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BAHIR DAR INSTITUTE OF TECHNOLOGY

SCHOOL OF GRADUATE STUDIES

FACULTY OF CHEMICAL AND FOOD ENGINEERING

PROCESS ENGINEERING PROGRAM

Master Thesis on:

Optimization and Investigation of Anti-Oxidative Activity of Crude Yield from Curcuma Longa (Turmeric Rhizome)

By

Gedam Tafere Taye

July, 2021

Bahir Dar, Ethiopia

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Optimization and Investigation of Anti-Oxidative Activity of Crude Yield from Curcuma Longa (Turmeric Rhizome)

By

Gedam Tafere Taye

A Thesis

submitted to the school of graduate studies of Bahir Dar Institute of Technology, Bahir Dar University in partial fulfillment of the requirements for the degree of Masters of Science in process engineering under faculty of chemical and food engineering.

Supervisor Dr. Metadel K.

July, 2021

Bahir Dar, Ethiopia

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Bahir Dar University

Bahir Dar Institute of Technology

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Final Thesis Approval Sheet

I hereby confirm that the changes required by the examiners have been carried out and incorporated in the final thesis

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As members of the board of examiners, we examined this thesis entitled "Optimization and Investigation of Anti-Oxidative Activity of Crude Yield from Curcuma Longa (Turmeric Rhizome)" by Gedam Tafere. We hereby certify that the thesis is accepted for fulfilling the requirements for the award of the degree of Masters of science in "Process engineering, chemical engineering"

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DECLARATION

This is to certify that the thesis entitled "Optimization and Investigation of Anti-Oxidative Activity of Crude Yield from Curcuma Longa (Turmeric Rhizome) ", submitted in partial fulfillment of the requirements for the degree of Master of Science in (Process engineering) under (Chemical and food engineering), Bahir Dar Institute of Technology, is a record of original work carried out by me and has never been submitted to this or any other institution to get any other degree or certificates. The assistance and help I received during the course of this investigation have been duly acknowledged.

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Name of the candidate	signature	Date

ACKNOWLEDGMENT

First, I express my heartiest devotion to the “Almighty God” and “His mother” for “Them” graceful blessings at every step. Without God nothing could have been accomplished.

I would like to express my thankfulness to my advisor Dr. Metadel Kassahun, for his expert supervision, encouragement and support throughout the period of this work. From the core of my heart, I owe my gratitude to my husband Dawit Bekalu and my friends Eskedar Adane, H/Woyn and Birtukan Minichil for their inspiring attitude, constant encouragement, and endless affection.

I gratefully acknowledge the support wholehearted help and co-operation provided by technical assistances, Alebel, Adisu, and Tadelle during my research work. I also acknowledge Mr. Adugna and Tewekel M. for their support.

My sincere thanks to all of them, whom I am not recalling this moment, who helped me in any way during my research work. Finally, my deepest appreciation goes to Bahir Dar institute of technology for offering this opportunity.

ABSTRACT

Free radicals generate in a living cells and is the part of normal cellular function. However, excess free radicals cause of oxidative damage to molecules and thus are involved the initiation phase of diseases. Here anti oxidative mechanism of these excess free radicals is required. Turmeric is one of the most important natural anti oxidative spices. Use this spices as alternative or supplementary medicine to reduce the burden of high cost, side effects and progressively increasing drug resistance of pathogens. This study was carried out to determine the optimal experimental conditions of maceration extraction method and to investigate the anti-oxidative activity of the bioactive compounds from turmeric using Design expert software. Three main factors effect on yield from dried turmeric that include: ethanol concentration (60, 70 and 80 %), duration (1, 2 and 3 hr) and extraction temperature (60, 70 and 80^{o}c). The ash, moisture, fiber, fat, protein and carbohydrate content were 5.359, 7.886, 15.95, 16.59, 5.552 and 48.663, respectively. Significance of the extraction parameters were analyzed by analysis of variance. Response surface method was applied to investigate their interaction effect on the crude extraction yield and to find the optimum yield. As the result of response surface method optimization, the optimum yield (40.2408%) was found at an optimum extraction temperature of 69.95 ^{o}C, and ethanol concentration of 78.87%, and extraction time of 2.32hr. At these optimum condition the turmeric phytopharmaceutical intermediate products such as total flavonoids, total phenol and antioxidant content were 71.678 mgQUE/g DW, 4.551mg GAE/g DW and 189.971mg AAE/g DW, respectively. The above evidences suggest that turmeric rhizome is a potential source of natural antioxidant and can be used to prevent diseases associated with free radicals.

Keywords: Curcuma longa linn, Surface response methodology, Maceration, Crude extraction yield and Antioxidant activity.

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LIST OF ABBREVIATIONS

AAEAC	Ascorbic acid equivalent antioxidant capacity
AAE	Ascorbic Acid Equivalent
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
DNA	Deoxyribonucleic acid
DPPH	1,1- diphenyl-2- picrylhydrazyl
DW	Dry Weight
FCR	Folin Ciocalteu Reagent
FRAP	Ferric Reducing Antioxidant Power
GAE	Galic acid equivalent
MAE	Microwave assisted extraction
QUE	Quercetin acid equivalent
RNA	Ribonucleic acid
SFE	Supercritical fluid extraction
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
UAE	Ultrasound assisted extraction
UV- Vis	Ultra- violet visible

1 INTRODUCTION

1.1 Background

Growth of chemical and phytochemical analysis has run to the increasing use of herbal medicine for the treatment of human diseases and many of the pharmaceuticals currently accessible to physicians have a long history of use as herbal remedies, such as opium, aspirin, digitalis and quinine (Garg et al., 2016). The use of, search for drugs and dietary supplements derived from plants has accelerated in recent years.

Herbal medicine used as self-care options for sleep, stress and mood disorders to digestive ailments, colds and flu. It is also used to treat many diseases including asthma, eczema, premenstrual syndrome, rheumatoid arthritis, migraine, menopausal symptoms, chronic fatigue, irritable bowel syndrome and cancer. Herbal supplements are best taken under the guidance of an accomplished healthcare provider. For example, one study initiate that 90 % of arthritic patients use alternative therapies like herbal medicine (Garg et al., 2016). Herbal medicine utilized as food additives or food spices. Both herbs and spices are generally used in the preparation of food mostly to improve in their color and flavour. They are also used as preservative agents to extend the shelf life of foods (Djikeng & Kingne, 2019).

Among herbal medicines plant, curcuma longa Linn, turmeric, is the most vital one that used as a golden spices, food preservatives, flavouring agent, dyes, cosmetics, starch and ornamentals (Gupta et al., 2013; Xiang et al., 2011). It is used as a remedy for diarrhea, rheumatism and is also used to relieve cough and tuberculosis (Sirirugsa, 1999). Its rhizome is stomachic, stimulant and carminative. This spice is potential sources of natural anti oxidative agents as well. It produces various anti-oxidative compounds to counteract reactive oxygen species to survive. Antioxidant is a molecule which preventing the oxidation of other molecules. Oxidation is a chemical reaction which transfers electrons from a substance to an oxidizing agent. During this reaction excess free radicals such as superoxide radical, hydroxyl radical, peroxy free radical, hydrogen peroxide, lipid peroxides and nitric oxide radical produced (Jan et al., 2013). These free radicals start to form chain reactions and damage cells and then they are responsible for aging and causing various human diseases such as heart disease, ageing,

diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others (Mahfuza khatoon et al., 2013).

In fact, antioxidants possess terminate these chain reactions by scavenging free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents like thiols or polyphenols (Sies, 1996). Mainly the antioxidant activity of phenolic is due to their redox properties that allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers. The importance of natural phenolic compounds from plants materials is raising interest in scientists, food manufacturers and consumers due to functional food with specific health effects. So it is vital to evaluate and quantify effective antioxidant principles of medicinally or economically viable plant materials.

1.2 Statement of the Problem

The oxidative disintegration of lipids, proteins and nucleic acids decrease the shelf life of foods because it reduces food safety and wholesome quality. It also initiation phase of some degenerative diseases. Due to this, antioxidants are required to prevent those oxidations. In food processing and preservation, synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole are commonly used but they have been found to have side effects and have been proved carcinogenic (Jan et al., 2013). Thus there has been increased interest in natural antioxidants, especially those of plant origin (herbs and spices). Even if many people believe that products labeled “natural” are always safe and good for them, unfortunately, the use of antioxidant herbs traditionally has its own significant drawback like health crisis, due to the contamination, dosage and presence of toxic compounds. There was also problem that facing the use of ant oxidative herbs or spices is the proof requirement that the active components contained in them are useful, safe and effective. To mitigate such bottlenecked problem, attention is being focused on the investigation of the better understood on their properties, safety and the efficacy of bioactive constitutes in the traditional medicine, ant oxidative herbs and spices. The efficacy of bioactive constitutes can be significantly improved by studying the influence of extraction parameters (Giacometti et al., 2018). Thus, the primary aim of the present study was optimizing the main extraction parameters (extraction temperature, time and solvent

concentration) of turmeric rhizome on yield and then to determine the anti-oxidant activity of its bioactive compounds such as total phenol and total flavonoid.

1.3 Objectives

1.3.1 General Objective

The main objective of the present work was to optimize and investigate the anti-oxidant activity for crude extraction yield from turmeric rhizome.

1.3.2 Specific Objectives

Its specific objectives of this work were:

- To determine physiochemical properties and identify the bioactive metabolites with a chemical test.
- To investigate the effect of extraction parameters on ethanoic crude extract yield.
- To characterize the ethanoic crude extract.

1.4 Scope of Study

The scope of this research was focused on extraction, optimization of basic extraction parameters on extraction yield, determination of the total phenolic content (TPC) and total flavonoids content (TFC) and investigation of the antioxidant activity of phenol and flavonoids. The total phenolic, total flavonoid content and antioxidant content were determined by using UV-Vis spectroscopy with standard procedures.

1.5 Significance of the Study

The significance in this study is high total phenolic compounds and flavonoids contain in curcuma longa act as natural antioxidant. The extract phenolic and flavonoid compounds of curcuma longa promise high potential to be used in food industry as spices and food additives as well. Turmeric used as source antioxidants may have also considerable economic benefit to food processors and it may develop the economy of country and help to increase the farmer's income by creating job opportunity around the rural areas. This work prompted us to examine the possibility of turmeric extract itself as candidate agent for pharmacologic evaluation (especially COVID 19 and HIV) (Zahedipour et al., 2019).

2 LITERATURE REVIEW

2.1 Traditional Medicinal Plants

2.1.1 Traditional Medicinal Plants in Africa

(Okigbo et al., 2008) studied about traditional medicine. Based on their study, it is as a main African socio cultural heritage that practices for a long period of time the first choice of health care treatment for at least 80% of Africans who suffer fever and other common ailment. They also use herbal medicines in order to improve their health, to keep their beauty and to spice their diet, because of the occurrence of bioactive constituents of plants such as alkaloids, tannins, flavonoid and phenolic compounds (Pawar et al., 2015; Vasu et al., 2009). However, there was problem that facing the use of traditional medicine is the proof requirement that these active components contained in medicinal plants are useful, safe and effective. The proofs of pharmacology activity that are available at present are mostly based on empirical experience. The scientific proof then becomes the most important things to eliminate the concern of using medicinal plants as drug alternative treatment unfortunately, most African countries are notable to conduct researchers provides scientific proof pharmacology. International collaboration is important for African countries, as it would enhance the development of drugs obtained from medicinal plants to their benefits.

2.1.2 History of Medicinal Plants in Ethiopia

Ethiopia is a country that has a wide range of climate and ecological condition. It has also owns huge diversity of flora and fauna that has more than 6,500 species of vascular plants out of which an estimated 12% are endemic and about 887 species are used as medicinal plants (Zerabruk & Yirga, 2012). 80% of Ethiopian people be influenced by traditional medicine for their health care and more than 95% of traditional medicinal preparations made from plant origin. Ethiopia is also a home for many languages, cultures and beliefs that have in turn contributed to the high diversity of traditional knowledge and practice of the people, which, among others include the use of medicinal plants.

Popular knowledge of such medicinal plant used by human is based on thousands of years of practice by trial and error people learn how to recognize and use plants including those with a magic- religious function. Although Ethiopian traditional

medicinal practitioners are the best sources of information secret, it passed only orally to their older son, at their oldest age (Janzen, 1981). But between the 17 & 18 centuries few medicinal text books written in Geez and Arabic employs that plants have been used as a source of traditional medicine in Ethiopia health care system (DEMISIE, 2016). The majority of Ethiopian still depends on traditional medicine due to inadequate coverage of modern medical system, shortage of pharmaceuticals and high-priced of modern drugs as well as lack of effectiveness of modern drugs.

2.1.3 Zingiberaceae Family Species

Based on Pitopang and his colleagues study, the ginger family (Zingiberaceae) is the largest family that containing more than 52 generas and 1600 species and these aromatic herbs with creeping horizontal grow in moist areas including some regions that are seasonably dry (Pitopang et al., 2019).

2.1.4 Curcuma longa Linn

The dried rhizome of Curcuma longa L. known as turmeric belongs to the ginger, Zingiberaceae, family and a natural colorant (Jain et al., 2007; Laokuldilok et al., 2015; Pan et al., 2020; Rajagopal, 2020) in pharmacy, confectionery and food industries. It is an important spice that grows to about 2 feet in length and is widely distributed in tropical and subtropical regions of the world (Pawar et al., 2015). It provides a variety of physiological functions, such as, antimicrobial (Abdel-lateef & Hammam, 2016; Pan et al., 2020; Rasmussen et al., 2000), anti-inflammatory (Abdel-lateef & Hammam, 2016; Farhood et al., 2019; Jain et al., 2007; Pan et al., 2020; Zahedipour et al., 2019), antioxidant (Abdel-lateef & Hammam, 2016; Mhillaj et al., 2019; Pan et al., 2020; Reddy et al., 2018), antiarthritic (Reddy et al., 2018), anticancer (Abdel-lateef & Hammam, 2016; Grabarska et al., 2018; Huang et al., 2013; Zahedipour et al., 2019), hepato-protective (Jain et al., 2007), cardio-protective (Zahedipour et al., 2019), antidiabetic (Abdel-lateef & Hammam, 2016; Liu et al., 2012), immune-modulartory (Abdel-lateef & Hammam, 2016), anti-alzheimer (Fang et al., 2014; Reddy et al., 2018; X. Wang et al., 2014), anticoagulant (Abdel-lateef & Hammam, 2016) and anti-tumor (Abdel-lateef & Hammam, 2016).

Figure 2.1: Curcuma longa plant

(Source: ((Chairman K et al., 2015))

2.1.5 Anti-Oxidative Bioactive Compounds of Turmeric

2.1.5.1 Polyphenols

To date, at least 235 compounds are existing in turmeric plant. Primarily phenolic compounds and terpenoids have been identified from this species, such as 22 diarylheptanoids and diarylpentanoids, 8 phenylpropene and other phenolic compounds, 68 monoterpenes, 109 sesquiterpenes, 5 diterpenes, 3 triterpenoids, 5 sterols, 2 alkaloids, and 14 other compounds (Li & Wang, 2011). Among the phytochemicals, polyphenolic compounds are the major contributor of antioxidant activity in the extract. Polyphenolic compounds, are also primarily accumulated in rhizomes, are major bioactive ingredients showing various bioactivities in *in vitro* and *in vivo* bioassays. These compound occur in food plants as esters or glycosides conjugated with other natural compounds such as flavonoids, alcohols, hydroxyl fatty acids, sterols, glucosides and soon (Taylor S.L, Wang J, 2011)

Polyphenol compounds that contain phenol rings and known also as phenolics (Garg et al., 2016).They have also received greater attention due to its biological response modifiers and that have several functions such as metal chelators and free radical terminators. The simplest of the class is phenol (C_6H_5OH) as shown as Figure 2.2.

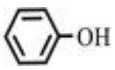


Figure 2.2: Structure of phenol

(Source: (Garg et al., 2016))

Besides these, these compounds are different in structure but are characterized by hydroxylated aromatic rings for example flavan-3-ols. They classified as secondary metabolites and their function in plants is often poorly understood. Many of them are polymerized into larger molecules like the proanthocyanidins and lignins. The phenolic compounds of turmeric are name curcuminoids that include mainly curcumin, demethoxycurcumin (1-(4-hydroxypheny1)-7-(4-hydroxy-3 methoxypheny1)hepta-1,6-diene-3,5-dion and bisdemethoxycurcumin (1,7-bis(4 hydroxypheny1)hepta- 1,6-diene -3,5- dione) (Revathy et al., 2011). Curcumin is a yellow crystalline natural polyphenolic compound that could be a potential treatment option for patients with coronavirus and HIV disease by molecular docking (Zahedipour et al., 2019). It against the virus that arise from its ability to interact with various molecular targets thereby triggering cellular signaling pathways such as apoptosis and inflammation. Curcumin ($C_{21}H_{20}O_5$) (1), also known as diferuloyl methane or 1,6-heptadiene-3,5-dione-1,7-bis(4-hydroxy-3-methoxyphenyl) (Wakte et al., (2011) and (Revathy et al., 2011).

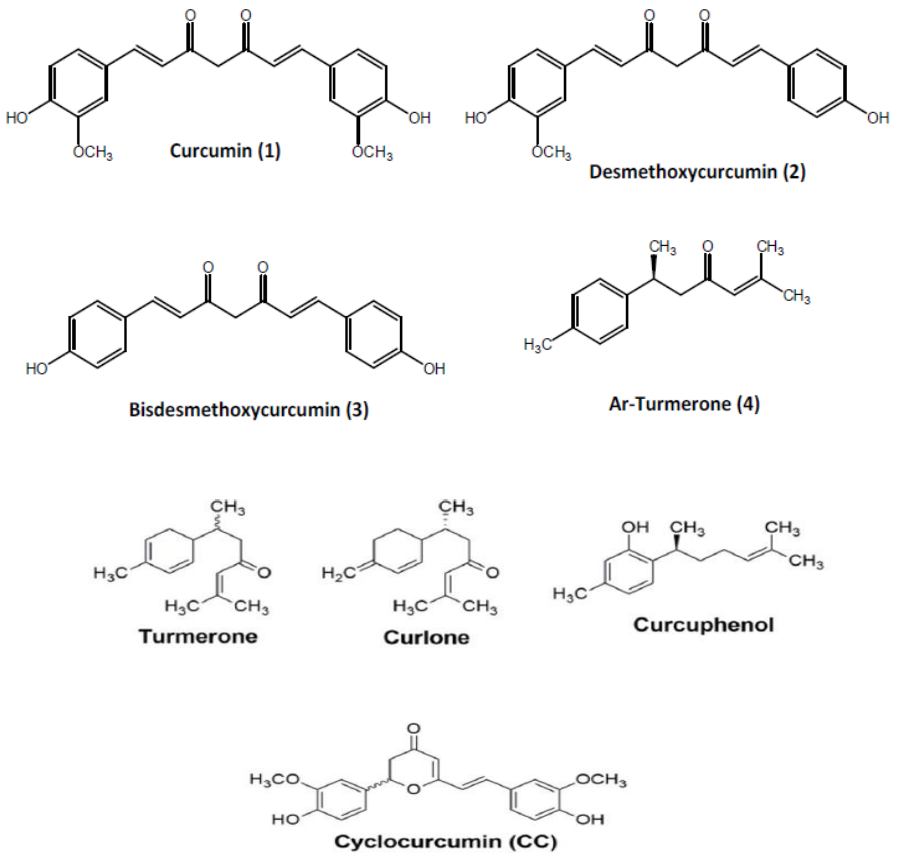


Figure 2.3: Chemical Structure of selected major bioactive Constituents from *C. longa* L.

(Source: ((Avula et al., 2012))

Turmeric rhizome contains 4-6% Curcuminoids, 2-4% essential oil, 2-3% of fixed oil, various volatile oils including turmerone, atlantone, and zingiberone and other constituents include sugars, proteins and resins (Kuttigounder et al., 2011).

Phenolic compounds include phenolic acids, flavonoids, terpenes, annins, stilbenes and lignans but high molecular weight phenolic such as condensed and hydrolysable tannins have no antioxidant activity.

2.1.5.2 Antioxidant Mechanisms of Phenolics

Phenolic are able to act as antioxidants in different ways (Pereira et al., 2009). The first mechanism is by donating the hydrogen from hydroxyl groups. Hydrogen-donating antioxidants react with reactive oxygen and reactive nitrogen species in a termination the reaction that breaks the cycle of generation of new radicals. Their antioxidant activity is depending on the number and position of hydroxyl groups in the molecule (Taylor S.L, Wang J, 2011). The antioxidant efficiency of mono-phenols is strongly enhanced by introducing a second hydroxyl group at the ortho- or para- positions and is increased by one or two methoxy substitutions in ortho- position with respect to the hydroxyl group. The second ways are due to their ability to chelate metal ions involved in the creation of free radicals but phenolics can act as pro-oxidants by chelating metals in a manner that increases their catalytic activity or by reducing metals, thus increasing their ability to form free radicals.

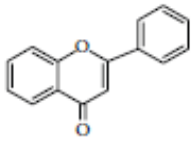
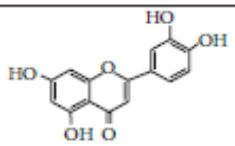
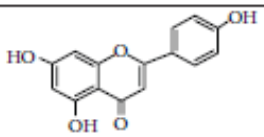
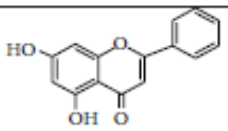
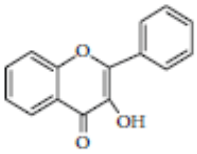
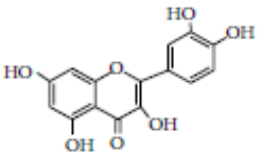
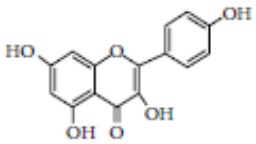
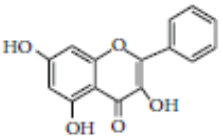
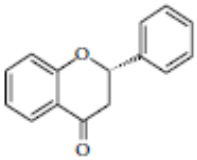
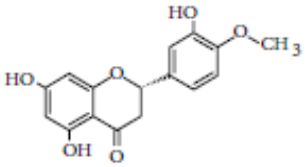
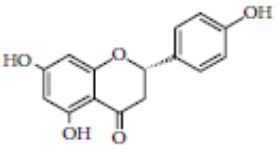
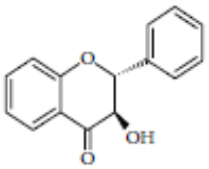
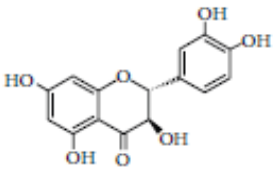
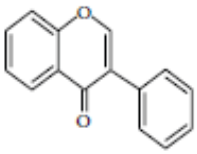
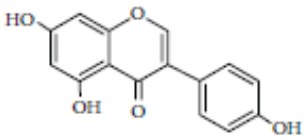
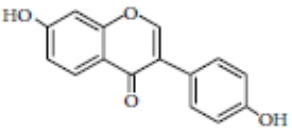
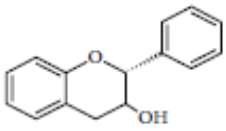
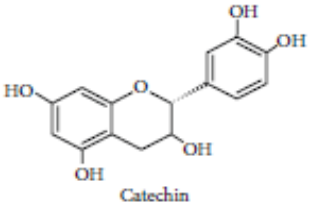
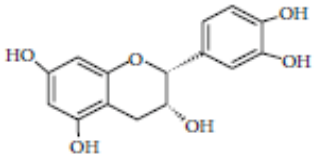
2.1.5.3 Flavonoids

Flavonoids contain of a large group of polyphenolic compounds having a benzo-γ-pyrone structure. They are synthesized by phenylpropanoid pathway. Their general structures are shown in Table 2.1.

Many flavonoids have antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepato-protective, anti-inflammatory, and anticancer activities, while some flavonoids exhibit potential antiviral activities. Anti-oxidative activity of flavonoids depend upon the configuration, substitution of functional groups about their nuclear structure and total number of hydroxyl groups (Kočevar et al., 2007). The hydroxyl groups in flavonoids facilitate their antioxidant effects by scavenging free radicals and by chelating metal ions. The chelation of metals prevents the free radical generation which damage target biomolecules.

Due to this this study focus on investigation of anti-oxidative activity of these phenol and flavonoids after optimize the extraction parameters.

Table 2.1: Structure of flavonoids

Group of flavanoid	Structure backbone	Examples		
Flavones		 Luteolin	 Apigenin	 Chrysin
Flavonols		 Quercetin	 Kaempferol	 Galangin
Flavanones		 Hesperetin	 Naringenin	
Flavanonol		 Taxifolin		
Isoflavones		 Genistein	 Daidzein	
Flavan-3-ols		 Catechin	 Epicatechin	

2.2 Factors that Affect Extraction Process

The yield, the activity of the obtained extracts and efficiency of extraction be influenced by on the solvent with varying polarity, temperature, agitation speed, extraction time, pH, size of the particle and composition of the sample (Paulucci et al., 2013). Under thus, solvent concentration, extraction time and extraction temperature is known as the most important parameters (HİN, 2018). The polyphenolic compounds are poorly

soluble in the hydrocarbon solvents but they are soluble in polar solvents including aqueous mixtures containing methanol, acetone, ethanol and ethyl acetate. Methanol is suit in extraction of lower molecular weight polyphenols but it is toxic whereas aqueous acetone is good extract higher molecular weight flavonoid. Due to the high solvent costs, the toxicity resulting from the solubility of the bioactive compounds in the solvent and low extraction efficiency, these solvents have been used regularly for the extracting antioxidant compounds. Therefore, ethanol is one of the appropriate extraction solvents for antioxidant compounds due to its low cost, "green" characteristics, ease of access and safety for direct use in foods and pharmaceuticals (Hİn, 2018).

Optimization extraction and purification of biological activity curcumin from Curcuma longa L by high-performance counter-current chromatography was premeditated by (Pan et al., 2020). They have seen the effect of the concentration of ethanol (at 70, 80 & 90 %), extraction temperature (at 60, 70 & 80 ^{o}C), the ratio of liquid to material (at 15, 20 & 25 ml/g) and extraction time (at 1, 2 & 3 hour) on a crude extract. From 97mg crude sample, the purities of the isolated curcumin, demethoxycurcumin and bisdemethoxycurcumin can reach 98.26, 97.39, and 98.67%, respectively, while their contents in the crude extract were 68.56, 19.01, and 10.32%, respectively, were obtained within 70 min.

Another researcher has also seen three main factors effect on total polyphenol content from dried turmeric leaves that include extraction temperature (50, 60, and 70 ^{o}C), duration (40, 50, and 60 minutes) and solvent concentration (40, 50, and 60 %). From the results the optimal extraction condition of polyphenol from turmeric leaves was temperature at 68.3 ^{o}C for 59.9 minutes and 40 % ethanol that would give the highest polyphenols content 64.2 mg gallic acid equivalents (GAE) per g of dry weight at absorbance 765 nm by using a Cary 50 UV-Visible spectrophotometer (Thach, 2020).

The others researchers have also seen the effect of extraction solvent types (chloroform, acetone, ethyl acetate, butanol and methanol) (Hamed et al., 2019) on optimization of Curcuminoids Extraction for Evaluation Against Parkinson's Disease in Rats. From the result, the highest extraction yield was found in methanol (74 % w/w of defatted powder) and acetone (65% w/w of defatted powder) extracts, while the highest curcuminoids content (566 mg/g extract) was found in the ethyl acetate extract and

High performance liquid chromatography (HPLC) analysis showed the poly phenolic compounds in terms of three major compounds; curcumin (68 %), demethoxycurcumin (19 %) and bisdemethoxycurcumin (11 %) of total curcuminoids.

(Wakte et al., 2011) studied on the optimization of micro wave, ultra- sonic and supercritical carbon dioxide assisted extraction techniques for curcumin from curcuma longa. The critical parameters viz. effect of microwave pre-irradiation time and soaking solvent at constant microwave power on the curcumin yield were studied. The extraction efficiency of all the above-described techniques were established in terms of percent curcumin yields and extraction rate constants. The percent curcumin yields of microwave and ultra-sonic irradiation of dry curcuma powder resulted in 68.57 and 40.00%, respectively, whereas water-soaked irradiated curcuma powder yielded 90.47 and 71.42% curcumin recovery respectively, during a total extraction period of five minutes. The microwave extraction parameters were microwave pre-irradiation time (1, 3, 5 or 7 min) and soaking solvent (water and ethanol) at 400rpm stirring speed throughout the extraction period. The water soaked powder was irradiated at 270W whereas ethanol soaked powder was irradiated at 50W microwave power. The extraction was performed at 90W (extraction solvent: ethanol) and 60W (extraction solvent: acetone) microwave power.

(Ahaotu & Lawal, 2019) examined the effect of dynamic maceration factors, namely agitation speed, time, drug to solvent weight ratio, extraction temperature and ethanolic strength on the curcumin. The most influential variable was the ethanolic strength of the solvent and the optimized condition involves extraction temperature of 80 ºC, an extraction time of 12 h, agitation speed of 30 rpm, drug to solvent ratio of 1/6, and the solvent with concentration of 70%.

Table 2.2: The polarities of different extraction solvent types

Solvent	Relative Polarity
hexane	0.009
p-xylene	0.074
toluene	0.099
benzene	0.111
ether	0.117
methyl t-butyl ether (MTBE)	0.124
diethylamine	0.145
dioxane	0.164
chlorobenzene	0.188
tetrahydrofuran (THF)	0.207
ODCB (orthodichlorobenzene)	0.225
dimethoxyethane (DME; glyme)	0.231
pyridine	0.302
methylene chloride	0.309
HMPT	0.315
1,2-dichloroethane	0.327
DMPU	0.352
acetone	0.355
dimethylformamide (DMF)	0.386
kt-butyl alcohol	0.389
sulfolane	0.41
dimethylsulfoxide (DMSO)	0.444
acetonitrile	0.46
nitromethane	0.481
2-propanol	0.546
benzyl alcohol	0.608

Continued from table 2.2	
Solvent	Relative Polarity
acetic acid	0.648
ethanol	0.654
methanol	0.762
ethylene glycol	0.79
trifluoroethanol	0.898
hexa fluoroisopropanol	0.969
water	1

(Source: ((Avula et al., 2012))

(Şahin, 2018) studied on the optimization of ultrasonic-assisted extraction parameters for antioxidants from turmeric rhizome. Based his study, three basic parameters were examined. The response predictions obtained at optimum extraction conditions of ethanol concentration of 82% (v/v), time of 64min, and temperature of 32°C were 47.32 mg GAE/g (for Folin-Ciocalteu), 29.15 (for ABTS) and 5.17mg TE/g (for CHROMAC) with 95% confidence level.

2.3 Extraction Methods

2.3.1 Soxhlet Extraction

(Handa SS et al., 2008) in this method, the finely ground crude is placed in thimble which is placed in chamber of the Soxhlet apparatus. The extracting solvent in flask is heated to reflux and its vapors condense in condenser. The condensed extractant drips into the thimble containing the crude powder and extracts it by contact. When the level of liquid in chamber rises to the top of siphon tube, the liquid contents of chamber siphon into flask. This process is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated.

This type method of extraction is commonly used at the small research setting as a batch process only. Its advantage is that large amounts of sample can be extracted with a much smaller quantity of solvent because many portions of warm solvent being passed via the sample just one batch of solvent is recycled. In fact, it can maintain a relatively high extraction temperature with heat from the distillation flask and no filtration of the extract is required.

The disadvantage of this procedure is poor extraction of polar lipids. Agitation is not possible in the Soxhlet device. Besides that, a long time needed for the extraction process. The possibility of thermal decomposition of the target compounds occurred due to the extraction usually take place at the boiling point of the solvent for a long time. The solute is rinsed at the boiling temperature of the solvent which may cause damage of thermolabile (a substance readily destroyed or deactivated by heat) compounds. It also involved large volumes of solvents and exposed to the hazards of boiling solvents.

2.3.2 Maceration, Infusion, Decoction and Percolation

It is a technique that widely used in medicinal plants research. It involved soaking plant materials with a solvent and allowed to stand at room temperature for a period of minimum 3 days with frequent agitation until the soluble matter has dissolved (Handa SS et al., 2008). After 3 days, the mixture is pressed or strained by filtration after standing. Both infusion and decoction use the same principle as maceration. Both are soaked in cold or boiled water but the maceration period for infusion is shorter whereas for decoction, the sample is boiled in specified volume of water (eg. 1:4 or 1:16) for a defined time; it is then cooled and strained or filtered (Handa SS et al., 2008). Another method that shares similar fundamental principle is percolator. In this case, dried powdered samples are packed in the percolator and added with boiling water and macerated for 2 hours. The percolation process is usually done at moderate rate (6 drops /min) until the extraction is completed before evaporation to get a concentrated extracts (Rathi et al., 2004).

This technique is the easiest and simple method. However, organic waste come into an issue as large volume of solvents is used and proper management of the waste is needed. Choice of solvents and alteration in temperature enhance the extraction process, reduce the volume needed for extraction and can be introduced in the maceration technique when such alteration is not objectionable (Nn, 2015). During this method, type of solvents used play a critical role in the soaking process.

2.3.3 Ultrasound Assisted Extraction (UAE) (Sonication)

Physical and chemical properties of the materials subjected to ultrasound. Cavitation bubbles are created near the sample tissue then they break the cell wall and therefore

cell content is released and enhancing mass transport of the solvents into the plant cells (Dhanani et al., 2017).

The benefits of UAE are mainly due reduction in extraction time and solvent consumption but it use of ultrasound energy more than 20 kHz may have an effect on the active phytochemicals through the formation of free radicals and consequently undesirable changes in the molecules and here, sound sensitive bioactive compounds are disturbed (Handa SS et al., 2008).

2.3.4 Supercritical Fluid Extraction (SFE)

Cylindrical extraction vessels are used for SFE and their performance is good beyond any doubt. The actual efficiency was poor due to the occurrence of significant analyte loss (Handa SS et al., 2008).

The advantage of CO_2 as the extracting fluid are inexpensive, abundant and safe but it possesses several polarity limitations. Solvent polarity is vital during extracting polar solutes and when strong analyte-matrix interactions are present. To alleviate such polarity limitations, organic solvents are frequently added to the carbon dioxide extracting fluid. We can also use argon instead of carbon dioxide because it is inexpensive and more inert

This method of extraction possesses distinct advantages including require lower extraction temperature, which strictly avoids damage from heat and some organic solvents, no solvent residues and environmentally friendly extraction procedure.

2.3.5 Microwave-Assisted Extraction (MAE)

Microwave is electromagnetic radiations with frequencies in the range of 30–300 MHz. It create heat because of the induction of molecular motions that causes the cell wall to be ruptured and then the active substance in cell is released (Mandal et al., 2008). It is superior to soxhlet and maceration methods of extraction with regard to time expended, more effective and economical (Laokuldilok et al., 2015). Those researchers were determined the components. The total phenolic contents of MAE ranged from 181.00 to 196.36 GAEmg/g extract; while soxhlet extraction and maceration were 180.10 and 170.44 GAE mg/g extract, respectively. The optimum condition of MAE was 900watt

for 1min of exposure time as it provided higher bioactive compounds and antioxidant activity than conventional methods.

The benefits of MAE were study by Zigoneanu and his colleagues as follows: it can be completed in minutes, polar or non-polar solvents can be used and all extraction parameters can be controlled by a software-based system (Zigoneanu et al., 2008). The extraction process also shows further advantages which described by Martino and his colleagues; Zhao as well as his colleagues, including higher recoveries, better reproducibility and lower amounts of solvent consumption compared to conventional extraction (Martino et al., 2006; Zhao et al., 2018). The system is designed to operate at elevated temperature monitored by a fiber optic temperature probe.

Based on the above literature point of view, here due to the specific requirements, such method can be time consuming, require the use of large amount of organic solvent and have lower extraction efficiencies, even with the same technique of extraction, for different source of marker compounds in both different and the same plant materials, different operating conditions many study must be required. So that the method and processing conditions that employed in the extraction play a greater role in determining the quality, cost and overall the efficacy of the standardized phytopharmaceutical intermediate product. Therefore, optimization of maceration extraction parameters of crude extract yield and evaluation of antioxidant activity from turmeric rhizome was selected with various experimental conditions, such as the concentration of ethanol (at 60, 70 & 80 %), extraction temperature (at 60, 70 & 80 ^{o}C) and extraction time (at 1, 2 & 3 hour) on a crude extract for this study. Solvent type and concentration changed phenolic content and antioxidant activity, enhancing with the increase of ethanol concentration in the solution, until the concentration reached 80% (Laroze et al., 2010). At higher temperature >70 ^{o}C with longer duration, the antioxidant activity of turmeric is decrease that caused by its inability to react with peroxyl/ -OH radicals from the phenolic compound (Réblová, 2012).

3 MATERIALS AND METHODS

3.1 Chemical and Reagents

Gallic acid (99%), Folin- Ciocalteau reagent (sodium tungstate, sodium molybdate, lithtium sulfate, phosphoric acid (85%) and HCl (37%)) and sodium carbonate (99.5%) were used for total phenolic content determination. Ethanol (97%) was used for extracting the active component from the curcuma longa. Quercetin acid (99.8%), sodium acetate (99.5%), aluminum chloride (99%) was used for total flavonoid content determination. Diethyl ether used to determine fat content of the crude sample. 1.25% H_2SO_4 (98%) and 1.25% NaOH (98.8%) were used for determination of fiber content of the crude turmeric. 1% aqueous $FeC1_3$ used for testing phenol of the crude. NaOH *(ca.* 40%), 4% boric acid for determination of crude protein. 100 mg KBr pellet, in order to prepare the translucent sample disks for FTIR analysis. All chemicals and reagents used in the current study are analytical reagent grades (A range of high purity reagents suitable for a variety of analytical techniques).

3.2 Materials

For this study I used the following tools: Test tube for solution preparation of phenolic and flavonoid analysis, cylinder to measure samples. Aluminum foil laminated polythene bags to store the sample powder, Whatman filter which used to separate residue from extract crude, Rotary evaporator (Rota-vapor, RE300, UK) for concentrated the filtrate of the extract, flask for holding the sample during maceration extraction, thermometer to measure temperature, micropipette (10-100um and 100-1000um) to take sample, mass balance for measuring sample, FTIR - to identify the functional groups that are present in the compound and UV-visible spectrophotometer instrument (Perkin Elmer, Lambda 35 UV/Vis spectrophotometer) for measuring of the sample absorbance.

3.3 Preparation of Plant Materials

Curcuma longa (turmeric) rhizomes (in Amharic Erd) was collected from the field in Amhara region, Ethiopia. These rhizomes were washed in tab water and then cut into smaller pieces and left to dry at room temperature for 4 days. The dried sample was

ground to powder form using a mortar and pestle and stored at room temperature in sealed plastic bags until it was used for further analysis.

3.4 Maceration Extraction Method and Yield Determination

The extraction method was based on (Maisuthisakul *et al*., 2007). It involved soaking 10g of dried sample (w) in a stoppered container with a solvent (60, 70 and 80% of ethanol), at various temperature (60, 70 & 80 ^{O}C) and for a period of 1, 2 & 3 hours with frequent agitation until the soluble matter has dissolved. And then solvent was evaporated under rotary evaporator at 40^{o}C after filtration through Whatman with diameter 90mm. The evaporated extracts (w1) were kept in air-tight amber bottles and stored at 4^{o}C until they were analyzed. Finally, the percentage of yield of the extracts was determined according to (Ahmad, 2016) as follow:

$$\text{The percentage of yield} = \frac{\mathbf{w1}}{\mathbf{w}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.1$$

3.5 Proximate Analysis

3.5.1 Moisture Content

Moisture content was determined using the standard the Association of Official Analytical Chemists (AOAC) methods (Helruch, 1990). This was determined by loss of mass on drying method. 5 g of turmeric powder was taken and then added in to the measured crucible then placed in an oven at 105^{o}C. After 3 hrs, the sample was weighed at regular intervals of 30 minutes by transferring the crucible containing the sample to desiccator with partially closed by the lid. When the replication was proceeded, the weighed observed become constant after 4 hours, the moisture content was calculated based on Helruch, 1990 using the equation 3.2.

$$\text{Moisture (\%)} = \frac{\mathbf{w1-w2}}{\mathbf{w1}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.2$$

Where W1 is weight of the sample and crucible before drying, W2 is weight of sample and crucible after drying.

3.5.2 Ash Content

Ash content determination was done according to the standard methods, the Association of Official Analytical Chemists (AOAC) (Helruch, 1990). Briefly, 5g of sample was placed in crucible and transferred in muffle furnace at a temperature of 550^{o}c for

overnight to ensure that impurities on the surface of crucible are burned off. Then the crucible was cooled at room temperature in desiccator and its weight was recorded. Crucible was kept in desiccator for 30 min for cooling after removed from the furnace. Percent of ash was calculated according to Helruch, 1990 studied by using Equation 3.3.

$$\text{Ash (\%)} = \frac{w2-w1}{w1} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.3$$

Where W1 is weight of crucible before igniting, W2 is weight of crucible and sample after igniting at 550 ^{o}c.

3.5.3 Fat Content

The crude fat was determined using the standard the Association of Official Analytical Chemists (AOAC) methods (Helruch, 1990) that described as follow: the round bottom extraction flask was cleaned, dried in drying oven at 92^{o}C for 1h, cooled in desiccators for 30 min, and then weighed (W1). Filter paper was folded into a thimble shape. 3g of powder sample was weighed (W0) and placed into the thimble, and the thimble with sample content was placed into Soxhlet extraction chamber. About 200ml of diethyl ether (BP 34.6 ^{o}C) was added to weighed, cleaned and dried round bottom flask and fitted to the apparatus, then cooling water was switched on. After extraction was conducted for about 4 hrs, the round bottom flasks with fat extract were removed from the extraction chamber and placed in the drying oven at 70 ^{o}C for about 30min, cooled to room temperature in the desiccators. When the extraction was conducted after 4 hrs, there was no any fat that can be extracted. Moreover, after 1:20 hrs extract, there was color change, since the permitted time for the extraction was 1.20 to 4 hrs. The extraction round bottom flask with fat extract was weighed immediately after it was taken out of the desiccators (W2).

The fat content that obtained was expressed as follow based on Helruch, 1990 equation

$$\text{Crude Fat (\%)} = \frac{w2-w1}{w0} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.4$$

Where, W1 = the weight of empty round bottom flask

W2 = weight of empty round bottom flask + the dried crude fat

W0 = weight of sample

3.5.4 Fiber Content

The crude fiber content was determined using the standard the Association of Official Analytical Chemists (AOAC) methods (Helruch, 1990). Clearly, crude fiber is the insoluble residue of an acid hydrolysis followed by an alkaline. About 2.0g (w) fat free turmeric rhizome powder sample (from crude fat determination above) were transferred into 250 ml flasks and was boiled in 150ml of 1.25% H_2SO4 solution for 30min. The boiled sample was filtered and washed several times with hot water. The residue then transferred to clean and dried flask and boiled again in 150ml of 1.25%NaOH for another 30min under same condition. After boiling the mixture was filter and washed several times with hot water. Thus, obtained residue was washed with hot water and followed by ethyl alcohol (15ml) before transferring it to a crucible. The crucible with residue was then dried in an oven at 105 ^oC and cooled in a desiccator and weighed. The sample was dried at 105^oC in an oven until constant weight is achieved (W1). After cooling, incinerate the contents in a muffle furnace to burn the carbonaceous. Finally, the content was cooled and weighed (W2). The loss in weight gave the crude fiber. Percent of fiber was calculated as following based on Helruch, 1990 studied:

$$\text{Fiber (\%)} = \frac{w1-w2}{w} \times 100 \quad \ldots\ldots 3.5$$

3.5.5 Color of Powder

The color of turmeric rhizome powder was measured in L* a* b* system by colorimeter Konica Minolta (CR-400 Series, Japan). Its color was expressed in Commission Internationale de l'Eclairage's (CIELAB) (Laokuldilok et al., 2015). The parameter a* positive values are the indication of reddish colors while negative values for the greenish ones, the positive value of b* takes for yellowish colours and negative values for the bluish ones and L* is an approximate measurement of luminosity, which is the property according to which each colour can be considered as equivalent to a member of the greyscale, between black and white (Masson, 2010).

Chroma (C*) considered the quantitative attribute of colourfulness and it is used to determine the degree of difference of a hue in comparison to a grey colour with the same lightness. The higher the chroma values, the higher is the colour intensity of samples perceived by humans. Chroma was calculated blow based on (Hirata et al., 2020) formula:

$$C^* = \sqrt{(a* \text{ ^2} + b* \text{ ^2})} \quad \ldots\ldots 3.6$$

3.6 Qualitative and Quantitative Analysis of Phytochemicals

3.6.1 Preliminary Qualitative Analysis

A. Chemical test: The chemical analysis of the extract was performed by following the protocol (Banu & Cathrine, 2015; Sawant & Godghate, 2013).

Test for Flavonoids: Alkaline reagent test: Few drops of aqueous (1M) NaOH and few drops of HCL was treated with 2 ml of plant extract until yellow orange color appear. This color indicates the presence of flavonoids.

Test for Glycosides: **Sodium hydroxide reagent**: 2 ml of alcoholic extract from each sample was mixed with 5ml of water and a few drops of sodium hydroxide were added. The appearance of a yellow color confirms as an indication glycoside.

Test for Saponins: Frothing test: To 10 ml of distilled water, 1 ml of filtrate from extract was added and shaken vigorously for 10 minutes in a gradual cylinder. The formations of stable foam confirm the presence of saponins.

Test for Phenols; Ferric chloride test: To 1ml of turmeric rhizome extract, 2.0ml of distilled water followed by drops of 1% aqueous $FeC1_3$ solution were added. The formation of bluish-black indicates to confirm the existing of phenols.

Test for Alkaloids

A few drops of Wagner‟s reagent are added to few ml of extract sample along the sides of test tube. A reddish- Brown precipitate confirms the existing of alkaloids.

B. Fourier transform infrared spectrophotometer (FT-IR) Analysis

It is the most powerful tool for identifying the type of chemical bonds that present in the plant extract. The chemical bonds in the explained spectrum characterized by the wavelength of light absorbed. Dried powder of turmeric was used for FT-IR analysis. A few mg of dried extract powder was encapsulated in small amount of KBr pellet in order to prepare the translucent sample disks. The powdered sample of extract was loaded in FT-IR spectroscopy with a scan range from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}.

3.6.2 Quantitative Analysis

3.6.2.1 Protein Content Analysis

The protein content of the turmeric was determined using the standard AOAC methods (Helruch, 1990). briefly, 0.5066g of the sample introduced into the digestion flask (Kjeldahl flask). Then the sample is digested by addition of small volume (5ml) of concentrated H_2SO_4 (an oxidizing agents which digests the food), anhydrous Na_2SO_4 that speed up the reaction by raising the boiling point of H_2SO_4 and a catalyst ($CuSO_4$) to speed the reaction. Often 1g of catalyst mixture made of Na_2SO_4 with anhydrous $CuSO_4$ in the ratio of 10:1 is used. In this step, nitrogen in the food was convert (other than that which is in the form of nitrates or nitrites) into ammonium sulphate salt or ammonia and other organic matter to CO_2 and H_2O. In the acid solution, ammonia is not liberated as gas because it exists as an ammonium sulfate salt. After digestion is completed, the content in the flask is diluted by water and a concentrated NaOH *(ca.* 40%) is added to neutralize the acid and to make the solution slightly alkaline. The ammonia is then distilled into receiving flask that consist a standardized with excess boric acid (4%) for reaction with ammonia. And then directly titrated with standard acid (0.1N HCI). Percent of Nitrogen was calculated as following based on Helruch, 1990 studied:

$$\text{Nitrogen } (\%) = \frac{V*N*(ca.0.1)*14.00}{\text{sample weight based on dry basis}} \times 100 \ldots\ldots\ldots\ldots\ldots 3.7$$

Where *V* is volume of HCI in liter consumed to the end point of the titration, N is the normality of HC1 used often is about 0.1N and 14.00 is the molecular weight of nitrogen. The % of nitrogen is converted to % of protein by using appropriate conversion factors as follows based on Helruch, 1990 studied:

$$\% \text{ Protein} = \% \text{ N x F} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.8$$

Where, F is the conversion factor in most cases is 6.25. Use this factor in this experiment.

3.6.2.2 Determination of Total Flavonoids Content

The total flavonoid content (TFC) was estimated using an aluminum chloride colorimetric assay and adopted by (Samara et al., 2014). A calibration curve was developed at quercetin concentrations of (0.04856mg/ml, 0.9712mg/ml, 1.4568mg/ml,

1.9424, 2.428 mg/ml and 2.9136mg/ml) and the blank sample. Blank contains of all the reagents, except for the extract or quercetin standard solution. For flavonoid determination, Briefly, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6ml of each extract with 0.2676, 0.5352, 1.0704, 1.6056, 2.1408 and 2.676mg/ml, respectively, 0.10 mL of 10% aluminum chloride ($AlCl_3.6H_2O$), 0.10 mL of sodium acetate ($NaC_2H_3O_2.3H_2O$) (1 M) and 2.80 mL of distilled water was added and the volume was made up to 4ml by adding 97% of ethanol. After incubation for 40 min, absorbance was read at 415 nm using a spectrophotometer. All extracts were performed in triplicates. The concentration of total flavonoid content was found from the calibration plot and expressed as mg of quercetin equivalents/ g of dry mass by using the following equation based on Samara et al., 2014 studied:

$$\text{Total flavonoids content (mg QE/g)} = \frac{F*V}{m} \quad \ldots\ldots 3.9$$

Where: F = Total flavonoids content calculated from calibration curve (mg QE/ml), V = Volume of a sample taken (ml), m = Fresh weight of sample (g).

3.6.2.3 Determination of Total Phenolic Content

The total phenol content (TPC) of the turmeric extracts was estimated spectro-metrically according to the Folin-Ciocalteu method and adopted by (Kamtekar et al., 2014). A calibration curve was developed at gallic acid concentrations of (10, 20, 40, 60, 80 & 100 µg/ml). Briefly, for the determination of phenolic content, in a 20 ml test tubes, 0.02, 0.4, 0.08, 0.12, 0.16 and 0.2 ml of each extract sample with the concentration of 0.2676, 0.5352, 1.0704, 1.6056, 2.1408 and 2.676 mg/ml respectively, 1ml of 1:10 (10-fold) diluted Folin Ciocalteu's reagent and 7ml of distilled water was added, shaken, and left to rest for 5 minutes. Then 1ml of 7.5% (w/v) sodium carbonate was added into the prepared solution and the volume was made up to 10ml by adding 97% of ethanol. The mixture was left to react in a cool dry place (dark) for 2 hours at room temperature (in a water bath at 50°C for 5 min). The Folin-Ciocalteau reagent is sensitive to reducing compounds of phenols thereby producing a blue color upon reaction. The intensity of the blue-colored complex was formed and measured at 760 nm against a blank using a UV-visible spectrophotometer instrument (Perkin-Elmer UV-Vis spectrometer, Lambda 35). The measurements were done in triplicate The data for total phenolic contents was expressed as mg of Gallic acid equivalent weight

(GAE)/g of dry mass by using the following equation based on Kamtekar et al., 2014 studied:

$$\text{Total phenolic content (mg GAE/g)} = \frac{P*V}{m} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.10$$

Where: P = Total phenolic content calculated from calibration curve (mg GAE/ml), V = Volume of a sample taken (ml), m = Fresh weight of sample (g).

Reagent blank was prepared from (1 mL FCR + 1 mL 7.5% Na_2CO_3 + 7ml distilled water + 1ml ethanol) and sample solution (0.02ml crude extract + 1 mL FCR + 1 mL 7.5 % Na_2CO_3 + 7ml distilled water + 0.98ml of 97% ethanol). Standard solution (0.02ml Gallic acid + 1 mL FCR + 1 mL 7.5 % Na_2CO_3 + 7ml distilled water + 0.960 ml of 97% ethanol).

3.6.2.4 Antioxidant Activity of Bioactive Components of Turmeric Rhizome

3.6.2.4.1 DPPH Free Radical-Scavenging Activity

The antioxidant activities of turmeric extract were measured by evaluating the free radical-scavenging effect of the samples with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical using the method reported by (Pan et al., 2020). Ascorbic acid was used as standard with 0.50692mg/ml, 1.01384mg/ml, 2.02768mg/ml, 3.041252mg/ml, 4.05536mg/ml and 5.0692mg/ml concentration. Briefly, from each extract sample 0.50692mg/ml, 1.01384mg/ml, 2.02768mg/ml, 3.041252mg/ml, 4.05536mg/ml and 5.0692mg/ml concentration added 1mL of 0.1mM DPPH. Then the samples were prepared and left in a dark room for 30 minutes to change the purple color into yellowish. The reduction of the DPPH radical was then determined by measuring the absorbance of the mixture at 517 nm with UV-visible spectrophotometer instrument. The percentage of DPPH scavenging activity was calculated using the following equation using the method reported by (Pan et al., 2020):

$$\text{DPPH scavenging Activity (inhibition ratio) (\%)} = \frac{A_{DPPH} - A_S}{A_{DPPH}} \times 100 \quad \ldots\ldots\ldots\ldots 3.11$$

Where A_{DPPH} is the absorbance of DPPH at the addition of ethanol instead of sample (control solution: 1ml of 0.1mM DPPH and 1ml of ethanol) and A_S is the absorbance of DPPH in the presence of either a sample or standard. Here the blank is only 2ml of ethanol.

3.6.2.4.2 Calculation of Half Maximal Inhibitory Concentration

The efficient concentration (EC50) or half maximal inhibitory concentration (IC50) value, defined as the concentration of the sample leading to 50 % reduction of the initial DPPH concentration, was calculated from the linear regression of plots of percentage inhibition (% DPPH scavenging activity) against concentration of turmeric rhizome extracts (Aykul & Martinez-Hackert, 2016) and IC_{50} calculate in the follow:

$$IC_{50} = \frac{50-intercept}{slope} \quad \text{.....3.12}$$

3.7 Statistical Analysis

Each experiment was performed in two replicates and the data was subjected to calculations of mean ± standard deviation. The mean values were used for drawing the graphs. Data analysis for this study was carried out by DESIGN EXPERT 7.0.0 software with 20 experimental run, three factor, three level face-centered cube design and 6 replications at the center point to determine the experimental conditions that maximize the extraction yield and to evaluate the effects of the process variables such as, extraction temperature (60, 70 and 80 ^{o}C), extraction time (1, 2 and 3hr) and ethanol concentration (60, 70 and 80%). The Process Parameters were optimized Using Response Surface Methodology (RSM), by central composite design (CCD). The significance of the equation parameters for each response variable was analyzed by F-test. Only the factors with significance higher than or equal to 5% ($p \leq 0.05$) were considered. The model adequacy was checked accounting for the coefficient of determination (R^2). Finally, simultaneous optimization of the multiple responses was performed using the same software. All the independent variables were kept within range while the response (yield) was maximized.

4 RESULTS AND DISCUSSION

4.1 Physical and Chemical Analysis of Dried Turmeric

The physical and chemical qualities of dried turmeric are shown in Table 4.1. The obtained turmeric powder exhibited moisture content of 7.886% on dry weight basis implies that the turmeric rhizome is properly dried and stored. Higher moisture levels result in deterioration of quality due to microbial growth. (Hodgson, 1976) said that moisture content up to 13% was allowed for safe storage. Climatic factors play a crucial vital role in regulation of moisture content.

The value of ash found in this study was 5.359 % by weight, slightly different from the earlier record (Ahaotu & Lawal, 2019) that was 7.87 % wt. The maximum amount of ash among Curcuma species was also recorded 11.45% by weight by (Sheeja et al., 2015); minimum in C. longa (0.36 ± 0.001 %wt) by (Tejavathi et al., 2020). In this study the ash value (5.359 %wt) is also between 0.32 ± 0.02 %wt and 11.45 %wt. Ash is an important as it shows the purity of extract because it tells the presence or absence of foreign material like metallic, salts etc. The polyphenolic compounds that presented at low ash content which could positively influence the antioxidant capacity of the extraction, since ashes contain minerals and heavy metals, including iron that can act as pro-oxidants (Maisuthisakul et al., 2008). Furthermore, low ash content could be beneficial in reducing the risk of enzymatic action inhibition (Liu et al., 2012). Therefore, the low ash content is an indication of the good quality of the poly phenolic compounds. The ash value of this study lies within limit implies purity and quality of crude extract.

Table 4.1: Proximate analysis of dried turmeric

pH	Moisture (%)	Ash (%)	Fat (%)	Fiber (%)	Protein (%)	Carbohydrate (%)
7.640 ±0.11	7.886 ±0.13	5.359 ±0.21	16.590 ±0.54	15.950 ±0.73	5.552 ±0.23	48.663

(Source: Taken by author)

The fiber and fat contents of sample were, 16.59 % and 15.95 % respectively, slightly different from the earlier record (Ogbuehi, H.C., Ukaoma, 2018) that was 8.63% for

fiber and (Ahaotu & Lawal, 2019) that was 6.29% for fat. The variation comes from many factors such as water supply handling, harvesting and storage management. In general, fiber reduces the incidence of cardiovascular diseases and obesity and is effective for insulin sensitivity (Slavin, 2013) and lower body weights because the crude fiber that consists of a heterogeneous mixture of non-starch polysaccharides including cellulose, hemicellulose, pectin, β-glucans, gums, and lignin and contains frequently polyphenols associated with non-starch polysaccharides, that could be released from the vegetal matrix conferring antioxidant activity (Valdivia-l, 2005).

Higher amount of fat contents leads to diabetes, hypertension, cardiovascular disease, non-alcoholic fatty liver disease and even some forms of cancer (Bhatt & Smith, 2015). The above health issues as well as cosmetic reasons have led to the need for weight loss in society today. But in the case of this studied the fat content was low, preventing the rancidity and favoring the stability of this by-product. The pH of the sample was found to be acidic and closer to the pH range of 3–9 required for use in the pharmaceutical, cosmetic and food industries (Ragper & Coursey, 1967). The pH of the sample was 7.64 which was similar with Tejavathi et al., (2020) that was 7.6. The metal chaleting of phenol can occur at phsicological pH (5-8) (Hider et al., 2001).

The carbohydrate and crude protein contents of sample were 5.552 ±0.23 and 48.663% respectively which is similar with (Ahaotu & Lawal, 2019) studied that was 6.54±0.07% of protein and (Maniglia et al., 2015) studied that was 48.16±0.15 for carbohydrate. (Arueya et al., 2017) reported that some carbohydrate types do contribute to antioxidant activities. Polysaccharide has two mechanisms of clearing the hydroxyl radical: the first is to remove the already-generated hydroxyl radicals and the second is to inhibit the generation of hydroxyl radicals. The former mechanism is related to that hydroxyl radical can quickly capture the hydrogen atom of the C-H chain of polysaccharide and form H_2O. In addition, the acid group of polysaccharide (such as −COOH) can decrease the production of hydroxyl radicals through the inactivating Fenton reaction. The carboxyl group content has considerable effect on scavenging •OH radicals. The greater the molecular weight of polysaccharides is, the lesser the reduction and non-reducing end contents are and the weaker the antioxidant activity is (Dimitrova et al., 2015; J. Wang et al., 2016).

Polyphenols are susceptible to precipitate coupled to proteins via different mechanisms, such as hydrophobic benzenoid rings and ionic interactions, hydrogen and covalent bindings; these interactions can diminish free phenolic concentration reducing their antioxidant efficacy (Maestri et al., 2008). These implies there was no positive correlation between protein content and the ant oxidative properties in this study.

Sample size can be decreased through grinding and milling to increase contact surface area and interaction between the sample and the extraction solvent (Orsat & Routray, 2017). The size of the powder was less than 0.5mm which is the recommendable size in order to extract active component from the raw material (Figure 4.1).

Figure 4.1:: Curcuma longa rhizome powder

Figure 4.2:Protein Analysis A) Kjldahl Digestion Unit B) Kjaldahl distillation unit C) titration step

(source: taken by author)

Observation

The extraction was carried out by maceration method with ethanol solvents. The result showed a significant difference in extraction yield and time length as well as there is change in color of extract solution (from reddish brown to yellow).

Quantification of Colour

Colour measurement of food products has been used as an indirect measure of other quality attributes such as flavour and contents of pigments poly phenolic compounds

(curcumin) because it is simpler, faster and correlates well with other physicochemical properties. The most popular systems are RGB (red, green and blue).

The color of turmeric powder was expressed in CIELAB. The CIELAB coordinates (L*, a*, b*) were directly read. It was considered the CIELAB (Commission Internationale de l'Eclairage's) uniform space in which two colour coordinates, a* and b*, as well as a psychometric index of lightness, L*, were measured as 13.30, 16.21 and 48.13 respectively. Chroma (C*) was 20.968 that calculated based on the equation 3.6 from the method part.

Here the color value of turmeric powder showed a neutral or grey direction. The +a* direction showed redness and +b* movement represented a shift toward yellow, which is fit with the observation (it was reddish at the beginning and then change into yellowish colour finally) (Laokuldilok et al., 2015). It was also having higher color intensity during extraction.

4.2 Chemical Test for Phytochemical Analysis

In the present study, the chemical test analysis was conducted to evaluate the presence of major active constituents such as flavonoid, glycoside, phenols and alkaloid. The data presented in Table 4.2, the results of the phytochemical analysis indicate the presence of flavonoid, glycoside, phenols and alkaloid and the absence of saponins in ethanoic rhizome extract of turmeric.

Table 4.2: Chemical test analysis of a major phytochemical component of turmeric

Phytochemical	Test	Color observed	Turmeric
Saponins	Frothing test		-
Glycosides	Alkaline	Yellow	+
Flavonoids	Alkaline	Intense yellow	+
Phenol	Ferric chloride test	Blue-black	+
Alkaloid	Wagner's test	reddish- Brown	+

Where (+) present and (-) absent (source: taken by author)

Figure 4.3: Chemical test A) Saponins B) phenol C) flavonoid test D) Alkaloid test E) glycoside test

(source: taken by author)

The present study also supported by the study conducted by (Chauhan et al., 2017; Sawant & Godghate, 2013; Taoheed et al., 2017) in which turmeric rhizome extract confirm the presence of flavonoids, phenols, glycoside, and alkaloids. The presence of phytochemical constituents like alkaloids, flavonoids, phenols and so on in the plants are known to be responsible for much pharmacological activity on based on their study. Flavonoid compounds are hydroxylated phenolic substances that occur as a C6-C3 unit linked to an aromatic ring. Their activity is due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. The more lipophilic flavonoids also disrupt the microbial membrane. Many researchers have reported that flavonoids possess antioxidant, anticancer and anti-inflammatory. (Zahedipour et al., 2019) explained that a natural polyphenolic compound could be a potential treatment option for patients with coronavirus disease. Alkaloids responsible for antibacterial and antifungal activities, and hypoglycemic activity as stated by (Chairman K, Jayamala M, 2015).

In conclusion, the phytochemical screening is very essential in identifying new sources of therapeutically and industrially important compounds as described by (Sahle & Okbatinsae, 2017). It is imperative to initiate urgent steps for a screening of plants and vegetables for secondary metabolites.

Fourier transform infrared spectroscopy analysis of powder and ethanoic extract turmeric rhizome

The FT-IR spectrum of powder and ethanoic extract of turmeric rhizome is given in Figure 4.4. The data on the peak values and the probable functional groups (obtained by FT-IR analysis) present in both the powder and the ethanoic extract of the rhizome of turmeric plant was presented in Table 4.3 The region of infrared radiation aids to identify the functional group of the active component present in both the powder and the extracts based on the peak value of the FTIR spectrum. When the powder and the extract was passed into the spectroscopy, the functional groups of the components were separated based on its peak's ratio.

The absorbance bands analysis in the bio-reduction was observed in the region 400-4000 cm^{-1} are (major peak) 612.51, 1110.27, 1630.64, 2932.23 and 3426.91 cm^{-1} for a powder of turmeric rhizome. For ethanoic extract one, the major peak was 612.51, 1110.27, 1630.64, 2932.23 and 3444.49 cm^{-1}. Those are the major peak observed that indicating the presence of C-H (bending) deformation, C-O stretching, C=C (symmetry reduces intensity), C (triple bond) stretch, respectively, and the last peak indicate the presence of phenol (O-H (H-bonded)) for both powder and ethanoic extract.

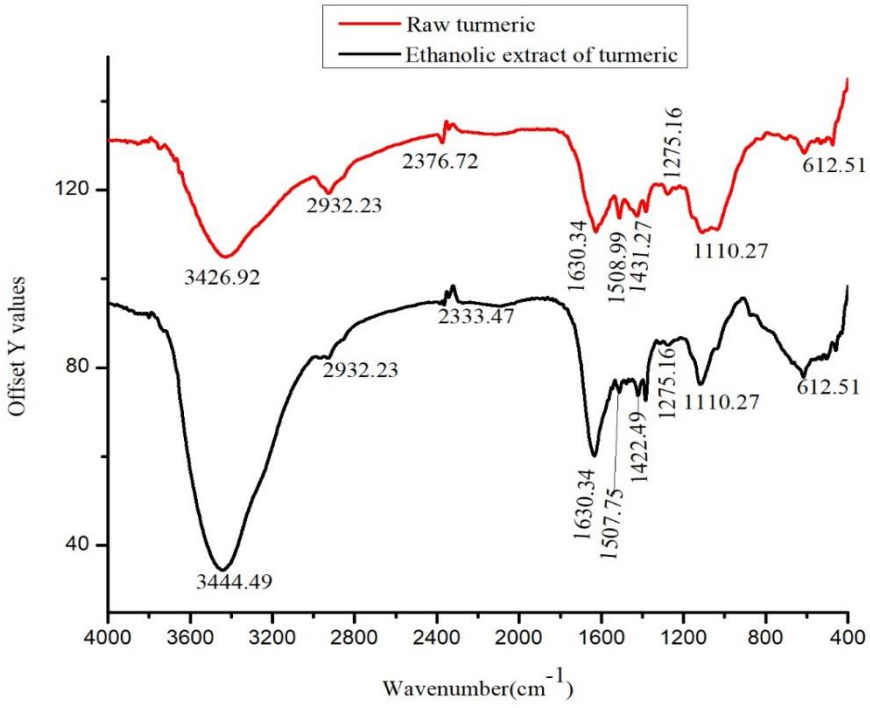


Figure 4.4: FTIR analysis for both powder and ethanoic extract of turmeric rhizome

(source: taken by author)

This FTIR was matching with the FTIR spectrum reported in literature (Harshal Ashock Pawar et al., 2017; Rohman et al., 2015). The 1628 cm^{-1} versus 1635 cm^{-1} difference differentiates C=C stretching at sites in yellow turmeric having trans-orientation of methane hydrogens from the cis-orientation in white turmeric, and the bands between 1600 and 1460 cm^{-1} are ring modes involving C-C partial double bonds of the aromatic ring (Dhakal et al., 2019). In general, the FT-IR analysis of both the powder and the ethanoic extract of turmeric rhizome revealed the presence of alkynes, alcohol (phenol), cyclic ether, aromatic ring, amine, alkene, nitrile, Carboxylic acid and derivatives and Alkanes. This result supports the presence of both flavonoids and phenols (due to the presence of hydroxylated aromatic rings) from FTIR analysis. This FTIR analysis helps to confirm the above qualitative chemical test of polyphenolic compounds in Table 4.2 and Figure 4.3. Investigation of FTIR spectra of powder turmeric and ethanoic extract turmeric clearly shows the similar peaks due to the similar chemical components contained. However, using detail scrutiny, there is a bit difference in terms of peak intensity (absorbance) or % of transmittance caused by different concentrations of components present in powder turmeric and ethanoic extract turmeric. The lower % of transmittance, the higher the intensity peak (absorbance). This indicated that the presence of more specified bond number in the sample due to extraction. The main components for both powder and ethanolic extract of C. longa is Curcuminoids which refer to phenolic compounds responsible for yellow color in turmeric. As consequence, there is no surprising if peaks coming from functional groups of curcuminoids dominate FTIR spectra of powder and ethanolic extract of turmeric. The functional groups responsible for infrared absorption of ethanolic extract of C. longa was compiled in Table 4.3.

Table 4.3: FT-IR spectral peak value (Stretching Vibrations), bond and functional groups obtained for both powder and an ethanoic extract of turmeric rhizome

Raw (powder) of turmeric			Ethanoic extract		
Peak valves cm^{-1}	Bond	Functional group	Peak valves cm^{-1}	Bond	Functional group
3426.92	O-H stretching	phenol	3444.49	O-H stretching	phenol
k2932.23	O-H (very broad) C-H stretching	Carboxylic acid and derivatives Alkanes	2932.23	O-H (very broad) C-H stretching	Carboxylic acid and derivatives Alkanes
2376.72	C (triple bond) N stretch	Alkynes Nitrile	2333.47	C (triple bond) N stretch	Alkynes Nitrile
1k630.64	C=C (symmetry reduces intensity)	Alkene	1630.64	C=C (symmetry reduces intensity)	Alkene
1508.99	Benzene ring bending vibration (Aromatic ring)		1507.75	Benzene ring bending vibration (Aromatic ring)	
1431.27	C-N CH_2 bending	amine	1422.49 CH_2 bending	C-N	amine
Peak valves cm^{-1}	Bond	Functional group	Peak valves cm^{-1}	Bond	Functional group
1275.16	C-O stretching	Cyclic ether	1275.16	C-O stretching	Cyclic ether
1110.27	C-O stretching	Cyclic ether	1110.27	C-O stretching	Cyclic ether
612.51	C-H (bending) deformation	Alkynes	612.51	C-H (bending) deformation	Alkynes

(source: taken by author)

4.3 Extraction yield

The data presented in Table 4.1A from Appendix 4.1 show the percentage yield obtained by the maceration extraction process under the present study. These results were inputs to the DESIGN EXPERT® software version 7.0. The percentage yield was determined using the equation (3.1) described in the methodology part. Results revealed in table 4.5 that the amount of yield obtained for ethanoic extract of turmeric rhizome was 33.30 ± 0.76% that have a better yield as compared to the yield described by (Sawant & Godghate, 2013) which were 15.42%, 15.50%, 16.10%, and 25.75 %, methanol, chloroform, acetone and ethanol solvents, respectively. This variation came due to the extraction time, type of solvent used, mass liquor ratio and the demographic area in which the plant material harvested.

4.4 Statistical Analysis of the Experimental Results

4.4.1 Analysis of Variance (ANOVA)

The significance of the equation parameters for response variable (extraction yield) was analyzed by F-test. Only the factors with significance higher than or equal to 5% ($p \leq 0.05$) were considered. In Table 4.4, the F-value of this study model was 112.75 which implies the model is significant. There is only a 0.01% chance that a model F-value this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, AC, BC, B^2 and C^2 are significant model terms. The "Lack of Fit F-value" of 1.30 implies the Lack of Fit is not significant relative to the pure error. There is a 38.97% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good we want the model to fit.

Table 4.4: Analysis of variance (ANOVA) for Response Surface Quadratic Model showed the effect of extraction conditions on yield

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob >F	
Model	585.84	9	65.09	112.75	< 0.0001	Significant
A-solvent concentration	22.29	1	22.29	38.61	< 0.0001	
B-time	74.69	1	74.69	129.38	< 0.0001	
C- temperature	28.76	1	28.76	49.82	< 0.0001	
AB	7.16	1	7.16	12.41	0.0055	
AC	3.34	1	3.34	5.79	0.0370	
BC	7.66	1	7.66	13.27	0.0045	
A^2	0.87	1	0.87	1.50	0.2486	
B^2	25.77	1	25.77	44.64	< 0.0001	
C^2	128.90	1	128.90	223.27	< 0.0001	
Residual	5.77	10	0.58			
Lack of Fit	3.26	5	0.65	1.30	0.3897	not significant
Pure Error	2.51	5	0.50			
Cor Total	591.61	19				

4.4.2 Adequacy Check for the Developed Response Surface Quadratic Models

The model adequacy (model's goodness of fit) was checked accounting for the coefficient of determination (R^2).

Table 4.5: Model adequacy measures

Std. Dev.	0.76	R-Squared	0.9902
Mean	33.30	Adj R-Squared	0.9815
C.V. %	2.28	Pred R-Squared	0.9190
PRESS	47.91	Adeq Precision	31.470

The regression coefficient (R^2) quantitatively evaluates the correlation between the experimental data and the predicted responses. As shown in Table 4.5, Results of R^2 = 0.9902 and Adj- R^2 = 0.9815 obtained explains that the predicted values were found to be in good agreement with experimental values. Since the R^2 value is closer to 1.0 it indicates that the regression line perfectly fits the data. Similar to that in this investigation, R^2 obtained was 0.9902, which was close to 1. Results imply that the predicted values were found to be in good agreement with experimental values (R^2 = 0.9902 and Adj- R^2 = 0.9815), indicating the achievement of the RSM. In this case, the value the regression coefficient from Table 4.5 indicated that only 0.98 % of the total variance was not explained by the developed regression model. The obtained R^2 values suggest good adjustments to the experimental results. The adjusted determination coefficient (Adj-R^2) was also satisfactory for confirming the significance of the model. Pred R-Squared indicating that the model will probably explain a high percentage (about 91.90 %) of the variability in new data. To accept the model, the difference between the "Adj R-Squared" and Pred R-Squared" less than 0.2. here the "Pred R-Squared" of 0.9190 is in reasonable agreement with the "Adj R-Squared" of 0.9815. “Adeq precision” is a measure of the range in predicted response relative to its associated error in other word it measures the signal to noise ratio. It could be used to navigate the design space or decide whether the model can be used or not. A ratio greater than 4 is desirable. In this study 31.470 indicates an adequate signal.

The coefficient of variation (CV) indicates the degree of precision with which the experiments are compared. Generally, the higher the value of the CV is, the lower the reliability of the experiment. Here a lower value of CV (2.28) indicates greater reliability of the experiments performed. PRESS is the Predicted Residual Sum of

Squares for the model which is a measure of how well a particular model fits each point in the design which is 47.91. Model adequacy measures for yield shown in Table 4.5.

As shown in figure 4.5 below, the normal probability plot indicates the residuals following by the normal % probability distribution, in the case of this experimental data the points in the plots shows fitted to the straight line in the figure, this shows that the quadratic polynomial model satisfies the assumptions analysis of variance (ANOVA) i.e. the error distribution is approximately normal. If the model is correct and the assumptions are satisfied, the points in the plots shows fitted to the straight line in normal probability plot of residuals. Here the plot is almost fitted (figure 4.5). The plot of actual and predicated value also in the straight line which is the points fitted (figure 4.6).

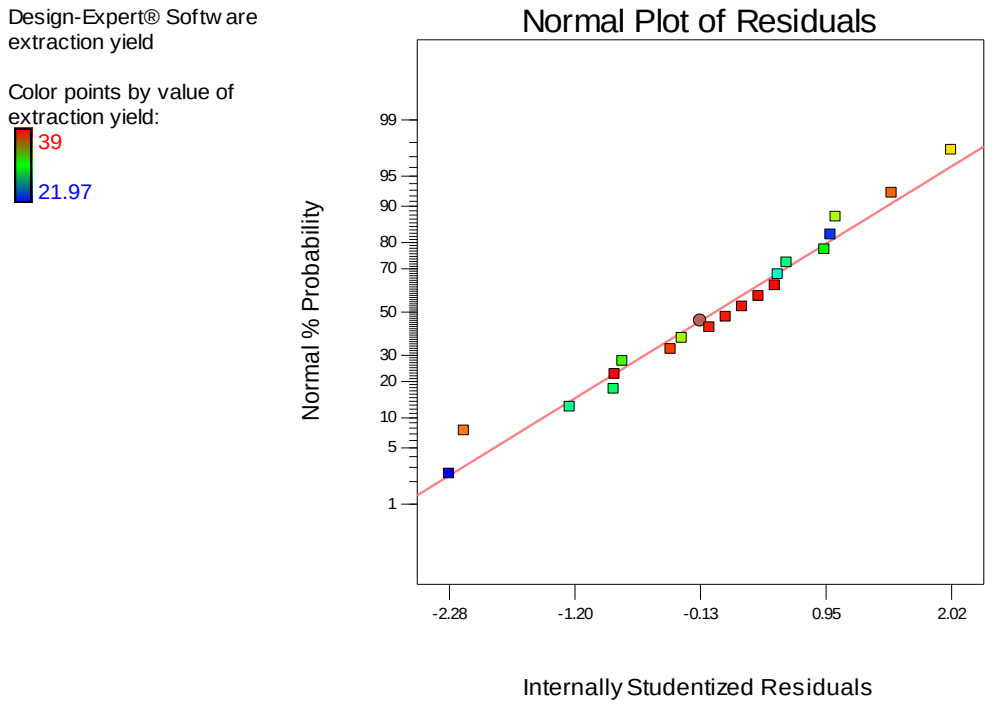


Figure 4.5: Normal probability plot of residuals

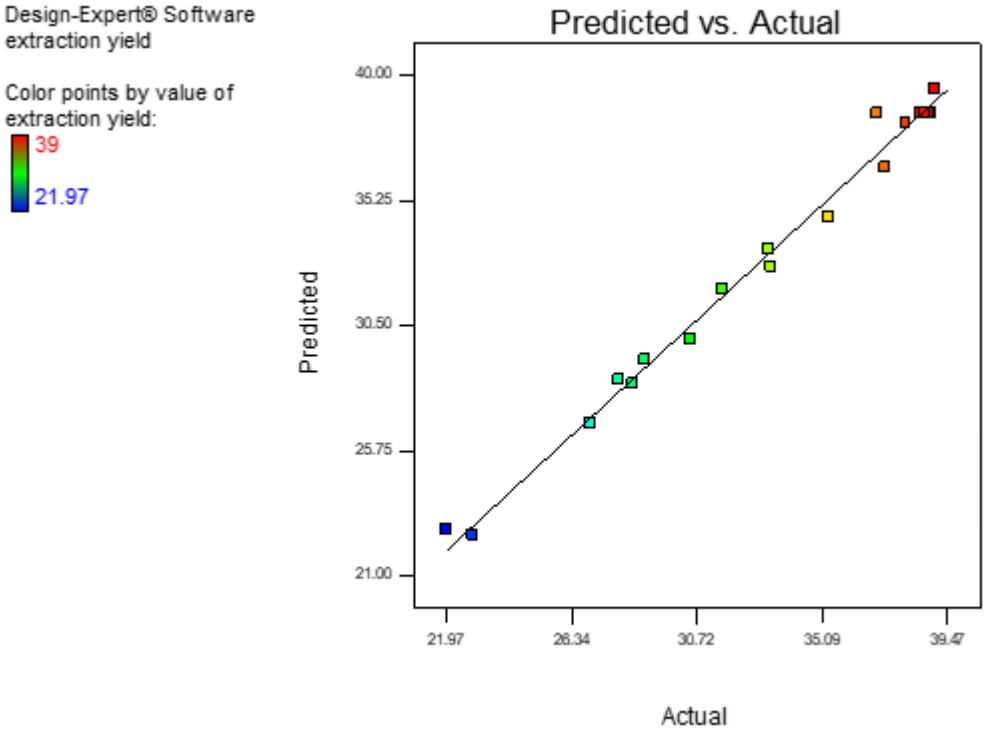


Figure 4.6: Predicted and actual value

4.5 Individual Effect of Experimental Variables on the Yield

4.5.1 Effect of Solvent Concentration

From Table 4.4 all the independent variables had a significant effect ($p < 0.05$), Prob > F is less than 0.0001, on all the responses which indicated that all extraction parameters employed in this study were important factors in the optimization process. All factors also have the positive effect on the extraction yield as shown in Table 4.6. The concentration of ethanol could influence the polarity of solvent, which was critical for the solubility of phenolic compounds (Orsat & Routray, 2017). In this part, the effect of different concentrations of ethanol on crude yield was analyzed, and other conditions remained constant as follows: 2hr and 70^{o}c. As shown below Figure 4.7, concentration varied from 60 to 80%. As the solvent concentration is increasing with that of increasing extraction yield up to 80%. But it requires further study at what solvent concentration maximum extraction yield obtained.

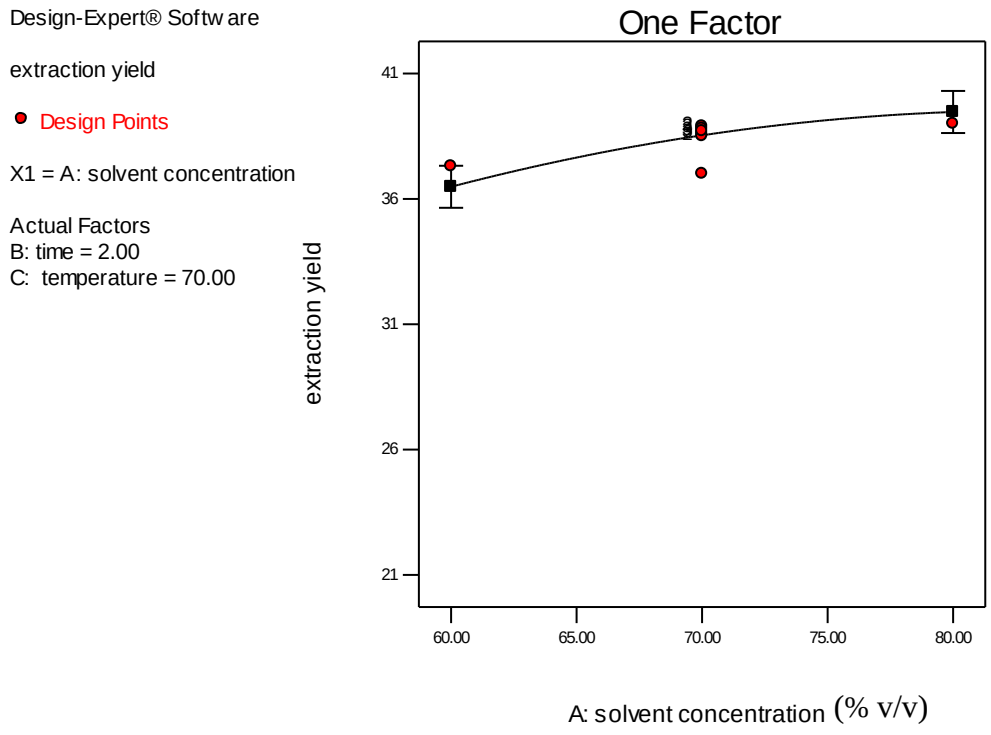


Figure 4.7: The effect of solvent concentration on yield@ constant extraction temperature (70 ^{o}C) and time (2hr)

4.5.2 Effect of Extraction Time

The extraction time had the most positive effect on the extraction yield (Table 4.6). As shown in Figure 4.8, as extraction time is increasing, at constant temperature (70^{o}c) and solvent concentration (70%), the yield is also sharply increasing. The maximum extraction yield obtained around 2hr (Figure 4.8 and Table 4.1A). A longer extraction time generally led to a higher percentage yield of extract. This could have been due to the longer amount of time the solute and solvent were in contact with each other. Longer contact time favored the system to have more mass transfer. However, excessive extraction time would be unnecessary as the solvent and sample would be in final equilibrium after certain duration. This is based on Fick's second law of diffusion by then; the rate of extraction of compounds would decelerate (Teixeira *et al*., 2014). Fick's 2nd law of diffusion describes the rate of accumulation (or depletion) of concentration within the volume / time as proportional to the local curvature of the concentration gradient (Sandberg, 1996).

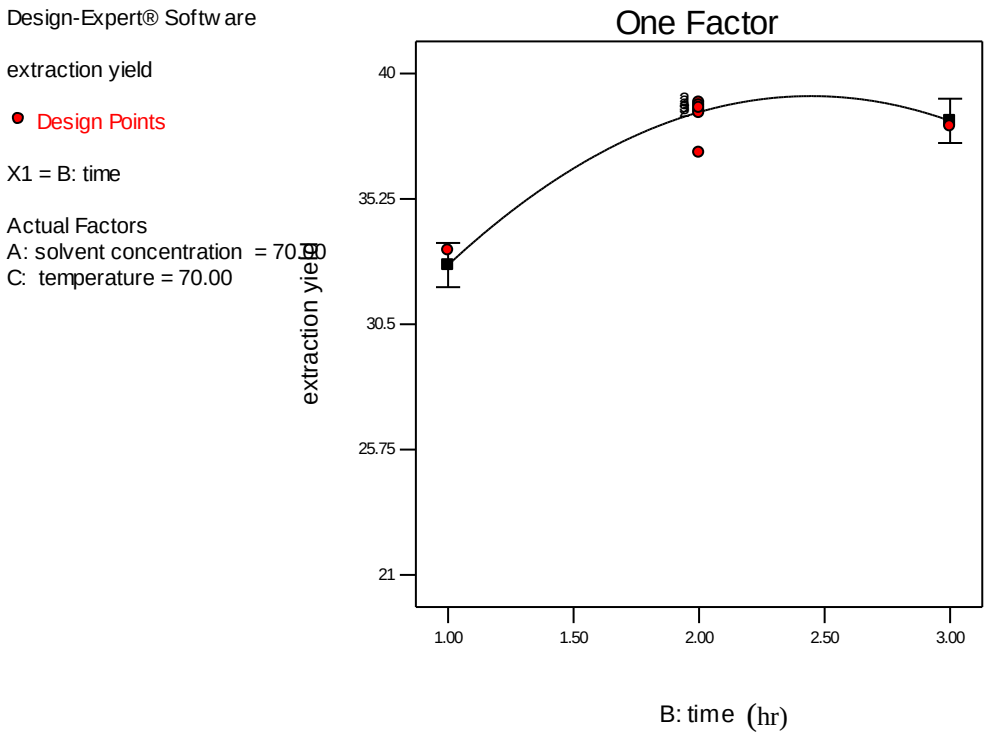


Figure 4.8: The effect of extraction time on yield@ constant solvent concentration (70%) and extraction temperature (70^{o}C)

4.5.3 Effect of Extraction Temperature

Here the positive effect of extraction temperature on the yield at constant ethanol concentration (70%) and extraction time (2hr) was shown in as shown in Table 4.6 and in Figure 4.9. The yield was highly increased as temperature increased from 60 ^{o}C to 70 ^{o}C due to cell bursting. This is occurring because of a sudden temperature increase inside cellular structures followed by leaching out of active compounds, thus leads to faster and efficient extraction. Maximum yield was obtained around 72.5^{o}C. When the temperature was increased from 72.5^{o}C to 80 ^{o}C the yield was decreased due to vaporization of ethanol. This higher temperature also induces oxidative condensation or decomposition of thermo-labile compounds like catechin. This decomposition is due to destruction of polyphenols or their conversion into non-antioxidant forms (Laokuldilok et al., 2015) and then the yield is decreasing.

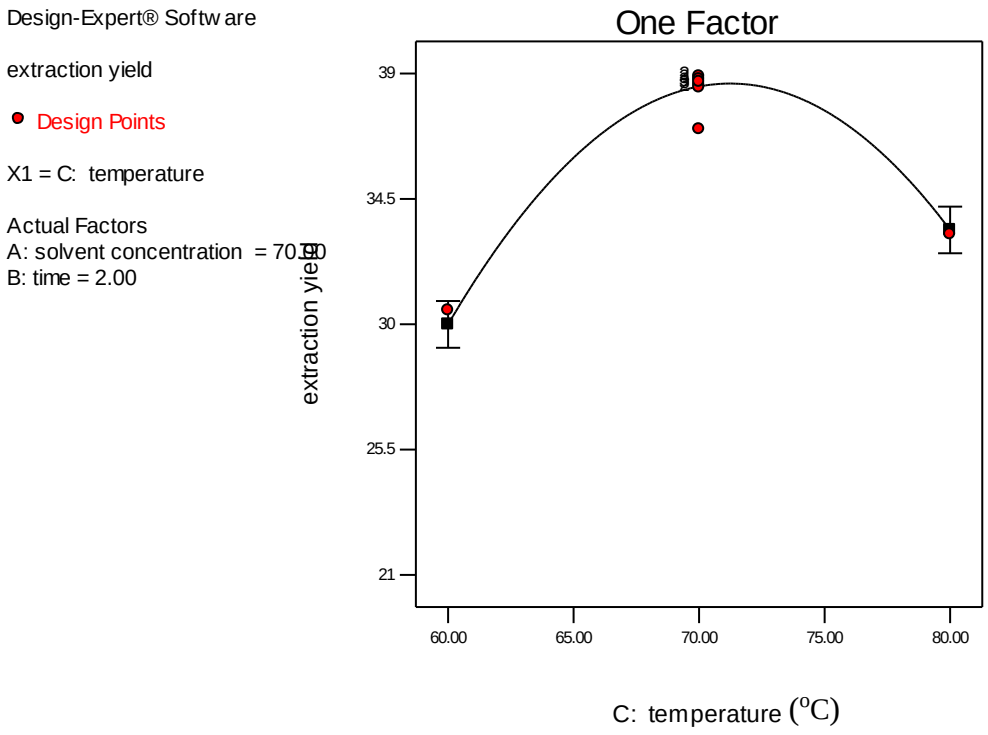


Figure 4.9: The effect of extraction temperature on yield@ constant solvent concentration (70%) and time (2hr)

4.6 Interaction Effect between Process Variables

In order to analyze the regression equation of the model, three-dimensional surface and 2D contour plots were obtained by plotting the response (yield of extraction) on the Z axis against any three variables while keeping the other variable at the center. These plots are created to analyze the change in the response surface. The response optimized value on extraction yield was based on the three process variables described on the response surface plot. The response surface plots of the quadratic model are shown in Figure (4.10, 4.13 and 4.16). It was reported that Response surface plots provide a method to predict the yield of extraction for different parameter values of the tested variables and the interaction plots help in identification of the type of interactions between these variables. There are three interaction factors analyzed by the model equation. This are: - AB (solvent concentration and extraction time), AC (solvent concentration and extraction temperature) and BC (extraction time and extraction temperature). Among these three interaction factors, interaction of extraction time and extraction temperature (BC) is the most significant interaction factor for yield as a response, because it is the highest interaction coefficient (0.98) of the rest. The sign of

the coefficient of the interaction factor indicates the effect of interaction factor on yield. Therefore, the interaction factors with positive signs have positive effect on yield. Whereas, interaction factors with negative signs have a negative effect on yield. The AB and AC interaction effect had positive on the extraction yield but BC had negative interaction effect based on analysis of variance (statically) Table 4.6.

4.6.1 Interaction Effect of Solvent Concentration and Extraction Time on the Yield

Figure 4.10 shows the response surface plots developed as a function of extraction time and solvent concentration while extraction temperature was kept constant at 70^{o}c. Upon increasing the extraction time from 1 to 2hr with an increase of ethanol concentration from 60 to 80, the yield of increased highly. Beyond this time, the yield of was gradually decreased. The highest yield was obtained at 80% ethanol and 2hr. The decrement of yield with increasing of time from 2 - 3hr and this was due to the oxidation of the extracted active components since the active components that was extracted at the lower time was oxidized with in the prolonged time.

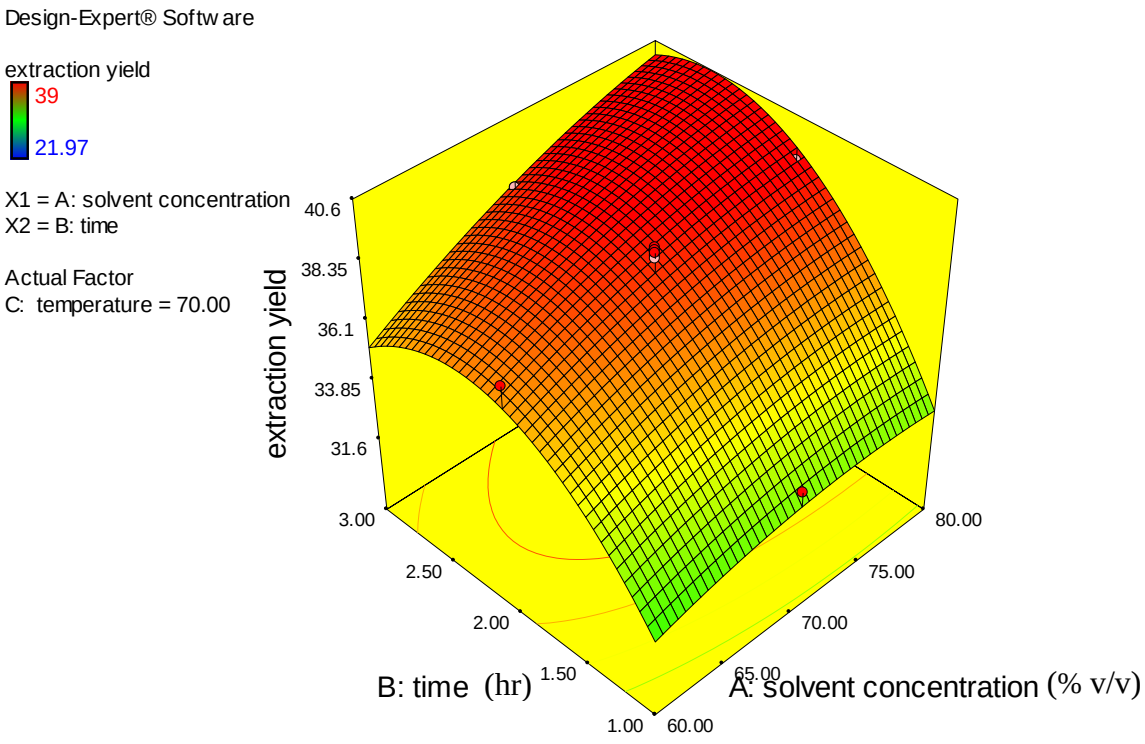


Figure 4.10: Response surface plots of the effect of solvent concentration and extraction time on the yield

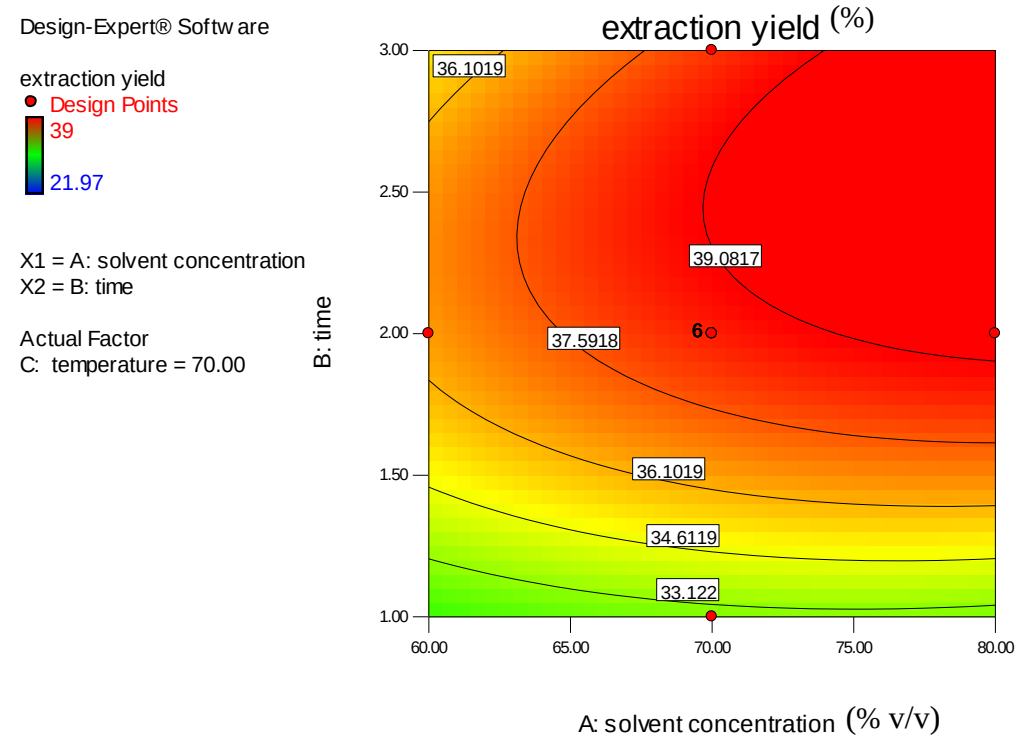


Figure 4.11: 2D contour plots of the effect of solvent concentration and extraction time on the yield

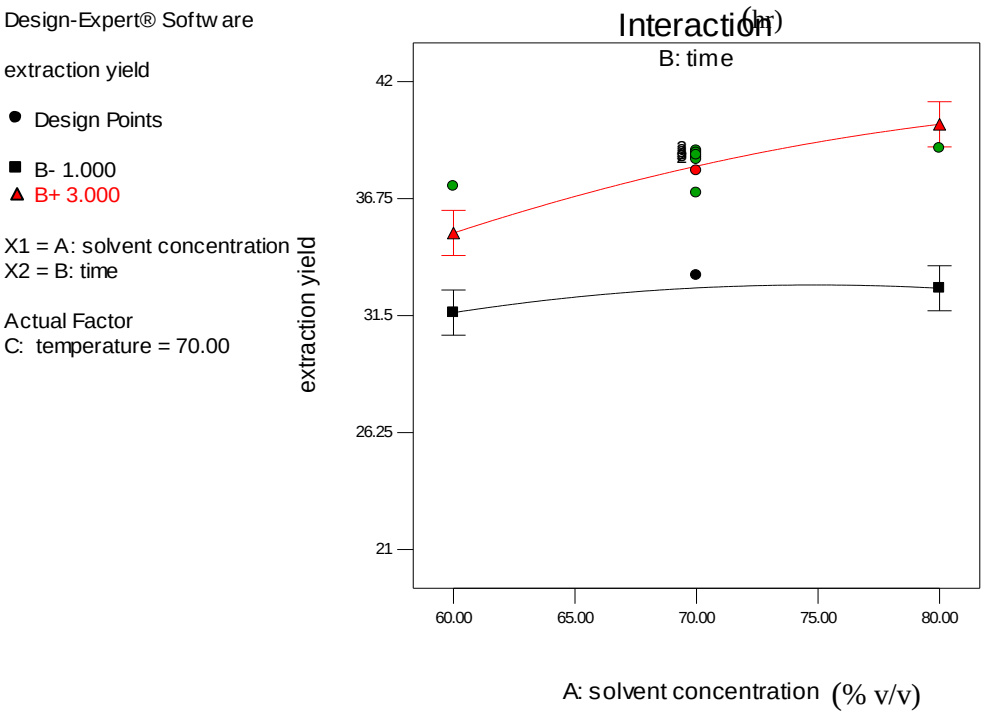


Figure 4.12: Interaction effect of solvent concentration and extraction time on the yield

4.6.2 Interaction Effect of Solvent Concentration and Extraction Temperature on the Yield

Figure 4.13 represents the effects of ethanol concentration with temperature and their mutual interaction on the extraction yield at constant extraction time (2hr). The increase in concentration and temperature enhanced the production of yield initially. With increasing ethanol concentration up to (80%) the yield had increased from 21.97 to 39%. However, as the temperature was further increased decreased the yield considerably this is due to the degradation of polyphenol.

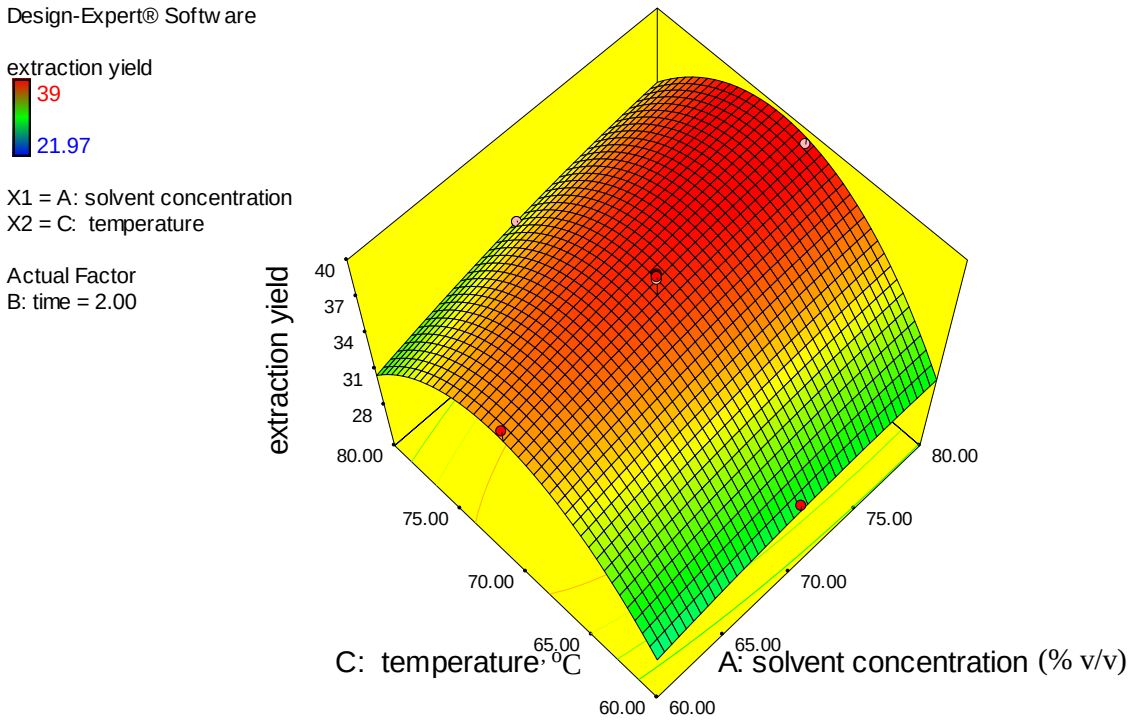


Figure 4.13: Response surface plots of the effect of extraction solvent concentration and temperature on the yield

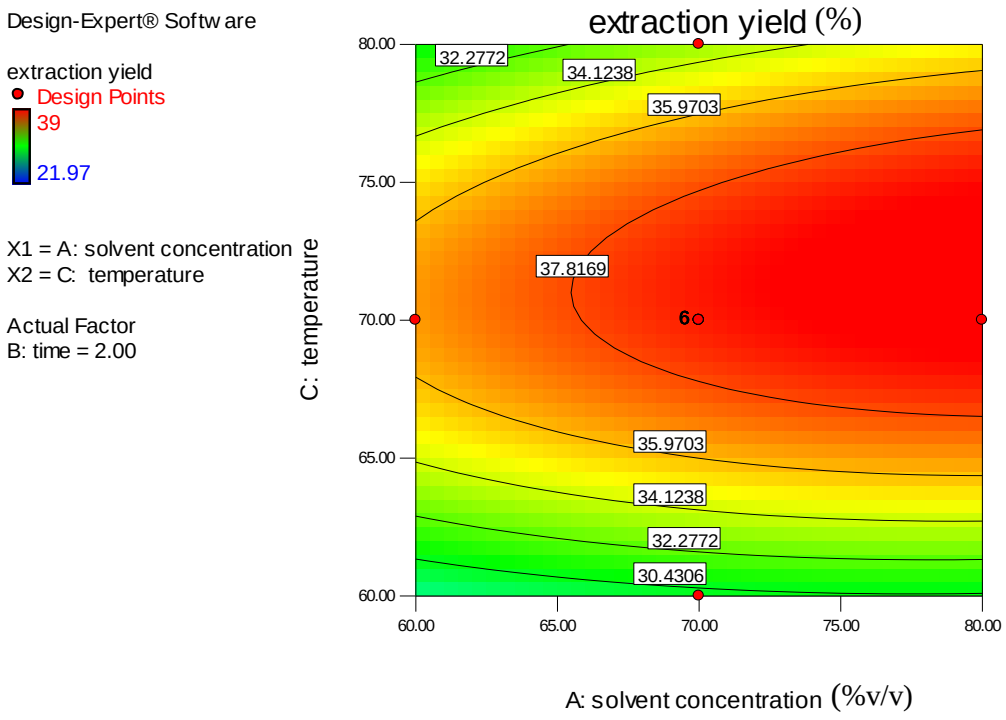


Figure 4.14: 2D contour plots of the effect of solvent concentration and extraction temperature on the yield

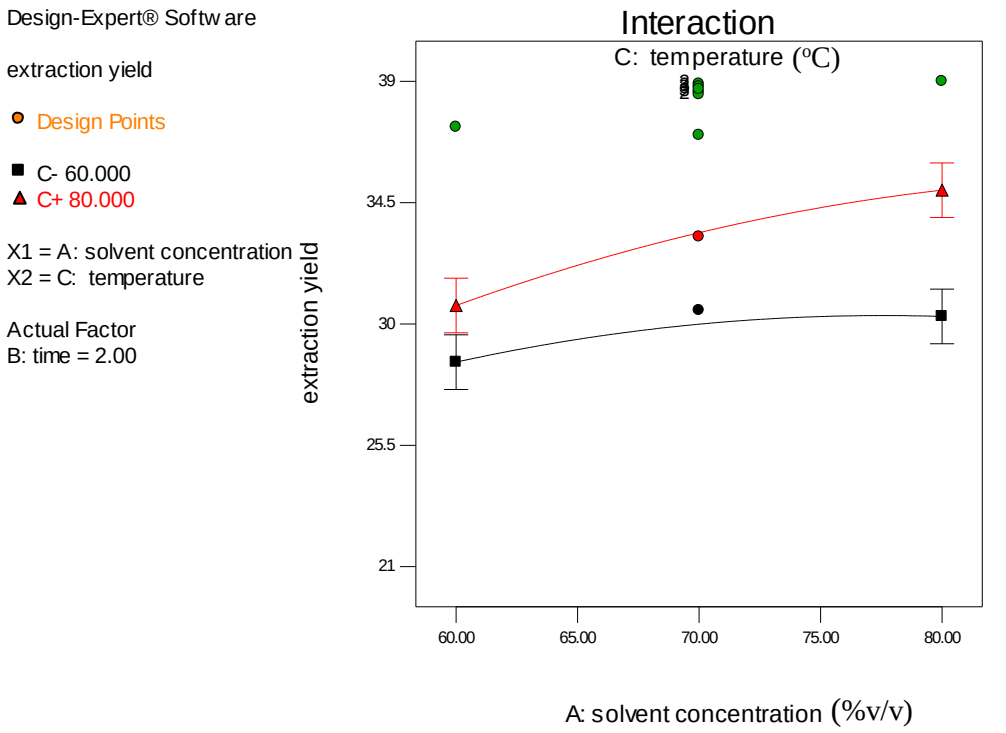


Figure 4.15: Effect of interaction of ethanol concentration and temperature on the yield

4.6.3 Interaction Effect of Extraction Time and Extraction Temperature on the Yield

As we observed from table 4.6 there was a positive interaction (+ 0.95) between extraction time and temperature on the yield at constant solvent concentration at 70%. Its viscosity and surface tension increases at higher temperatures, leading to significantly increased diffusivity of solvent into biological matrix, subsequently improving the retrieval of targeted analytes (crude yield) (Orsat & Routray, 2017). The maximum extraction yield for this study was observed both at moderate extraction time and extraction temperature value in figure 4.16. Too much extraction time and higher temperature also cause ethanol vaporization and solubility of solute this causes difficulties in extraction.

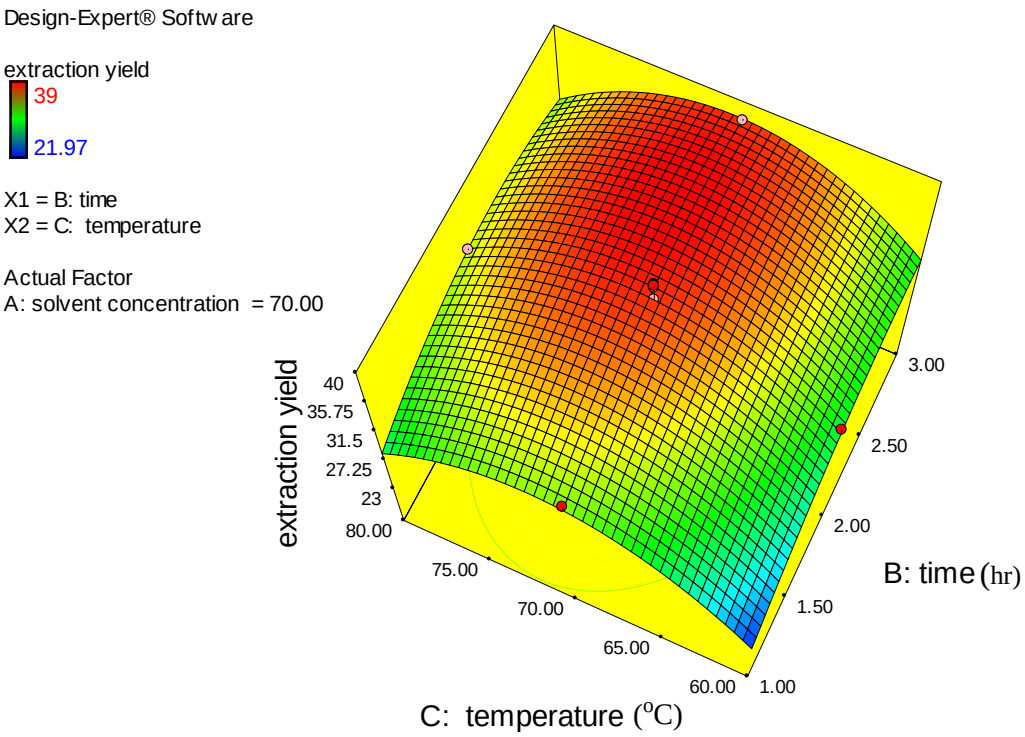


Figure 4.16: Response surface plots of the effect of extraction time and temperature on the yield

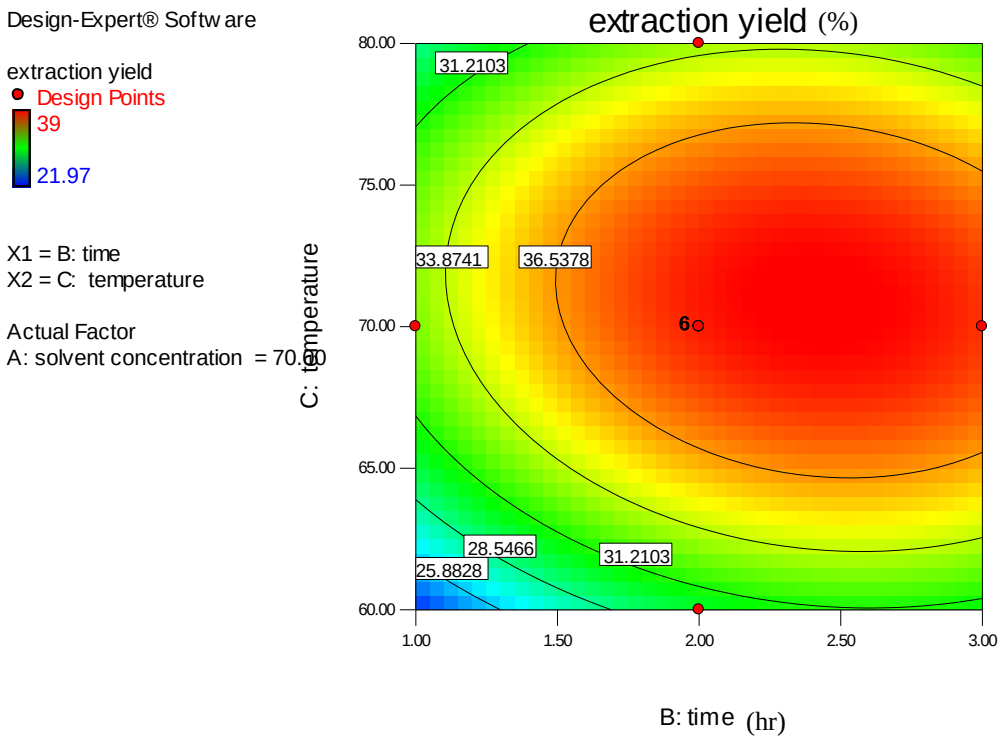


Figure 4.17: 2D contour plots of the effect of extraction time and extraction temperature on the yield @ constant concentration (70%)

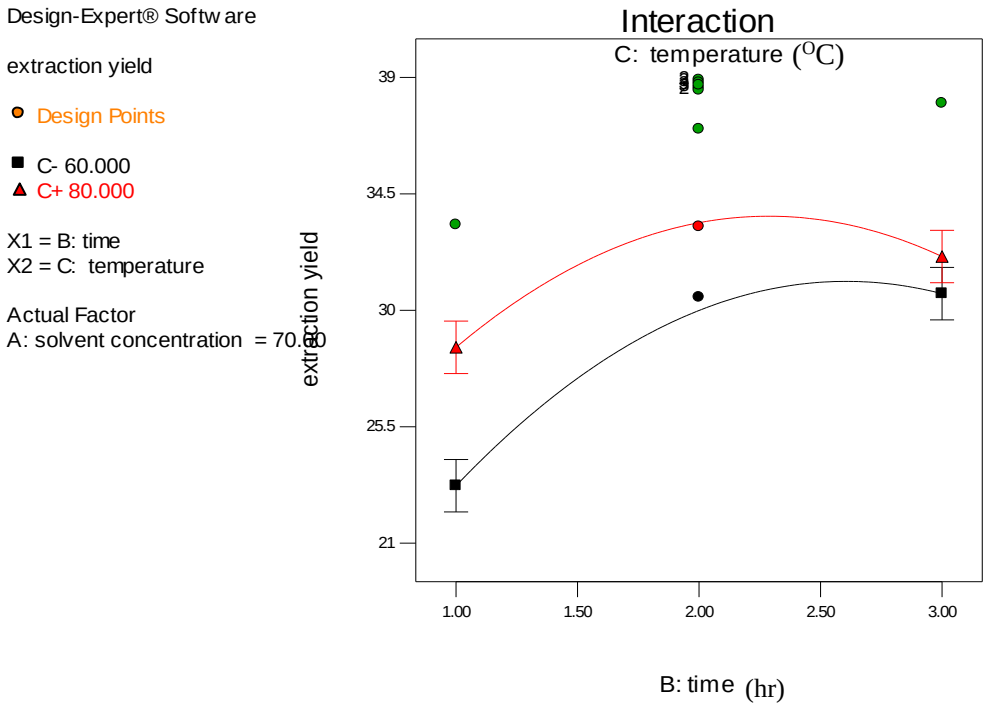


Figure 4.18: Effect of interaction of extraction temperature and time on the yield

Final Equation in Terms of Coded Factors:

Extraction yield = +38.53 + 1.49A + 2.73B + 1.70C + 0.95 AB + 0.65AC – 0.98BC – 0.56A^2 – 3.06B^2 – 6.85C^2……4.2

From Table 4.6, the final equation in terms of coded factors indicates the approximated predicted estimation in each parameter. It useful for identifying the relative impact of the factors by comparing factors coefficient while the final equation in terms of actual factors the levels should be specified in the original unit. This equation should not be used to determine the relative impact of each factor because the coefficient is scaled and the intercept is not at the center of the design.

Final Equation in Terms of Actual Factors

Extraction yield = −333.2670 + 0.29358 × ethanol $Concentration$ + 15.20595× $Time$ + 9.49788× $Temprature$ +0.094625 ethanol $Concentration$ × $Time$ +6.46250 × 10^3 × ethanol $Concentration$ × $Temprature$ − 0.097875 × $Temprature$ × $Time$ − 5.61364× 10^3 × ethanol $Concentration^2$ − 3.06136× $Time^2$ - 0.068464× $Temprature^2$ ……4.3

Table 4.6: Regression coefficients and the corresponding 95% CI High and Low

Factor	Coefficient Estimate	Df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	38.53	1	0.26	37.95	39.12	
A-solvent concentration	1.49	1	0.24	0.96	2.03	1.00
B-time	2.73	1	0.24	2.20	3.27	1.00
C- temperature	1.70	1	0.24	1.16	2.23	1.00
AB	0.95	1	0.27	0.35	1.54	1.00
AC	0.65	1	0.27	0.048	1.24	1.00
BC	-0.98	1	0.27	-1.58	-0.38	1.00
A^2	-0.56	1	0.46	-1.58	0.46	1.82
B^2	-3.06	1	0.46	-4.08	-2.04	1.82
C^2	-6.85	1	0.46	-7.87	-5.83	1.82

4.7 Optimization of Operating Process Variables Using RSM

Response surface methodology (RSM) is a systematic design for process development and optimization. The optimization was carried out using Design Expert 7.0, RSM, to obtain the criteria for maximum extraction yield. By applying a desirability function method, a total of 30 solutions were found to satisfy the goal. The predicted optimized condition with higher desirability (1) is shown in Appendix 4.1, Table 4.1B.

Table 4.7: Goal of optimization and limits of process parameters

Name	Goal	Lower Limit	Upper Limit
Solvent concentration (%)	In range	60	80
Extraction time (hr)	In range	1	3
Temperature(^{o}C)	In range	60	80
Yield(%)	maximize	21.97	39

The optimum possible solutions were presented in Table 4.1B from Appendix 4.1 and in Figure 4.20 - 25 below in the form of response surface plot. Based on desirability analysis a total of optimum points via numerical optimization generated from this the more preferred one is selected based on the operating cost and the product quantity as well as quality. Desirability function was used to identify the optimum levels of factors and to get maximum desirable responses and the optimized combinations of process variables was selected among the solutions generated with maximum combined desirability value i.e. 1 from shown in Table 4.1B from Appendix 4.1. In Figure 4.19, the predicted optimum yield (40.2408%) was observed at 78.87% of ethanol concentration, 2.32hr and 69.95 ^{o}C that have a better yield as compared to the yield described by (Hamed et al., 2019) which were 32.401 ± 1.2, 31.85 ± 1.4 and 31.67 ± 1.2 with chloroform, ethyl acetate and butanol solvents respectively. Determination of optimum yield of plant extract helps one to estimate the number of plant materials required to generate a required amount of active compound.

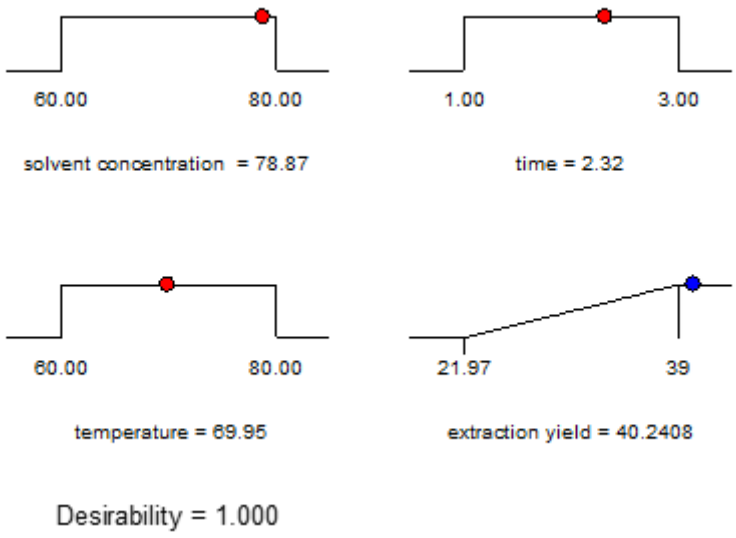


Figure 4.19: Ramps solutions

From Figure 4.20 – 4.25 shown that the 3D surface plot, contour plot and interaction effect of factors at the optimum operation condition (78.87% of ethanol concentration, 2.32hr and 69.95 ^{o}C) with predicted value. Figure 4.20 and 4.21 showed the effect of solvent concentration (A) and extraction time (B) on the yield of extract. Increase of solvent concentration from 60 to 80 % v/v and extraction time from 1 to 2.32 hr improve the extraction yield at extraction temperature was constant (69.95^{o}C). The optimum prediction (40.2408%) was obtained at 78.87% v/v of concentration and 2.32hr. However, when the extraction time over 2.32hr, did not show any obvious effect on extraction yield. The possible explanation could be that, increasing extraction time accelerate chemical degradation of bioactive compound in extraction process, which resulted in the lower extraction yield.

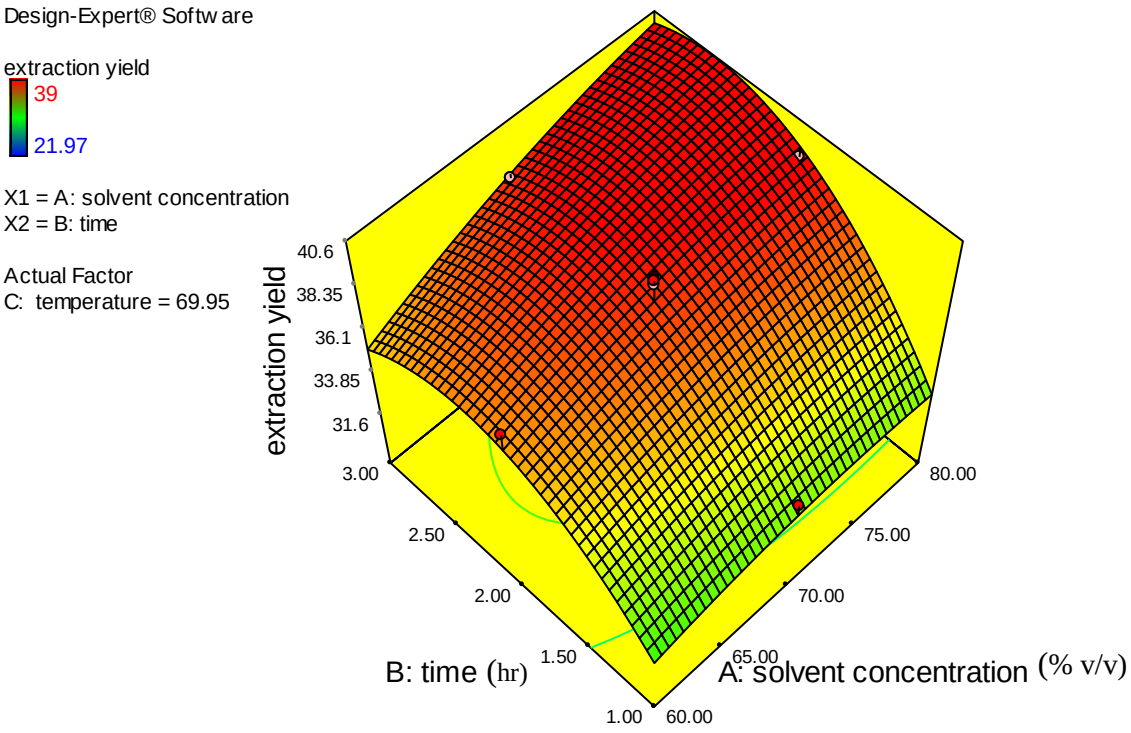


Figure 4.20: Response surfaces of predicted yield at fixed extraction temperature (69.95°c)

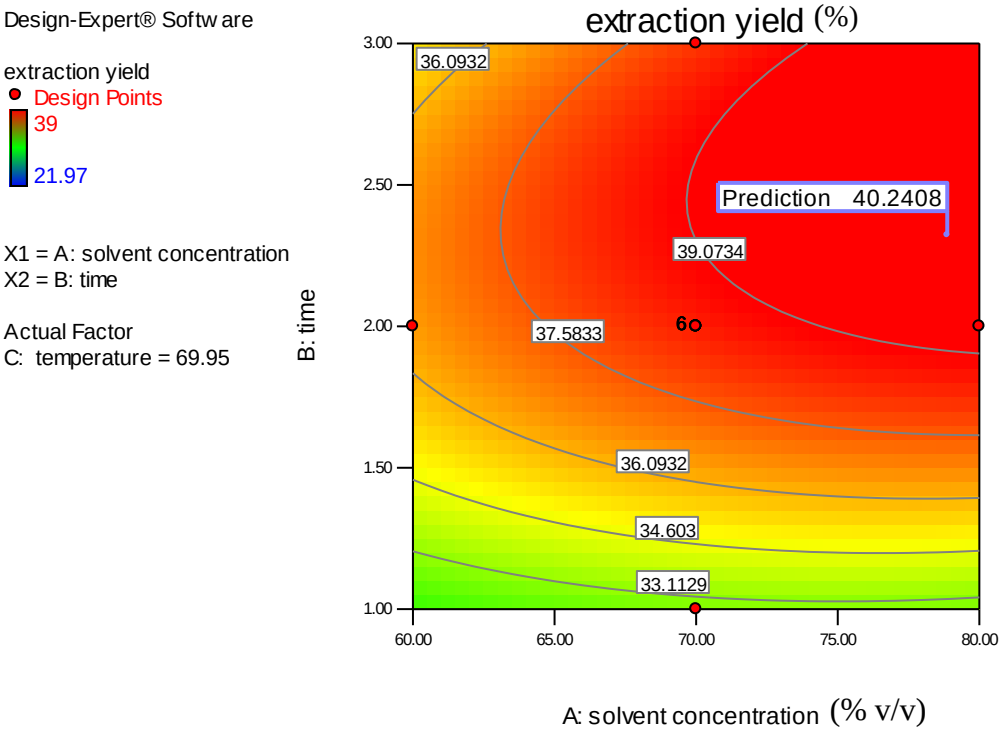


Figure 4.21**:** 2D contour plots of the effect of solvent concentration and extraction time on the yield @ constant extraction temperature (69.95°c)

Figure 4.22 and 4.23 showed the effect of solvent concentration (A) and extraction temperature (C) on extraction yield. It may be also observed that when extraction time(C) was fixed, extraction temperature (B) displayed a significant effect on the response yield. Varying solvent concentration from 60 to 80 %v/v with the increase of extraction temperature from 60 to 80℃, the target compounds' extraction yield was increasing up to 69.95℃ with the increase of solvent concentration, but extraction temperature beyond 69.95℃ extraction yield was decreasing.

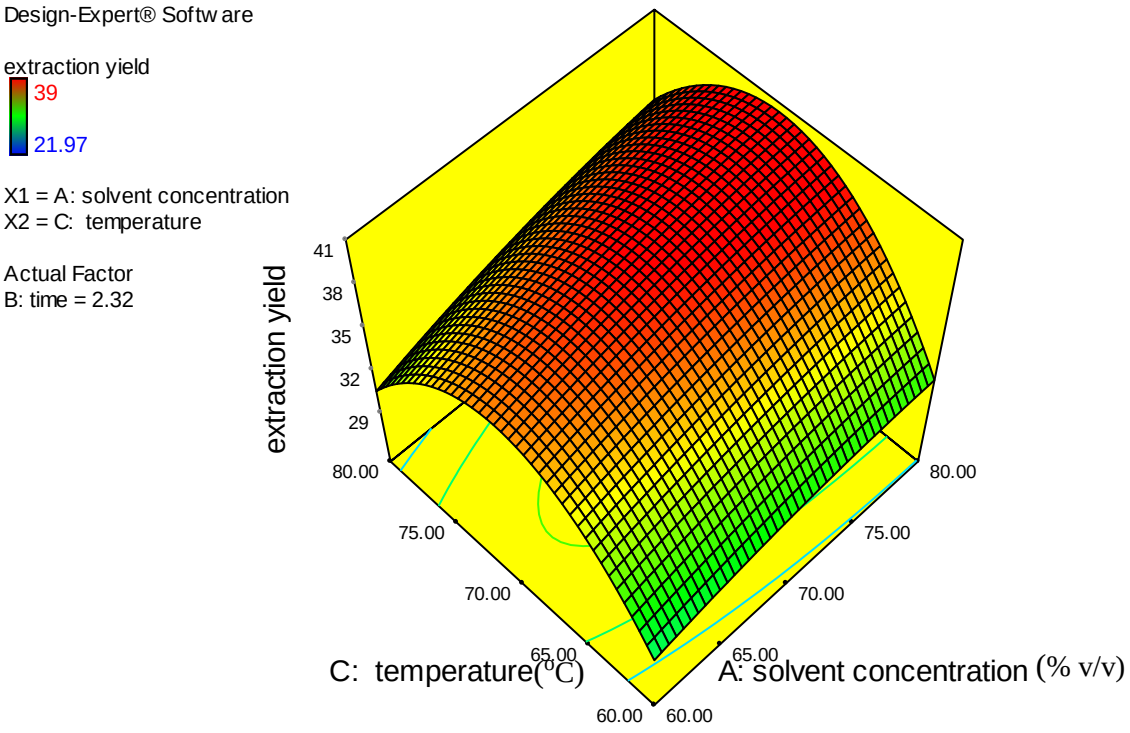


Figure 4.22: Response surfaces of predicted yield at fixed extraction time (2.32 hr)

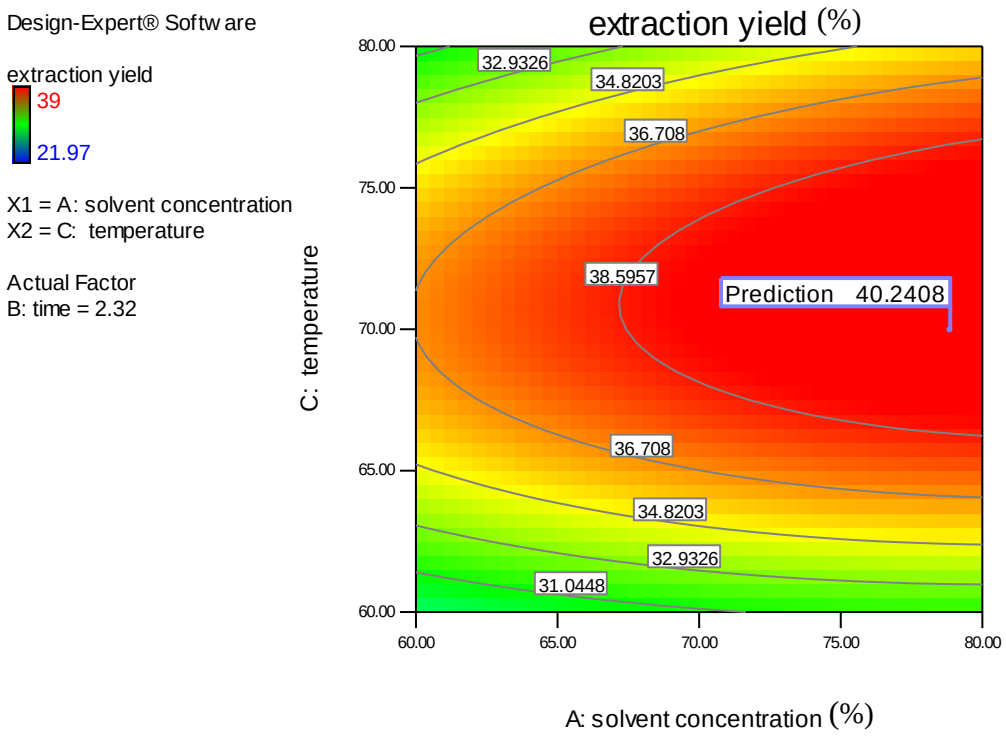


Figure 4.23:2D contour plots of the effect of solvent concentration and extraction temperature on the yield @ constant extraction time (2.32hr)

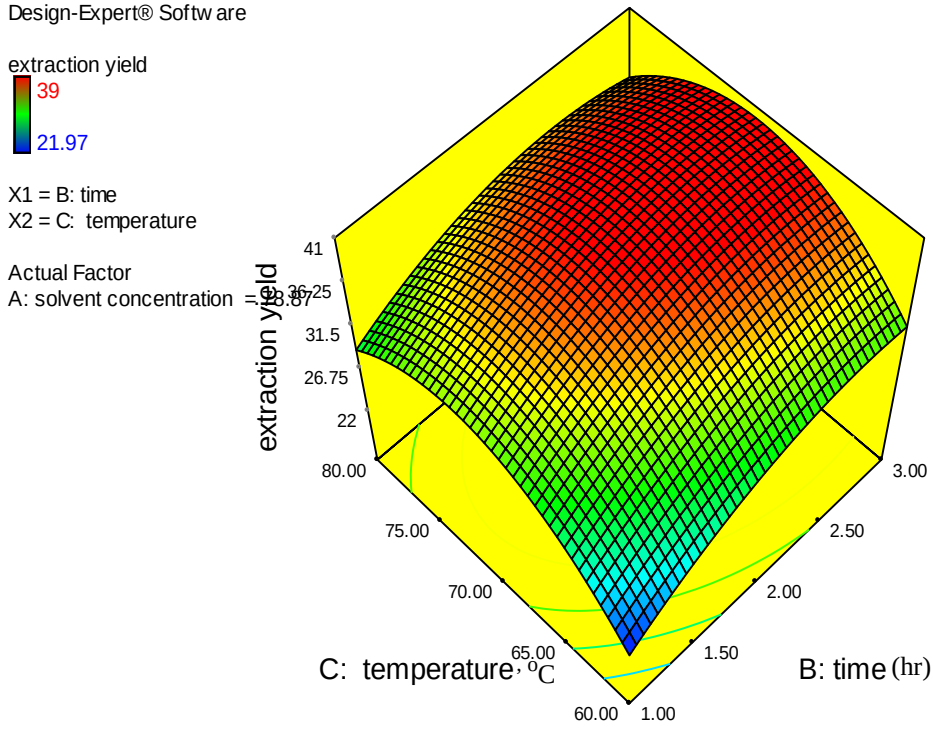


Figure 4.24: Response surfaces of predicted yield at fixed extraction solvent concentration (78.87%)

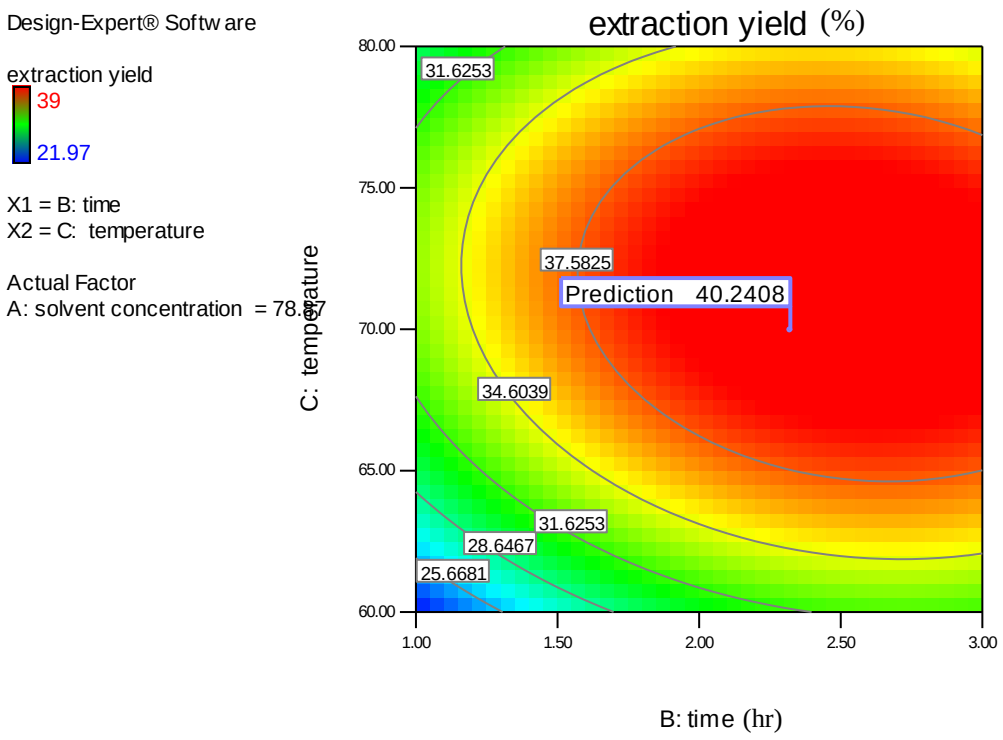


Figure 4.25: 2D contour plots of the effect of extraction temperature and extraction time on the yield @ constant extraction solvent concentration (78.87%)

4.8 Verification of the Model

The optimal process parameters were as follows: 78.87% ethanol concentration, 69.95ºC and 2.32hr. Under the optimal extraction conditions, the actual data of the crude yield was 40 $\pm$ 0.02% that comparable with the predicted value (40.2408%), which verified the accuracy of the model to predict the crude yield. In addition, the experimental crude yield value was increased to 40$\pm$ 0.02% (after optimization) from 39% (before optimization), which indicated that the optimizing process was necessary.

4.9 Quantification of Total Flavonoid Content, Total Phenol and Antioxidant Capacity of Turmeric Rhizome

4.9.1 Total Flavonoid Content

Flavonoids are the plant pigments responsible for plant colors and exert their health-promoting activities via their high pharmacological potentials as radical scavengers (Tanvir et al., 2017). The amount of total flavonoid content in extract was determined by the aluminum chloride colorimetric method described in the method section using quercetin acid as the standard. The absorbance values obtained at different

concentrations of quercetin indicated in Table 4.8 was used for the construction of a calibration curve from Appendix 4.2 (Figure 4.2A). Calibration curve of standard quercetin ((Figure 4.2A).**)** was obtained by plotting the values of different concentration of quercetin versus their absorbance for quantitative determination of content of flavonoids. The slope, intercept, correlation coefficient (R^2) and regression equation was obtained. The representative linear equation obtained was y = 0.9499x, where x is concentration and y is the absorbance. The correlation coefficient value obtained was 0.9979 which indicated good linearity in the range of 0.0 mg/ml to 2.9136 mg/ml. The absorbance value increased proportionally upon increasing the concentration of quercetin from 0.0 to 2.9136mg/ml.

Table 4.8: Quercetin standard solution preparation and the corresponding absorbance

Quercetin (µL)	ethanol (µL)	Concentration (mg/ml)	Sodium acetate (µL)	$AlCL_3$ (µL)	Distil water (µL)	absorbance @ 415nm	Absorbance Mean ± SD
0	1000	0	100	100	2800	0	0
100	900	0.04856	100	100	2800	0.08429	0.11643 ± 0.03
100	900	0.04856	100	100	2800	0.123	
100	900	0.04856	100	100	2800	0.142	
200	800	0.9712	100	100	2800	0.834	1.005 ± 0.288
200	800	0.9712	100	100	2800	0.843	
200	800	0.9712	100	100	2800	1.338	
300	700	1.4568	100	100	2800	1.340	1.403 ± 0.101
300	700	1.4568	100	100	2800	1.350	
300	700	1.4568	100	100	2800	1.519	
400	600	1.9424	100	100	2800	1.70	1.813± 0.132
400	600	1.9424	100	100	2800	1.781	
400	600	1.9424	100	100	2800	1.958	

Continued table from 4.8							
500	500	2.428	100	100	2800	2.084	2.316± 0.238
500	500	2.428	100	100	2800	2.303	
500	500	2.428	100	100	2800	2.561	
600	400	2.9136	100	100	2800	2.584	2.743±0.225
600	400	2.9136	100	100	2800	2.645	
600	400	2.9136	100	100	2800	3.00	

As the concentration of ethanoic extract turmeric rhizome is increasing from 0.056mg/ml to 0.959mg/ml with that of increasing total flavonoid content from 4.185 to 71.678 mg QUE/g DW from Table 4.10. The total flavonoid content that has been reported by (Azahar et al., 2017) was 6.12 ± 0.23 mg quercetin/g of extract from its leaves. According to (Sahu & Saxena, 2013) studied also total flavonoid content of curcuma rhizomes ranged from 22.52±0.015 to 79.36±0.01 mg quercetin equivalent/g with soxhlet extraction method and using methanol solvent. The maximum total flavonoid content in the present study was 71.678 mg QUE/g DW from 0.959 mg/ml which is in the range of the (Sahu & Saxena, 2013) studied. The variation was raised due to the extract sample concentration, type of solvent used, part of plant used, solvent strength, the technique of extraction and time of extraction.

The risk of heart disease, inhibit the initiation, promotion and progression of tumors can be reduced by flavonoid compounds because the functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions (Kočevar et al., 2007).

So it is importance to know its content/ presence of flavonoid in the extract. The quantification result of the flavonoid confirms the qualitative test of flavonoid in both chemical and FTIR analysis as described in Table 4.2 and Figure 4.3 and in Table 4.3 and Figure 4.4, respectively.

Table 4.9: Sample solution preparation and corresponding absorbance

sample (µL)	ethanol (µL)	Concentration (mg/ml)	Sodium acetate (µL)	$AlCL_3$ (µL)	Distil water (µL)	Absorbance @ 415nm	Absorbance Mean ± SD
100	900	1.338	100	100	2800	0.034	0.0534±0.025
100	900	1.338	100	100	2800	0.045	
100	900	1.338	100	100	2800	0.0812	
200	800	2.676	100	100	2800	0.57	0.595±0.028
200	800	2.676	100	100	2800	0.59	
200	800	2.676	100	100	2800	0.625	
300	700	4.014	100	100	2800	0.620	
300	700	4.014	100	100	2800	0.626	0.628±0.009
300	700	4.014	100	100	2800	0.638	
400	600	5.352	100	100	2800	0.690	0.709±0.021
400	600	5.352	100	100	2800	0.705	
400	600	5.352	100	100	2800	0.732	
500	500	6.69	100	100	2800	0.734	0.744±0.016
500	500	6.69	100	100	2800	0.736	
500	500	6.69	100	100	2800	0.762	
600	400	8.028	100	100	2800	0.765	0.911±0.240
600	400	8.028	100	100	2800	0.780	
600	400	8.028	100	100	2800	1.188	

As discussed in Table 4.2 and Figure 4.3, the color change observed for the flavonoid test in turmeric rhizome extract is confirmed the presence of flavonoid compound in turmeric rhizome ethanoic extract. This result reveals that the quantification result from the UV-Vis spectrometer supports the qualitative test.

Table 4.10: Total flavonoid content of turmeric rhizome

Absorbance of sample	Concentration (mg/ml) of sample C = (y)/slope	total flavonoid content (TFC) mg/g = C*Volume of stock sample solution	% of TFC (g/g)
0.0534	0.056	4.185	0.4185
0.595	0.626	46.786	4.6786
0.628	0.661	49.402	4.9402
0.709	0.746	55.755	5.5755
0.744	0.783	58.520	5.8520
0.911	0.959	71.678	7.1678

4.9.2 Total Polyphenol Content

The amount of total phenol content in extract was determined by Folin-Ciocalteu's reagent described in the method section using Gallic acid as the standard. The absorbance values obtained at different concentrations of Gallic acid presented in Table 4.11 were used for the construction of the calibration curve as presented in Appendix 4.2 (Figure 4.2B).). Calibration curve of standard gallic acid was obtained by plotting the values of different concentration of gallic acid versus their absorbance's for quantitative determination of content of polyphenol. The slope, intercept, correlation coefficient (R^2) and regression equation was obtained. The representative linear equation obtained was y = 10.001x where x is concentration and y is the absorbance, m is slope. The correlation coefficient value obtained was 0.992 which indicated good linearity in the range of 0.0mg/ml to 0.1 mg/ml. The results at 0.0091, 0.0199, 0.0351, 0.0452, 0.0539 and 0.0609 mg/ml concentration of sample, obtained are 0.68, 1.487, 2.623, 3.378, 4.028 and 4.551 mg GAE/g extract and total phenolic content of extract in turmeric were increasing with increasing the concentration of the extract in Table 4.13.

Table 4.11: Gallic acid standard solution preparation and corresponding absorbance

Gallic acid (µL)	ethanol (µL)	Concentration (mg/ml)	FC (ml)	Na_2CO_3 (ml)	Distil water (ml)	Absorbance @760nm	Absorbance Mean ± SD
0	1000	0	1	1	7	0	0
20	980	0.01	1	1	7	0.089	0.131±0.063
20	980	0.01	1	1	7	0.101	
20	980	0.01	1	1	7	0.203	
40	960	0.02	1	1	7	0.204	0.233±0.049
40	960	0.02	1	1	7	0.205	
40	960	0.02	1	1	7	0.290	
80	920	0.04	1	1	7	0.340	0.405±0.096
80	920	0.04	1	1	7	0.360	
80	920	0.04	1	1	7	0.515	
120	880	0.06	1	1	7	0.578	0.626±0.083
120	880	0.06	1	1	7	0.578	
120	880	0.06	1	1	7	0.722	
160	840	0.08	1	1	7	0.731	0.830±0.086
160	840	0.08	1	1	7	0.879	
160	840	0.08	1	1	7	0.880	
200	800	0.1	1	1	7	0.890	0.949±0.066
200	800	0.1	1	1	7	0.936	
200	800	0.1	1	1	7	1.021	

Table 4.12: Sample solution preparation and corresponding absorbance

Sample (μL)	ethanol (μL)	Concentration (mg/ml)	FC (ml)	Na_2CO_3 (ml)	Distil water (ml)	absorbance @760nm	Absorbance Mean ± SD
20	980	0.2676	1	1	7	0.05	0.0910±0.063
20	980	0.2676	1	1	7	0.06	
20	980	0.2676	1	1	7	0.163	
40	960	0.5352	1	1	7	0.11	0.1990±0.121
40	960	0.5352	1	1	7	0.15	
40	960	0.5352	1	1	7	0.337	
80	920	1.0704	1	1	7	0.291	0.3510±0.073
80	920	1.0704	1	1	7	0.33	
80	920	1.0704	1	1	7	0.432	
120	880	1.6056	1	1	7	0.421	0.4520±0.042
120	880	1.6056	1	1	7	0.435	
120	880	1.6056	1	1	7	0.500	
160	840	2.1408	1	1	7	0.505	0.539±0.055
160	840	2.1408	1	1	7	0.510	
160	840	2.1408	1	1	7	0.602	
200	800	2.676	1	1	7	0.603	0.609±0.0095
200	800	2.676	1	1	7	0.604	
200	800	2.676	1	1	7	0.620	

The higher total phenol content in the present study was 4.551 mg of gallic acid equivalent per gram of sample (45.51mg GAE/100g DW) that present at higher concentration of extract (0.0609mg/ml) which was higher than with that of the total phenol content has been reported by (Tanvir et al., 2017) which was 4.52 ± 0.250 mg GAE/100g sample. This is due to the type of herb parts used, the type of solvent used, solvent strength, the technique of extraction and time of extraction.

The quantification result of the phenolic compounds confirms the qualitative test of phenol as described in Table 4.2 and Figure 4.3 As discussed in Table 4.2 and Figure 4.3, the color change observed for the phenolic compound test in turmeric rhizome extract is confirmed the presence of phenolic compound in turmeric rhizome ethanoic extract. This result reveals that the quantification result from the UV-Vis spectrometer supports the qualitative test.

Plant phenolic are essential constituents that contribute to functional quality, color and flavor. They have significant roles both as singlet oxygen quenchers and free radical scavengers, helping to minimize molecular damage (Tanvir et al., 2017). The health benefits of phenolic are primarily derived from their antioxidant potentials because the radicals produced after hydrogen or electron donation are resonance stabilized and thus relatively stable.

Table 4.13: Total phenol content of ethanoic extract of turmeric rhizome

Absorbance of sample	Concentration (mg/ml) of sample C = (y)/slope	total polyphenol content (TPC) mg/g = C*Volume of stock sample solution	% of TPC, g/g	% of TPC, g/100g
0.0910	0.0091	0.680	0.068	6.8
0.1990	0.0199	1.487	0.1487	14.87
0.3510	0.0351	2.623	0.2623	26.23
0.4520	0.0452	3.378	0.3378	33.78
0.539	0.0539	4.028	0.4028	40.28
0.609	0.0609	4.551	0.4551	45.51

4.9.3 Antioxidant capacity of Phenol and Flavonoid of Turmeric Rhizome

4.9.3.1 Antioxidant Content of Turmeric Rhizome

The results for antioxidant activity clearly outline that turmeric rhizome could be one of the richest sources among plants in terms of antioxidant activity. The significant difference of turmeric rhizome for antioxidant activity across varieties is supposed to be the effect of change of ecological parameters, such as climatic conditions, seasonal variation, age of harvested cultivars, as well as physiochemical nature of the soil, which are responsible factors besides cultivar variation. The antioxidant activity of sample was estimated from the regression equation of ascorbic acid.

The ethanoic extract free radical scavenging activity of turmeric rhizome has been tested by DPPH radical procedure using ascorbic acid as a reference standard. The absorbance values obtained at different concentrations of ascorbic acid indicated in Table 4.14 was used for the construction of a calibration curve from appendix 4.2 (Figure 4.2C). Calibration curve of standard ascorbic acid (Appendix 4.2) (Figure 4.2C) was obtained by plotting the values of different concentration of ascorbic acid versus their absorbance for quantitative determination of content of antioxidant. The slope, intercept, correlation coefficient (R^2) and regression equation was obtained. The representative linear equation obtained was $y = - 0.2113x + 1.1175$, where x is concentration and y is the absorbance. The correlation coefficient value obtained was 0.9727 which indicated good linearity in the range of 0.50692 mg/ml to 5.0692 mg/ml.

Table 4.14: Ascorbic acid standard solution preparation and corresponding absorbance

Ascorbic acid (µL)	ethanol (µL)	Concentration (mg/ml)	DPPH (ml)	absorbance@ 517nm	Absorbance Mean ± SD
0	1000	0	1	1.480	1.480
20	980	0.50692	1	1.2573	1.0991±0.146
20	980	0.50692	1	1.07	
20	980	0.50692	1	0.970	
40	960	1.01384	1	0.945	0.859±0.075
40	960	1.01384	1	0.820	
40	960	1.01384	1	0.812	
80	920	2.02768	1	0.807	0.660±0.139
80	920	2.02768	1	0.643	
80	920	2.02768	1	0.530	
120	880	3.04152	1	0.533	0.427±0.107
120	880	3.04152	1	0.428	
120	880	3.04152	1	0.320	
160	840	4.05536	1	0.316	0.222 ±0.082
160	840	4.05536	1	0.18	
160	840	4.05536	1	0.170	
200	800	5.0692	1	0.154	0.118±0.032
200	800	5.0692	1	0.109	
200	800	5.0692	1	0.091	

Table 4.15: Prepared sample solution, corresponding absorbance and antioxidant content

Sample (µL)	ethanol (µL)	Concentration (mg/ml)	DPPH (ml)	absorbance @ 517nm	Absorbance Mean ± SD	Concentration of sample from calibration curve mg/ml	Antioxidant content mg ascorbic acid /g of sample
20	980	0	1	0.845	0.805±0.035	1.479	58.353
20	980	0.50692	1	0.790			
20	980	0.50692	1	0.780			
40	960	0.50692	1	0.779	0.678±091	2.08	82.064
40	960	1.01384	1	0.654			
40	960	1.01384	1	0.601			
80	920	1.01384	1	0.585	0.434±0.131	3.235	127.633
80	920	2.02768	1	0.360			
80	920	2.02768	1	0.357			
120	880	2.02768	1	0.354	0.275±0.069	3.987	157.303
120	880	3.04152	1	0.243			
120	880	3.04152	1	0.228			
160	840	3.04152	1	0.222	0.184±0.033	4.418	174.307
160	840	4.05536	1	0.170			
160	840	4.05536	1	0.160			
200	800	4.05536	1	0.164	0.099±0.057	4.815	189.971
200	800	5.0692	1	0.072			
200	800	5.0692	1	0.061			

As the concentration of ethanoic extract turmeric rhizome is increasing with that of increasing antioxidant content from Table 4.15. The maximum antioxidant content in the present study which was 189.971 mg ascorbic acid equivalent/g DW at 4.815mg/ml of maximum sample concentration.

4.9.3.2 The Half Maximal Inhibitory Concentration Values of Turmeric Rhizome

The efficient concentration (EC50) or half maximal inhibitory concentration (IC50) value was calculated from the linear regression of plots of percentage inhibition (% DPPH scavenging activity) against concentration of turmeric rhizome extracts (y = mx + b) where, y is the percentage of inhibition for extract, x is concentration (mg/ml), b is the y intercept and m is slope from equation 3.12 from method. The plot between percentage inhibition of ascorbic acid (Figure 4.26) and concentration of ascorbic acid was obtained with y = 14.679x + 22.933. The correlation coefficient value obtained was 0.9628 which indicated good linearity in the range of 0.50692 mg/ml to 5.0692 mg/ml.

The curve (Figure 4.27) was obtained by plotting the values of different concentration of ethanoic extract of turmeric versus their percentage inhibition for quantitative determination of IC_{50} values. The slope, intercept, correlation coefficient (R^2) and regression equation was obtained. The representative linear equation obtained was y = 10.47x + 44.706. The correlation coefficient value obtained was 0.9517 which indicated good linearity in the range of 0.50692 mg/ml to 5.0692mg/ml. Sharp increases in radical scavenging activity (% inhibition) with an increase in the concentration (Figure 4.26) of extracts were observed up to 5.0692 mg/ml concentration. The maximum percentage of scavenging activity value of ethanoic extract was 93.311 in Figure 4.27 at 5.0692 mg/ml.

As depicted in Figure 4.26 and Figure 4.27, and Table 4.1C from Appendix 4.1 at this concentration, the ethanoic extract showed significantly higher activity (93.3110%) than the ascorbic acid (92.027%). On the other hand, the higher activity of ethanoic extract than ascorbic acid can be attributed to a more elevated concentration of the TPC and antioxidants.

The results showed that, although the sample and ascorbic acid have noticeable effect on DPPH radical, ethanoic extract have sustainable hydrogen donating and radical scavenging ability than that of Ascorbic acid. Interestingly, ethanoic extract, which

exhibited the highest content of total phenolic, total flavonoid, registered the highest DPPH radical scavenging potential compared to the standard ascorbic acid.

A smaller IC_{50} value corresponds to a higher antioxidant activity. The IC_{50} (mg/ml) values obtained for the sample submitted to the DPPH assay ranged from 0.50692 to 5.0692 mg/ml. The values of IC50% of ethanoic extract was calculated based on equation 3.11 that was, expressed in mg/ml, 0.507 which lower than that of the control ascorbic acid (1.844mg/ml). That means it has higher antioxidant activity than that of ascorbic acid

Antiradical capacity (AAR) is defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% and is expressed as 1/IC50. A high value of AAR refers to high Antioxidant activity. For this study, the values of antiradical capacity of ethanoic extract and ascorbic acid were, expressed in mg/ml, 1.972 and 0.542, respectively. The highest value of AAR was found in the ethanoic extract (1.972), which was also the richest in phenolic, whereas ascorbic acid (0.542) exhibited the weakest activity. The larger the AAR, the more efficient the antioxidant, so that as can be found in the ethanoic extract more than in the ascorbic acid.

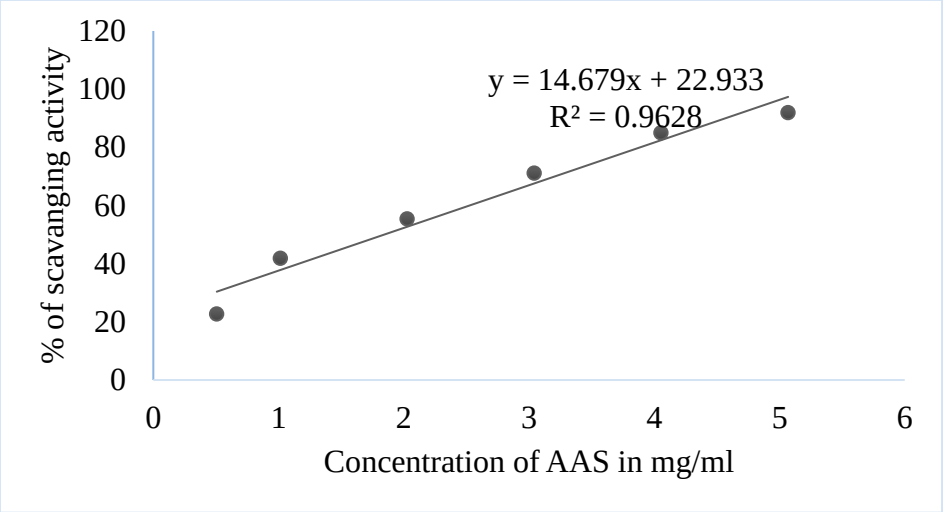


Figure 4.26: Plot of percentage of scavenging activities of ascorbic acid and concentration of ascorbic acid

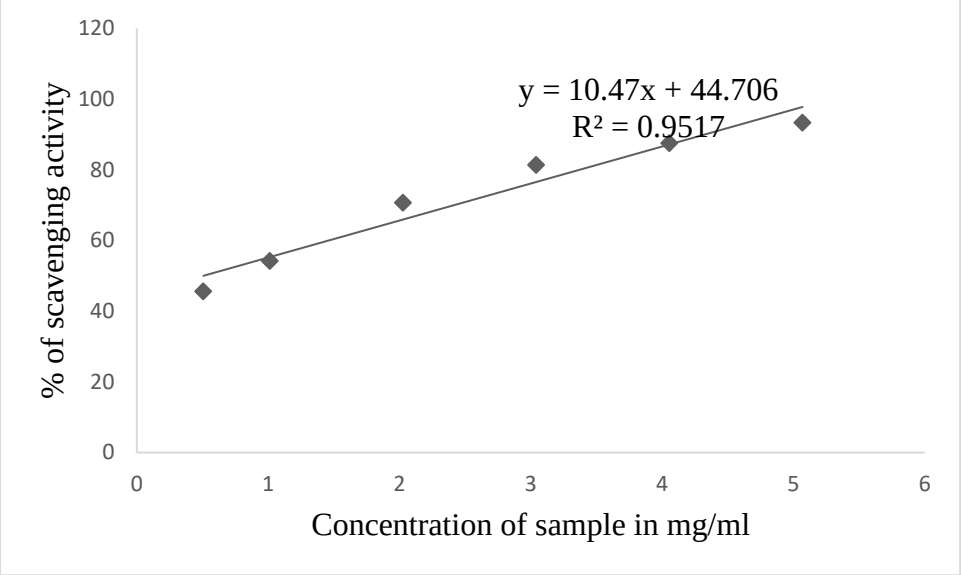


Figure 4.27: Plot of percentage of scavenging activities of ethanoic extract and its concentration

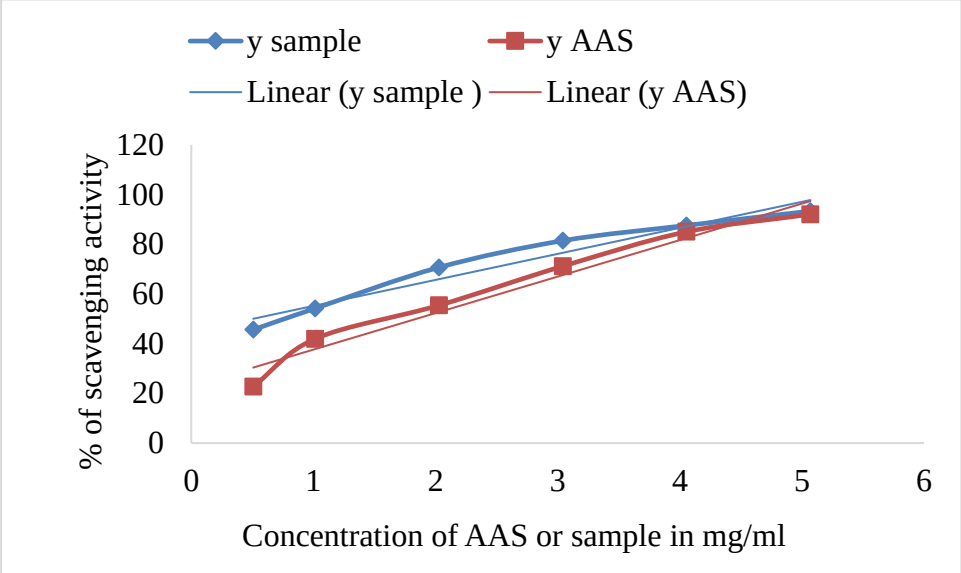


Figure 4.28: Plot of percentage of scavenging activities for both ethanoic extract and AAS.

As shown in the Figure 4.28 The inhibition activity for both the extract and ascorbic acid increase with increase the concentration of sample/ ascorbic acid.

5 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this research, extraction, proximate analysis, qualitative test for selected bioactive constitutes from turmeric rhizome were conducted. The effects of solvent concentration, extraction time and temperature on the yield of crude extract have been studied. The results of the experiments conducted have been analyzed by employing Design-Expert 7, three-level-three-factor Central Composite Design (CCD) and Response Surface Methodology (RSM). High extraction yield (39%) was obtained at 80% of ethanol concentration, 70℃ extraction temperature and 2hours extraction time. The CCD provided a powerful design for the optimization conditions using maceration method of extraction. Accordingly, the optimal predicted extraction yield was 40.2408% at 78.87% of ethanol concentration, 2.32hr and 69.95ºC whereas the actual or experimental optimal value of extraction yield was 40 $\pm$ 0.02% at those optimal parameters. In this study, crude extraction yield was strongly influenced on the extraction conditions. The determination of total flavonoid, total phenol, and antioxidants content were conducted from turmeric rhizome at the optimum condition that were 71.678mgQUE/g DW, 4.551mg GAE/g DW and 189.971mg AAE/g DW, respectively. I conclude that *Curcuma longa* is a good and reliable source of polyphenol, flavonoid and antioxidant compounds.

5.2 Recommendation

Given the demonstrated possibility of extracting antioxidants polyphenol compounds from Ethiopian turmeric rhizome further works are still necessary. Future researches that would be complementary to the present study aiming at investigation of the process are and therefore, strongly recommended to put due focus on the following points:

Antioxidants are sensitive in heat, metal and oxygen. For better potent of antioxidant activity, future studies should focus on working condition in an inert atmosphere and away from light in order to greatly prevent oxidation of polyphenols, flavonoids and addition of antioxidant compounds such as ascorbic acid and SO_2 has been proposed and also freeze drying, with taking a care that they should be dried away from oxidation

facilitating factors like heat, presence of oxygen and metal ions since in this study fresh turmeric only frozen transported to laboratory and also it dried in the oven.

This study leads to the further research in the way of isolation and identification of curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) that are good candidates for further development as a potential treatment option for patients with coronavirus and HIV disease besides antioxidant remedies with the consideration of economic benefits by higher Performance Liquid Chromatograph.

The metal chelating, singlet oxygen quenching activity of ethanoic extract of turmeric rhizome should be studied.

Other antioxidant determining methods including Phosphomolybdenum assay, Super oxide anion radical scavenging activity and Hydroxyl radical scavenging activity should also be applied for further investigation of the antioxidant activity of the extract.

The optimal storage conditions and shelf-life of extracts as a function of antioxidant activity has to be investigated and established in the future.

Future researches should pay a great attention to innovative food applications into meat, fish, cereal, fruit-based and dairy products, with a focus on the type of recovered ingredient and dosage level achieved by the application of this extract.

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APPENDIX

Appendix 3.1: Reagent preparation for phytochemical test

Wagner's reagent: it is used for alkaloids detection.

Solution (a) 2 g of iodine was dissolved in 60 ml of distilled water.

(b) 6 g of potassium iodide was dissolved in 20 ml of distilled water. Solution (a) and (b) were mixed and the volume was adjusted to 100 ml with distilled water.

1% aqueous $FeC1_3$:1 g of iron chloride was dissolved in 100 ml of distilled water.

Determination of protein content reagent

40% (w/v) NaOH: 40 g of sodium hydroxide was dissolved in 100 ml of distilled water

4%v/v boric acid: 4 ml of boric acid was dissolved in 96 ml of distilled water.

Determination of total flavonoids content reagent

Preparation of 10% aluminum chloride: 10 grams of $AlCl_3$ was taken in 100 ml of volumetric flask and a small amount of distilled water was added and dissolved in it. Then the final volume was made up to the mark by adding the required amount of distilled water.

Preparation of 1 M Sodium acetate solution: 8.2 grams of Sodium acetate was taken in 100 ml of volumetric flask and a small amount of distilled water was added and dissolved in it. Then the final volume was made up to the mark by adding the required amount of distilled water.

Preparation of standard quercetin acid solution: 0.4856 grams of quercetin was dissolved into 100ml 97% ethanol, so the concentration of the solution is 4.856mg/ml. This is called the stock solution. Then serial dilution was performed in order to prepare differently concentrated of solution (0.04856mg/ml, 0.9712mg/ml, 1.4568mg/ml, 1.9424, 2.428 mg/ml and 2.9136mg/ml) which is used for preparing a calibration curve.

Preparation of Blank: Blank consists of all the reagents, except for the extract or quercetin standard solution was substituted with 1ml of distilled water.

Figure 3.1A: Quercetin series solution

(source: taken by author)

Figure 3.1 B: Prepared sample for total flavonoid continent

(source: taken by author)

Preparation of Folin-Ciocalteau reagent

Folin-Ciocalteu's phenol reagent was prepared under the hood following the procedure described below: Into 100 mL of round bottom flask, 10 g of sodium tungstate (Na2WO4.2H2O**)** and 2.5 g of sodium molybdate (Na2MOO4.2H2O) were dissolved in 70 mL of distilled water. To the mixture 5 mL of 85% phosphoric acid and 10mL of concentrated hydrochloric acid were added. After the solution was refluxed at 100^{o}C for 2 hours, 15 g of lithium sulfate, 5 mL of distilled water and 1 drop of H_2O_2 were

sequentially added to the solution and refluxed for 15 min to remove the excess H_2O_2. The bromine oxidizes any trace of molybdenum-tungsten blue, and the final reagent should be yellow without any green. The refluxed solution was allowed to cool at room temperature and diluted to 100 ml by the addition of distilled water.

A B

Figure 3.1 C: A) FC reagent B) stock quercetin acid solution
(source: taken by author)

Figure 3.1D: Prepared sample for total phenol determination
(source: taken by author)

Preparation of standard galic acid solution: 0.058 grams of galic acid was dissolved into 100ml 97% ethanol, so the concentration of the solution is 0.5mg/ml. This is called the stock solution. Then serial dilution was performed in order to prepare differently concentrated of solution (0.01mg/ml, 0.02 mg/ml, 0.04 mg/ml, 0.06mg/ml, 0.08mg/ml and 0.1mg/ml) which is used for preparing a calibration curve.

Preparation of Blank: Blank consists of all the reagents, except for the extract or galic acid standard solution was substituted with 1ml of distilled water.

Figure 3.1E: Gallic acid serial dilution prepared solution
(source: taken by author)

Antioxidant Activity reagent

Preparation of 0.1mM DPPH: 4mg of DPPH was dissolved by 100ml of 97% ethanol.

Preparation of standard ascorbic acid solution: 2.5346 grams of ascorbic acid was dissolved into 100ml 97% ethanol, so the concentration of the solution is 25.346mg/ml. This is called the stock solution. Then serial dilution was performed to prepare differently concentrated of solution (0.50692mg/ml, 1.01384mg/ml, 2.02768mg/ml, 3.041252mg/ml, 4.05536mg/ml and 5.0692mg/ml) which is used for preparing a calibration curve.

Preparation of Blank: Blank consists of only ethanol while the control consists all reagent except ascorbic acid or extract sample.

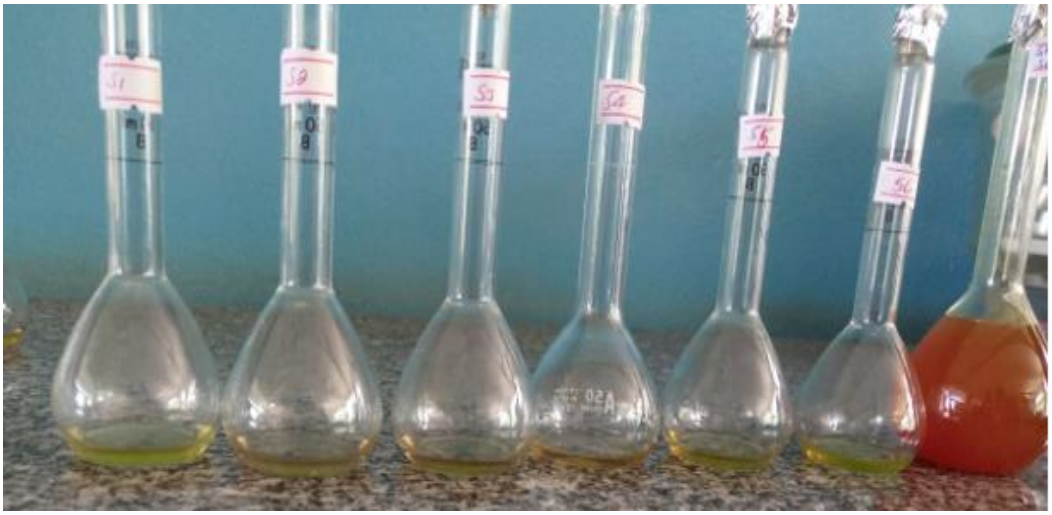


Figure 3.1F: Prepared sample for the determination of antioxidant activity
(source: taken by author)

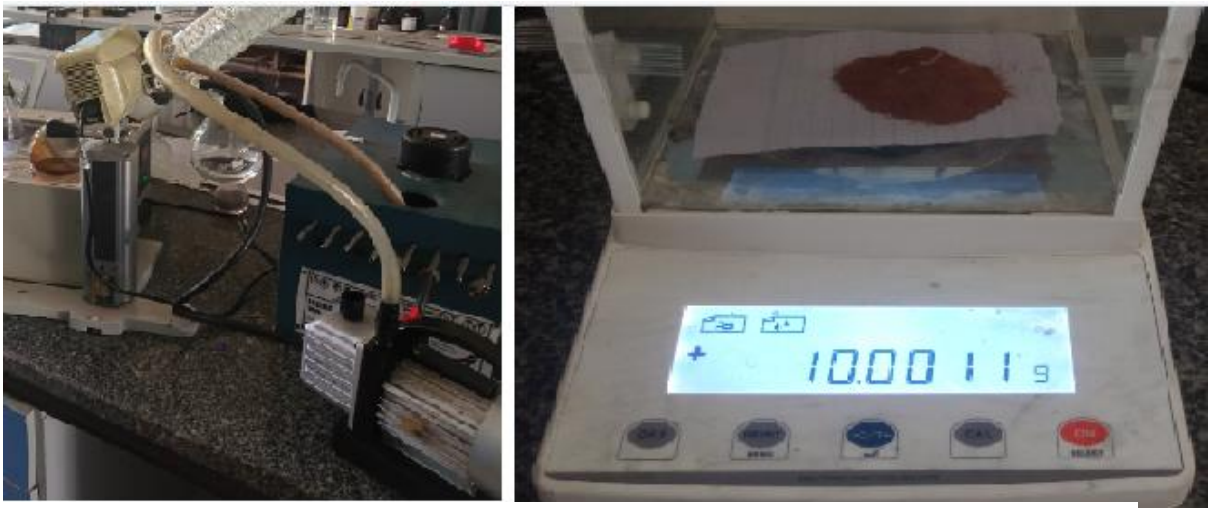
A B

Figure 3.1 G: A) DPPH stock solution and B) stock sample solution
(source: taken by author)

Appendix 3.2: Material used

A B

Figure 3.2 A: A) Perkin-Elmer UV-Vis spectrometer, Lambda 35 and B) funnels
(source: taken by author)



A B

Figure 3.2: A) Rotary evaporator B) Mass balance
(source: taken by author)

Appendix 4.1: Tables obtained from the experimental result

Table 0.1A: Determination of percentage yield of ethanolic extracts of turmeric rhizome using the software Design Expert® 7.0

Run	Factors			Reponses
	A:Concentration (% v/v): volume of ethanol per volume of water	B:Time (hr)	C:Temperature (°C)	Extraction yield (%)
1	60.00	1.00	60.00	21.97
2	60.00	3.00	60.00	28.5
3	60.00	2.00	70.00	37.3
4	60.00	1.00	80.00	27
5	60.00	3.00	80.00	28
6	70.00	2.00	60.00	30.5
7	70.00	1.00	70.00	33.3
8	70.00	2.00	70.00	38.9
9	70.00	2.00	70.00	38.8
10	70.00	2.00	70.00	38.6
11	70.00	2.00	70.00	38.5
12	70.00	2.00	70.00	37
13	70.00	2.00	70.00	38.7
14	70.00	3.00	70.00	38
15	70.00	2.00	80.00	33.23

Continued from Table 4.1A

Run	Factors			Reponses
	A:Concentration (% v/v): volume of ethanol per volume of water	B:Time (hr)	C:Temperature (^oC)	Extraction yield (%)
16	80.00	1.00	60.00	22.9
17	80.00	3.00	60.00	31.6
18	80.00	2.00	70.00	39
19	80.00	1.00	80.00	28.9
20	80.00	3.00	80.00	35.3

Table 4.1B: Optimum possible solutions on the crude ethanoic extract of turmeric rhizome

Number	solvent concentration %	Time, hr	Temperature, ^oc	extraction yield %	Desirability
1	72.36	2.72	71.23	39.4281	1.000
2	75.90	2.44	74.31	39.5131	1.000
3	76.08	2.53	73.29	39.9021	1.000
4	72.44	2.28	71.70	39.5219	1.000
5	79.62	2.48	73.49	40.3067	1.000
6	70.81	2.71	70.27	39.1245	1.000
7	73.26	2.35	73.15	39.4666	1.000
8	75.69	2.64	74.34	39.3788	1.000

Continued from Table 4.1A

9	75.34	2.37	67.67	39.1815	1.000	
10	71.91	2.52	71.95	39.4822	1.000	
11	69.44	2.44	71.29	39.0804	1.000	
12	73.78	2.37	71.94	39.7932	1.000	
13	78.81	2.41	69.54	40.2571	1.000	
14	73.29	2.44	69.46	39.6126	1.000	
15	75.75	2.73	69.37	39.8612	1.000	
16	79.44	2.72	67.52	39.6471	1.000	
17	71.91	2.26	72.06	39.3854	1.000	
18	75.46	2.72	70.55	39.9912	1.000	
19	71.29	2.57	70.25	39.3769	1.000	
20	79.22	2.01	70.50	39.5577	1.000	
21	78.87	2.32	69.95	40.2408	1.000	Selected
22	71.68	2.23	70.72	39.351	1.000	
23	73.91	2.38	73.27	39.5537	1.000	
24	72.50	2.60	70.07	39.5592	1.000	
25	70.56	2.26	71.96	39.1587	1.000	
26	77.21	2.32	74.87	39.3737	1.000	
27	76.80	2.43	74.78	39.4289	1.000	
28	76.17	2.55	70.17	40.1654	1.000	
29	78.27	2.15	72.84	39.8936	1.000	
30	78.95	2.13	71.80	40.0009	1.000	

4.1 C: Percentage of scavenging activities of ascorbic acid and ethanoic extract of turmeric rhizome

Concentration (mg/ml) of AAS/ sample	Absorbance of AAS at 517nm	Absorbance of Sample at 517nm	% SCV of AAS	% SCV of sample
0.50692	1.0991±0.146	0.805±0.035	22.736	45.608
1.01384	0.859±0.075	0.678±009	41.959	54.189
2.02768	0.660±0.139	0.434±0.131	55.405	70.676
3.04152	0.427±0.107	0.275±0.069	71.149	81.419
4.05536	0.222 ±0.082	0.184±0.033	85	87.568
5.0692	0.118±0.032	0.099±0.057	92.027	93.311
Control	1.480	1.480	0	0

Appendix 4.2: Calibration curve for total flavonoid, total phenol and antioxidant determinations

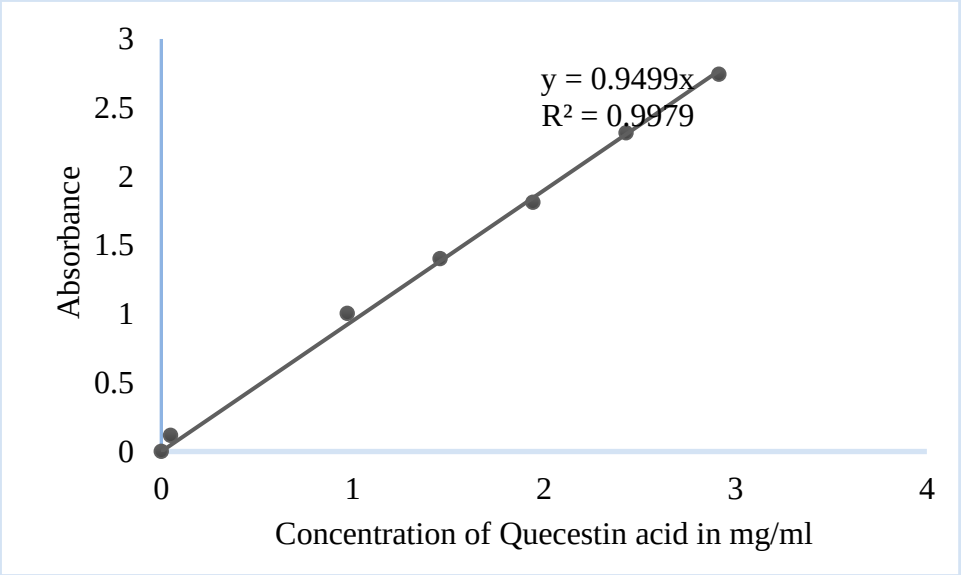


Figure 4.2A: Calibration curve for standard Quercetin

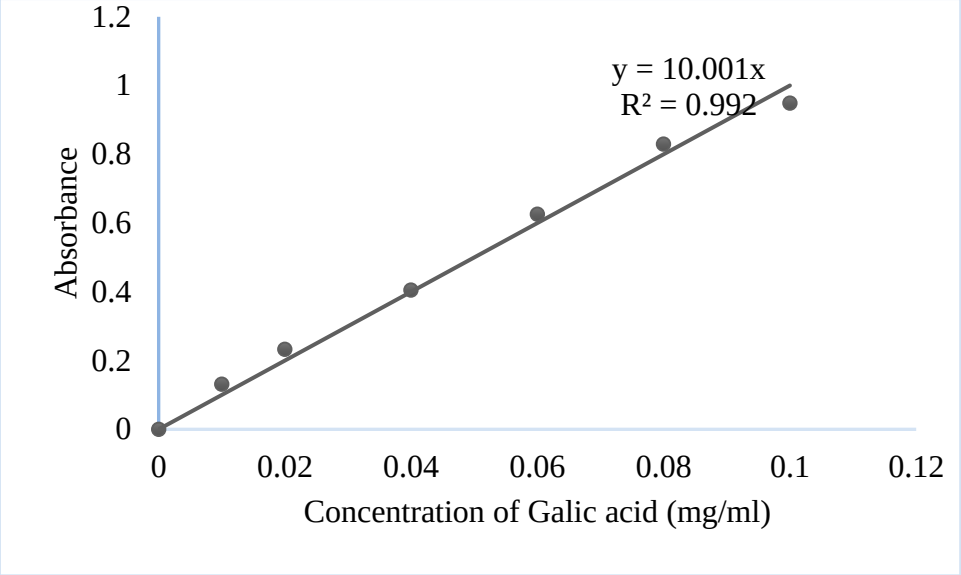


Figure 4.2B: Calibration curve for standard Gallic acid

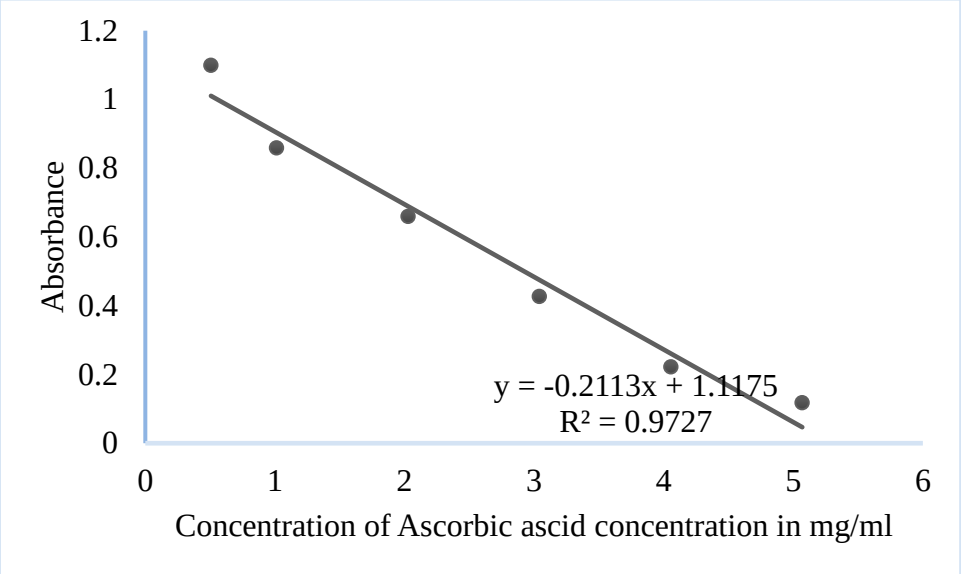


Figure 4.2C: Calibration curve for standard ascorbic acid