a stable emulsion, as well as to replace more expensive emulsifiers with cheaper alternatives. The result indicates that saponin was fairly efficient to prevent droplet aggregation of oil phase. According to the figure 4.18, complete separation of the emulsion did not occur even after 30days. This indicates that prepared emulsion was stable without separation of oil phase for 30days.

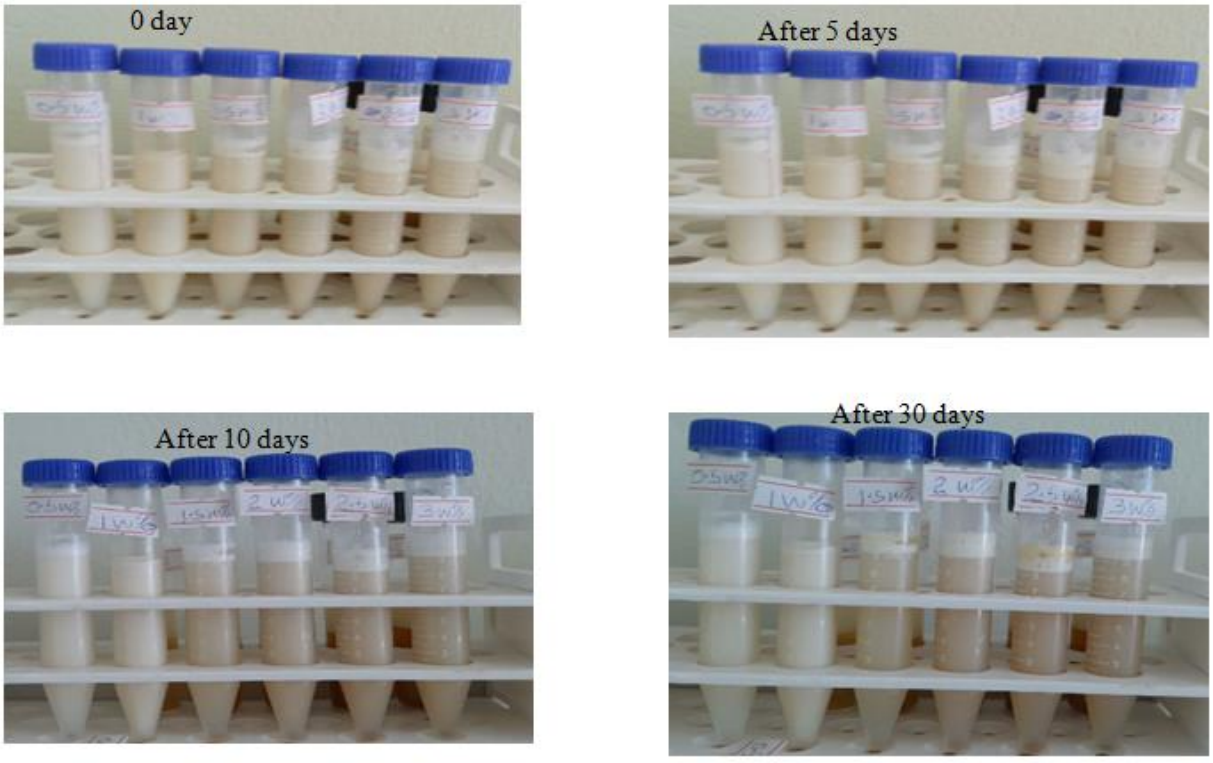

Figure 4.20 Evaluation of emulsion stability after 5, 10 and 30 days

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Extraction of plant based saponin from Endod (*phytolacca dodecandra*) berries as an emulsifier and foam stabilizer and emulsion preparation. The effect of temperature, time and sample to solvent ratio for extraction were investigated and optimum values were determined. The effect of diethyl ether and buthanol amount in the purification were also determined. Selection of distilled water/ethanol ratio for the extraction of saponin was performed. The extract was characterized using FT-IR and NMR analysis and test properties and performance of the extract based on foaming property, surface tension and critical micelle concentration, emulsification index. Solvent type for the extraction of saponin from this plant was distilled water ethanol mixture with the distilled water ethanol ratio of (80/20V/v) respectively. Optimum saponin extract yield was achieved, using sample to solvent ratio 1:10g/mL for 3hr at temperature of 70℃ and diethyl ether and buthanol amount used was 50mL for each. The FT-IR results confirmed the presence of O-H at 3281 cm^{-1}, C-H at 2922 cm^{-1}, C-O-C stretching at 1014 cm^{-1}, C=O at 1734 cm-1 and the C=C stretching at 1612 cm^{-1}. The extracted saponin showed stable foam at room temperature which increased as the concentration of saponin solution increases. Saponin concentration for attaining maximum foam stability was at 0.4% at room condition and saponin-stabilized foam was very stable at high temperature (60℃) with CMC saponin concentration. Whereas the stability of foam decreased as the temperature increased above this temperature. Foam stability of saponin concentration at pH=3 was high than pH=9 at CMC concentration.

Surface tension measurements indicated that the extracted saponin exhibits CMC value of 1.2g/L and surface tension value 40.04 mN/m under CMC concentration at room condition. The surface tension of saponin was measured with different pH at room condition and the surface tension at pH=3 only very small effects on CMC value were observed. Emulsion was prepared from saponin extracted from Endod and Paraffin oil. The stability of emulsion was determined using long-time storage stability test for 30 days after emulsion was prepared and the prepared emulsion was stabile for 30 days without completely separating the oil phase from the aqueous phase.

5.2 Recommendation

Some recommendations for future work are listed as follows:-

- It is proposed to modify and improve the emulsification activity of saponin extracted from Endod berries using different oils, study the effect of oil type on emulsification activity and the effect of (pH, temperature and ionic strength) may be investigated for further study.
- Saponin is a natural emulsifier used for preparation and stability of emulsion, emulsion preparation using high pressure homogenizer method and the stability of prepared emulsion using particle size of droplet and zeta potential activity of emulsion may be investigated in detail.
- Further study can be explored on the TGA analysis of saponin extract from this plant can be used to understand the thermal stability of saponin extract from this plant.

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