PHYTO CONSTITUENTS AND THEIR ANTIOXIDANT ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF FRUITS AND BARK OF TIMUR

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A dissertation submitted to the Department of Nutrition and Dietetics, Central Campus of Technology, Tribhuuvan University, in partial fulfillment of the requirements for the degree of BSC Nutrition and Dietetics.

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Approval Letter

The ***dissertation*** entitled "***Phyto Constituents and Their Antioxidant Activity of Different Solvent Extracts of Fruit and Bark of-Timur***" presented by **Rabiraj Poudel** has been accepted as the partial fulfillment of the requirement for **Bachelor degree in Nutrition and Dietetics.**

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Abstract

This study was aimed to assess the phyto constituents and antioxidant activity of fruit and bark of boke and sil timur, which were collected from village of Dhankuta. These sample were milled to fine powder and extracted with soxhlet apparatus in three different solvents. Then extracts were concentrated in rotary vacuum evaporator at 65°C under reduced pressure and stored at 2-8°C until analysis. The analysis was made for total phenol, total flavonoid, total tannin and DPPH free radical scavenging activity.

From analysis it was found that, the total phenol content was found highest in boke timur (fruit) in methanolic extract (175.06 mg GAE/g), whereas lowest was found in sil timur (bark) in petroleum ether extract (19.33 mg GAE/g). The total flavonoid content was found highest in boke timur (fruit) in methanolic extract (91.42 mg QE/g), whereas lowest was found in boke timur (bark) in petroleum ether extract (10.29 mg QE/g). The total tannin content was found highest in sil timur (fruit) in methanolic extract (44.04 mg TAE/g), whereas lowest was found in sil timur (bark) in methanolic extract (6.46 mg TAE/g) and tannin was not detected in sil timur (bark) in ethanolic and petroleum ether extract. The DPPH radical scavenging activity was found highest in boke timur (fruit) in methanolic extract (IC_{50} =47.64 μg/mL), whereas lowest was found in sil timur (bark) in petroleum ether extract (IC_{50} =210.06 μg/mL. So extraction of phytochemical and antioxidant can be seen high in methanol and in boke timur (fruit).

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Abbreviation	Full form
ANOVA	Analysis of Varience
CCT	Central Campus of Technology
CVD	Cardio Vascular Disease
DFTQC	Department of Food Technology and Quality Control
DPPH	Diphenyl picryl hydrazyl
Etc.	Et cetera
IC_{50}	Inhibitory concentration
GAE	Gallic Acid Equivalent
MC	Moisture Content
QE	Quercetin Equivalent
RSA	Radical scavenging activity
ROS	Reactive Oxygen Species
TAE	Tannic acid Equivalent
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
WHO	World Health Organization

Part I

Introduction

1.1 General introduction

Globally, medicinal plants are extensively used for the treatment of various diseases (Grover *et al.*, 2002). According to the World Health Organization (WHO), medicinal plants are the great source to offer a diverse range of potential therapeutic drugs and these drugs can be relatively safe and economical as compared to the synthetic medicines (Mekuria *et al.*, 2017), (Ekor, 2014), (Bahmani *et al.*, 2014). Since the last many years, herbal drugs are increasingly focused by the researchers and several plants are being monitored for their prospective therapeutic effects (Uprety *et al.*, 2010).

Timur is one of the prioritized medicinal plants for economic development in Nepal. Owing to its diverse applications, the species can be developed as an important commodity for alleviation of poverty in rural areas. The various ethno-pharmacological applications of timur have been verified by several related researches. More extensive study on the individual specific phyto-component can lead to novel innovations for the well-being of mankind (Phuyal *et al.*, 2019b). Mostly two types i.e. boke and sil timur is found in Nepal.

Zanthoxylum armatum, locally known as Boke Timur and Aankhe Timur, is a deciduous shrub or a small tree, around six meters tall or higher, with dense foliage, armed branched flattened prickles, yellow flowers and red seeds. Belonging to Rutaceae family, it naturally occurs in Nepalese forests and on open sites at altitudes ranging from 1000 to 2100 meters and can be found in different management systems (Paul *et al.*, 2018). It is distributed from Kashmir to Bhutan, north-east India and Pakistan, Laos, Myanmar, Thailand, China, Japan, North and South Korea, North Vietnam, and Taiwan (Phuyal *et al.*, 2019a). It is used in traditional medicinal systems for various ailments such as cholera, diabetes, cough, diarrhea, fever, headache, microbial infections, and toothache (Kalia *et al.*, 1999; Manandhar, 2002; Tiwary *et al.*, 2007). The dried fruits of the plant are used as condiment and have excellent spice value. The bark is ground and taken for relief fever, cholera and stomach disorder. Fruits

are chewed in toothache and stomachic. Fruits are widely used for making pickles. Fruits and thorns are used for fish poisoning (Malla *et al.*, 2014).

L. neesiana (Lauraceae), popularly known as Siltimur in Nepal, is a small tree that grows in the temperate Himalayan regions (Comai *et al.*, 2010). The fruits are aromatic and are used as a spice in preparation of various food products as well as traditional medicines to treat diarrhea, tooth pain, headache, and gastric disorders. In addition, it is used to eliminate intestinal parasites, and to treat plant poisoning in cattle (Manandhar, 2002; Subedi *et al.*, 2016; Watanabe *et al.*, 2013). Essential oils from *L neesiana* fruit consisted mainly of Z-citral, E-citral, eucalyptol, citronellal, α pinene and β pinene, and showed potent antibacterial and antifungal effects (Comai *et al.*, 2010). The fruits are chewed to cure diarrhoea and toothache. Leaves are used to cure skin diseases. Fruit juice is used to treat stomach disorder of livestock if they eat poisonous plants (Malla *et al.*, 2014).

An antioxidant is a substance that inhibit or delays oxidative damage to the cells of the organisms by scavenging the free radicals such as peroxide or hydroperoxide and thus reducing the risk of degenerative diseases (Yamagishi and Matsui, 2011). Phytochemicals are naturally occurring substances found in plants which provide health benefits. These are known as secondary metabolites and may often be created by modified synthetic pathways from primary metabolite or share substrates of primary metabolite origin (Kabera *et al.*, 2014). Different herbs and spices are the rich source of these phytochemicals which have powerful antioxidant activity Curcumin from turmeric, capsaicin from chili pepper and gingerol from ginger, eugenol from clove is some examples of bioactive compound with strong antioxidant activities (Guldiken *et al.*, 2018). Spices like clove, cumin coriander, nutmeg, pepper, cardamom etc. have a major antioxidant activity with their high content in phenolic and flavonoids compound (Slowianek and Leszczynska, 2016).

1.2 Statement of the problem

Timur is an indigenous plant whose different parts have medicinal value and are commonly used as spice in pickle. It is rich in phyto constituents and antioxidant such as phenol, flavonoid, tannin etc. Several studies reported that phyto constituents and antioxidant are good for disease prevention and overall health. But in Nepal, there is poor documentation and very

few researches were done previously on timur. Due to poor documentation and poor research there is less knowledge about timur in community. Therefore, the wastage of timur leads to poor production and utilization of it. Some experiments have been carried out regarding their phyto constituents and antioxidant properties. But the study from different habitats and different parts of the plants in different solvents is still meager, so the present study was carried out to quantify the total phenolic, total flavonoid and total tannin contents and evaluates the antioxidant properties in different extracts of the fruits, and bark of boke timur and sil timur.

1.3 Objectives of the study

1.3.1 General objective

The general objective of this dissertation is antioxidant and phytochemical activities of fruit and stem bark of boke and sil timur in different solvent.

1.3.2 Specific objectives

- To extract phyto constituents of fruit and bark of timur by using methanol, ethanol and petroleum ether solvents.
- To determine phytochemical constituents and antioxidant capacity of fruit and bark of timur.
- To compare phytochemical composition and antioxidant capacity of fruit and bark of timur.

1.4 Significance of the research

Timur is the medicinal herb, having large significance in people, containing phytochemicals and antioxidant, which shows beneficial effect in health. In the present scenario, the anti-diabetic, anti- hyper cholesterolemic, anti-carcinogenic, anti-inflammatory effect of different parts of medicinal herb have more importance, as a key health issue of mankind nowadays are diabetes, cardiovascular disease, arthritis and cancer.

This study will provide detailed information on phytochemicals and antioxidant activity of fruit and bark of boke and sil timur in methanol, ethanol and petroleum ether solvents. The outcomes of this study will be helpful to society/ community, further research purpose in

academic institution. It gives strong documentation and increasing productivity and utilization by providing information of timur.

1.5 Limitations of study

- Only methanolic, ethanolic, and petroleum ether extract were studied. Extraction in other solvent like chloroform, hexane etc. could not be performed.
- Other phyto constituents are not determined.

Part II

Literature review

2.1 Background

Medicinal plants have been used in several indigenous herbal practices since very old times to cure several diseases. Herbal medication still continues to serve as an important health care system even today despite the greater advancements in modern medication systems in the recent years. Their long uses in the folk medicine and their safer implications in human health have generated much interest in them, especially in developing countries. It has now been established that medicines derived from plant products are safer than their synthetic counterparts (Tejidos and Estral, 2007).

Zanthoxylum armatum is a small aromatic tree or large shrub up to 6m high. Branches are glabrous, usually armed with straight or slightly compressed, reddish brown stipular spines. The leaves are imparipinnate with 3–5 pairs of leaflets, elliptic-lanceolate, acuminate, base rounded or cuneate, sessile, margins usually entire, with a large gland associated with each tooth. The petiole and rachis are often winged between leaflets and sometimes bearing a spine at the point of insertion. Inflorescences are terminal panicles on short lateral shoots. Flowers are minute and polygamous, borne on short cymes. Male flower has 6–8 stamens, filaments 2mm arranged around globose pistillode. Female flower has 1–3 ovoid-subglobose carpels with two ovules attached to inner angle of axis. Fruit is a small drupe, reddish, ovoid and glandular warted, splitting into two when ripe. Fruits contain single rounded and shining black seeds, 2–3mm in size(Hedge and Long, 1994). The different parts of the plants: leaves, fruits, stem, bark, seeds have been used in several indigenous medicinal practices as carminative, antipyretic, appetizer, stomachic, toothache, dyspepsia (Kala *et al.*, 2005). The plant is used for treatment of pneumonia and tick infestation (Zia-ud-Din *et al.*, 2010). Powdered fruit, mixed with Menthaspp and table salt is eaten with boiled egg for chest infection and other digestive problems (Islam *et al.*, 2009).

Lindera neesiana Kurz (Lauraceae), popularly known as Siltimur in Nepal, is an aromatic and spicy plant with edible fruits. It is a traditional herbal medicine widely used for the

treatment of diarrhea, tooth pain, headache, and gastric disorders and is also used as a stimulant (Subedi *et al.*, 2016). Fruits of *Lindera neesiana* were used to treat diarrhoea in cattle (Pradhan and Badola, 2008).

Sil timur is a medium size deciduous tree growing upto 4m in height. It is distributed in Himalayan region from 1800-2700m; mainly in the eastern and central regions of Nepal (Polunin and Stainton, 1984; Press *et al.*, 2000; Sacherer, 1977).The fruits of this plant are aromatic and local people has been consuming for years as pickle and chewed for treating stomachache due to indigestion, diarrhea , toothache, nausea as anthelmintic and flatulence (Gurung, 2003; Manandhar, 2002; Pohle, 1990). Fruits are also given to cattle if they eat poisonous plants (Manandhar, 2002). Leaf is a good source fodder for cattle and goats. Leaves and branches are aromatic when crushed. Fruits, leaves and barks contain an essential oil.(RS Singh *et al.*, 1995). Essential oils from *L. neesiana* fruit consisted mainly of Z-citral, E-citral, eucalyptol, citronellal, *a* pinene and *b* pinene, and showed potent antibacterial and antifungal effects (Comai *et al.*, 2010).

2.2 Phytochemicals

Phytochemicals consist of a large group of naturally occurring non nutrient, biologically active compounds found in plants. As implied by the prefix “phyto” in the name, phytochemicals are basically produced only by plants. Phytochemicals acts as natural defense system for the host plants and in addition provide colour, aroma and flavor. Plants use phytochemicals as a natural protection from bacteria, fungi, and viruses (Ramanthan *et al.*, 1989). More than 4000 of these compounds have been discovered and it is expected that scientists will get discover many more phytochemicals in plant foods such as fruits, vegetables, legumes, cereals, herbs, and spices (Rowland, 1999).

A number of phytochemical are known, some of which include: alkaloids, saponins, flavonoids, tannins, glycosides, anthraquinones, steroids and terpenoids. They do not only protect the plants but have enormous physiological activities in humans and animals. These include cancer prevention, antibacterial, antifungal, antioxidative, hormonal action, enzyme stimulation and many more (Doss and Anand, 2012). Phytochemicals give hot pepper the burning sensation, onions and garlic the pungent flavor and tomatoes their red color

(Lesschaeve and Noble, 2005). Phytochemicals can have profound physiological effects, act as antioxidants, mimic body hormones and suppress development of disease in the body (Hayes, 2005).

2.2.1 Phenolics / Polyphenols

Polyphenols, which include more than 8000 compounds, are a family of natural compounds widely distributed in the outer layers of plant as suspected from their protective function in the plants (Manach *et al.*, 2004). Polyphenols occurs in all the plants foods and contribute to the beneficial health effects of vegetables and fruit (Balch and Balch, 2000). They range from simple molecules such as phenolic acid to highly polymerized compounds, such as tannins. Phenolic acids account for about one third of the total intake of polyphenols in human diet. These compounds are capable of removing free radicals, chelating, metal catalysts; active antioxidant enzymes, reducing α-tocopherol radicals, and inhibiting oxidases s(Oboh, 2006). As a result, they neutralize free radicals formed during normal physiological functioning of human body (Burns *et al.*, 2001). The antioxidant activity of phenols is due to their redox properties through which they act as hydrogen donors, singlet oxygen quenchers, reducing and metal chelating agents. There is a highly positive relationship between total phenols and antioxidant activity of many plant materials (I. Gülçin *et al.*, 2004).

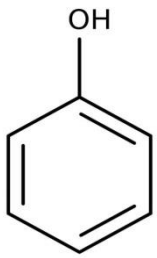

Fig.2.1. Structure of Phenol

In recent years, much attention has been paid by nutritionist on the dietary polyphenols due to their potent antioxidative effects and their credible effects in the prevention of various oxidative stress associated diseases. Oxidation process is one of the most important ways for producing free radicals in food and even in living systems. Free radicals cause many human

diseases like cancer, Alzheimer's, cardiac, kidney and liver diseases, fibrosis, atherosclerosis, arthritis, neurodegenerative disorders and aging (Halliwell and Gutteridge, 1990).

2.2.2 Flavonoids

Flavonoids are the largest group of phenolic compounds and have a basic skeleton composed of three rings (C_6-C_3-C_6). They are classified into six major classes according to their substitution pattern in the B-and C- rings, which are flavan-3-ols, anthocyanins, flavones, isoflavones, flavanones and flavonols (J. B. Harborne and Baxter, 1999). The flavonoid polymers are also known as proanthocyanidins. Flavonoids occur as plant secondary metabolites that are involved in pigmentation, antioxidants, antimicrobials, antistressors, and UV irradiation protection (Vaya and Aviram, 2001). More than 4000 flavonoids have been described so far within the parts of plants normally consumed by humans and approximately 650 flavones and 1030 flavanols are known (Ghasemzadeh *et al.*, 2010). Flavonoids are found in almost all plant based food and beverages, but the levels vary, depending on the degree of ripeness of fruits, variety and processing. Most flavonoids enhance the potency of vitamin C (ascorbic acid) and function as antioxidants. Antioxidant activity of flavonoids is believed to be due to their ability to act as free radical acceptor and to complex metal ions (Hertog *et al.*, 1992).

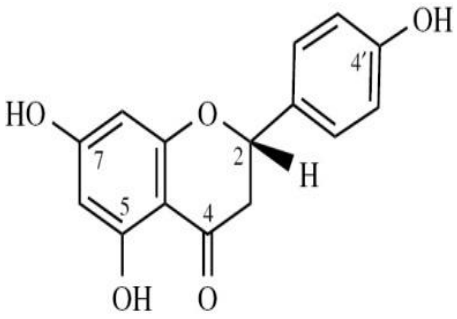

Fig.2.2. Structure of flavonoid

The previous studies showed that the ingestion of flavonoids reduces the risk of cardiovascular diseases, metabolic disorders, and certain types of cancer. These effects are due to the physiological activity of flavonoids in the reduction of oxidative stress, inhibiting low-density lipoproteins oxidation and platelet aggregation, and acting as vasodilators in blood vessels. Free radicals are constantly generated resulting in extensive damage to tissues leading

to various disease conditions such as cancer, Alzheimer's, renal diseases, cardiac abnormalities, etc., Medicinal plants with antioxidant properties play a vital functions in exhibiting beneficial effects and employed as an alternative source of medicine to mitigate the disease associated with oxidative stress. Flavonoids have existed over one billion years and possess wide spectrum of biological activities that might be able to influence processes which are dysregulated in a disease (David *et al.*, 2016).

2.2.3 Tannins

Tannins are polyphenols sometimes called plant polyphenols although originally the name tannin was given to the plant extracts exhibiting astringency, without knowing their chemical structures (Haslam, 1989). The features distinguishing tannins from plant polyphenols of other types are basically the properties of the former: binding to proteins, basic compounds, pigments, large-molecular compounds and metallic ions, and also antioxidant activities, etc (Okuda and Ito, 2011). These are widely distributed in plant flora. They are phenolic compounds of high molecular weight. Tannin are soluble in water and alcohol and are found in root, bark, stem and outer layers of plant tissue. They form complexes with proteins, carbohydrates, gelatin and alkaloids. On the basis of their structural characteristics it is therefore possible to divide the tannins into four major groups: Gallotannins, ellagitannins, complex tannins and condensed tannins (Saxena *et al.*, 2013).

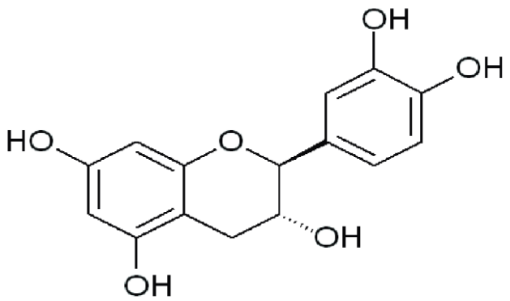

Fig.2.3. Structure of tannin

Tannins have diverse effect on biological system since they are potential metal ion chelators, protein precipitating agents and biological antioxidants. Because of varied biological roles that tannin can play and because of the enormous structural variation, it has

become difficult to develop models that would allow an accurate prediction of their effects in any system (Skowyra, 2014).

The tannin-containing plant extracts are used as astringents, against diarrhea, as diuretics, against stomach and duodenal tumors and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals (Dolara *et al.*, 2005). Recently the tannins have attracted scientific interest, especially due to the increased incidence of deadly illness such as AIDS and various cancers. The search for new compounds for the development of novel pharmaceuticals has become increasingly important, especially as the biological action of tannin-containing plant extracts has been well documented (Saxena *et al.*, 2013).

2.2.4 Antioxidants

An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property (Halliwell, 1995). These low-molecular-weight antioxidants can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Some of such antioxidants, including glutathione, ubiquinol, and uric acid, are produced during normal metabolism in the body (Shi *et al.*, 1999). Other lighter antioxidants are found in the diet. Although there are several enzymes system within the body that scavenge free radicals, the principle micronutrient (vitamins) antioxidants are vitamin E (α-tocopherol), vitamin C (ascorbic acid), and B-carotene(Levine *et al.*, 1999). The body cannot manufacture these micronutrients, so they must be supplied in the diet.

Antioxidants are the compound that inhibit free radicals and prevent from the oxidative damage of a molecule. Oxidation can produce free radical which can damage the cells of organism leading various chronic diseases such as cancer, diabetes, cardiovascular and neurological diseases, etc. Oxidative stress can be induced by many negative factors like unhealthy diet, radiations, adverse environmental condition, and psycho-emotional stress (Devkota *et al.*, 2006; Serafini and Peluso, 2016). Abnormal production of free radicals may cause several severe human diseases such as cancer; Alzheimer's disease; cardiac, kidney, and liver diseases; fibrosis; atherosclerosis; arthritis; neurodegenerative disorders; and aging.

Several medicinal plants have been screened for their antioxidant and other biological activities (Malesh and Satish, 2008; Martin and Ernst, 2003; Upadhyay *et al.*, 2010). Antioxidant is a chemical compound either synthesized or naturally isolated that inhibits the oxidation process happening in the body of living organisms. Oxidation is a chemical process that can generate free radicals that cause chain reactions and damage the body cells. Antioxidants are synthetic and natural and can terminate the chain reactions occurring in the body of living organisms (Dhakal and Sharma, 2020).

2.2.4.1 Mechanism of action of antioxidants

Two principle mechanisms of action have been proposed for antioxidants (Rice-Evans and Diplock, 1993) The first is a chain- breaking mechanism by which the primary antioxidant donates an electron to the free radical present in the systems. The second mechanism involves removal of ROS/reactive nitrogen species initiators (secondary antioxidants) by quenching chain-initiating catalyst. Antioxidants may exert their effect on biological systems by different mechanisms including electron donation, metal ion chelation, co-antioxidants, or by gene expression regulation (Krinsky, 1992).

2.3 Phytochemical metabolism in human

Most phytochemicals found in foods exist in a variety of forms which influence their digestion and absorption. Most common ones are the polyphenols which exist as glycoside conjugates. Some glycosides must be digested to aglycones (unconjugated forms) before being absorbed. Some other forms of phytochemicals are thought to be absorbed in the intestines without intensive digestion. The absorption of most phytochemicals is thought to involve a carrier. Also, many glycosides are neither digested nor absorbed in the small intestine. Such phytochemicals not absorbed in the small intestine have been shown to undergo microbial degeneration by colonic microflora (Ross and Kasum, 2002). The bacteria hydrolyse the glycosides, generating aglycones which may undergo further metabolism to form various aromatic compounds (Bradlow *et al.*, 1999).

Once absorbed, most phytochemical metabolites get conjugated in the small intestine or in the liver (Rhodes, 1996). Conjugation most often involves methylation, sulfating or glucunnidation. These conjugated metabolites are then bound to plasma proteins such as

albumin and are transported through the blood to various parts of the body. The amount of these conjugated metabolites in the plasma varies considerably win the type of polyphenol consumed, the food source, and the amount ingested (Kris-Etherton *et al.*, 2002). However after consumption of specific polyphenols, little is known about the metabolism of the different polyphenols in the body, and also about what metabolites are present in the plasma (Briskin, 2000).

2.4 Proximate Constitutes:

2.4.1 Proximate composition of boke timur (*Zanthoxylum armatum)*.

Table 2.1 Proximate composition of boke timur (on dry basis):

Constituents (%)	Fruit	Bark
Carbohydrates	59.59± 0.06	38.64± 0.11
Protein	7.74± 0.04	10.98± 0.01
Fat	8.90± 0.03	1.22± 0.05
Fiber	10.50±0.17	30.83± 0.10
Ash	11.90± 0.30	13.49± 0.01

(Barkatullah *et al.*, 2015)

2.4.2 Proximate composition of sil timur (*lindera neesiana*).

Table 2.2 Proximate composition of sil timur (on dry basis %):

Constituents (%)	Fruit
Carbohydrates	26.3
Protein	7.1
Fat	42.15
Fiber	14.4

Nutrient content per 100 gm of sample

Source: (DFTQC Food Composition Table 2017)

2.5 Effect of proximate constitutes:

Proximate and nutrient analyses of plants are important for determination of nutritional value. Various medicinal plant species are utilized for curing various ailments. Besides medicinal value, proximate analysis is also important, to ascertain nutritional worth of these plant as well.

Carbohydrates are considered the primary source of energy for all organisms, playing nutritional as well as structural role. High carbohydrates contents suggest suitability of the plant as feed. It is imperative to increase protein production by utilizing all the available ways and means as they play both curative and nutritive role.

Crude fibers are found in higher amount in non-starchy materials 23 and are considered good for the treatment of diseases like diabetes, gastrointestinal disorders, obesity and cancer (Ayoola *et al.*, 2010) .

High ash content is an indication of the mineral stuffing available in the plant materials. Ash values ascertained high deposit of minerals in the plant tissue, already explored in the present study.

2.6 Phytochemical Composition of Timur:

Phytochemicals are bioactive non-nutrient plant compounds in fruits, vegetables, whole grains, and other plant foods that have been hypothesized to reduce the risk of major chronic diseases (Liu, 2004). *Zanthoxylum armatum* consists of the following constituents extracted from different parts of the plant i.e. seed, leaves, fruit, root and barks etc. which are alkaloids, flavonoids, saponin, tannins, steroids, terpenes, glycosides, carbohydrates, phenolic, proteins, essential oil and amino acids, Viz., arginine, aspartic and glutamic acid, glycine, histidine, threonine, tyrosine.

For *Zanthoxylum armatum* , Alkaloids (19.60±0.10mg/g), sterols (33.83±0.29mg/g), saponins (14.78±0.10mg/g), tannins (8.62±0.13mg/g), phenols (91.20±1.33mg/g) and flavonoids (18.33±1.22mg/g) were reported form the bark. While fruits were reported to have alkaloids (25.07±0.21mg/g), sterols (164.92±0.14mg/g), saponins (28.60±0.10mg/g), tannins (25.5±0.5mg/g), phenols (110.67±0.44mg/g) and flavonoids (22.8±1.33mg/g) which is done in ethanolic extract (Ibrar *et al.*, 2017).

In methanol extract, TPC value of that for wild fruit was (185.02 ± 2.15 mg GAE/g). Similarly, the value was (185.15 ± 1.22 mg GAE/g) for wild bark. TFC value for wild fruit, it was (103.7 ± 1.39 mg QE/g) and the TFC values were (91.27 ± 3.13 mg QE/g) for wild barks (Phuyal *et al.*, 2020) . The total flavonoid content of ethanolic extracts of *Z. armatum* fruit was 22.8 ± 1.33 mg/g and that of bark was 18.33 ± 1.22 mg/g (Ibrar *et al.*, 2012). TPC values was found highest in fruit of boke timur (187.32 ± 1.43 mg GAE/g) than bark of boke timur (173.22± 3.15 mg GAE/g) , TFC of boke timur (fruit) was (108.21±2.32 mg QE/g) and (91.42±1.21 mg QE/g) for boke timur (bark) (Alam and Ashraf, 2019). TPC content of sil timur fruit was found to be (82.54 mg GAE/g*) and (53.28 mg GAE/g*) for bark of sil timur, TFC of sil timur (fruit) was (24.34 mg QE/g) and (78.22 mg QE/g) for Sil timur (bark) (Joshi *et al.*, 2016).

Total tannin content in methanolic extract of boke timur (fruit) was found to be (29.21±2.23 mg TAE/g) and boke timur (bark) was found to be (9.45±4.32 mg TAE/g) Cai *et al.* (2004). Total tannin content in sil timur (fruit) was found to be (45.66±1.54 mg TAE/g) and sil timur (bark) was found to be (9.43±3.12 mg TAE/g) Agbo *et al.* (2015). Sil timur

(fruit) (48.34±1.56 mg TAE/g) have high tannin content among selected plants (Batubara *et al.*, 2012). Tannin content of fruit and bark of boke and sil timur was (32.10±2.09 mg TAE/g), (11.21±1.02 mg TAE/g), (48.33±5.32 mg TAE/g) and (4.43±1.31 mg TAE/g) respectively (Khan *et al.*, 2011). Total tannin content in ethanolic extract of sil timur (fruit) was (31.25 mg TAE/g) and tannin was not present in ethanolic extract of sil timur (bark) (Soni and Sosa, 2013). In phytochemical screening tannin was not present in ethanolic and petroleum ether extract of sil timur bark (Chhetri and Khatri, 2017). (Kheroda Devi *et al.*, 2019) and (Adebola *et al.*, 2017).

The TPC values of boke timur (fruit) was (72.32±3.12 mg GAE/g) and boke timur (bark) was (46.58±1.05 mg GAE/g), TFC value of boke timur (fruit) was (19.21±1.11 mg QE/g) and boke timur (bark) was (13.01±2.43 mg QE/g) and tannin content of boke timur (fruit) was (19.89±3.32 mg TAE/g) and boke timur (bark) was (11.46±1.09 mg TAE/g) in petroleum ether extract (Mukhija and Kalia, 2014) and TPC of sil timur (bark) was (22.82±2.21 mg GAE/g) and sil timur (fruit) was (55.05±2.31 mg GAE/g), TFC of sil timur (bark) was (24.02±0.32 mg QE/g) and sil timur (fruit) was (15.32±1.43 mg QE/g) and tannin content of sil timur (fruit) was (32.67±2.56 mg TAE/g) (Cao *et al.*, 2016). Highest TPC content was found in fruit of boke timur (75.86±1.23 mg GAE/g) among selected herbs and spices (Sepahpour *et al.*, 2018).

For *lindera neesiana*, TPC content of sil timur fruit was found to be (82.54±2.19 mg GAE/g) and (53.28±3.12 mg GAE/g) for bark of sil timur in methanolic extract (Joshi *et al.*, 2016). TPC value of methanol extract for fruits was (83.95±0.93 mg GAE/g) and for TFC value of fruit was (9.13 mg QE/g). TPC value of ethanol extract of stem bark was (68.34±0.38 mg GAE/g dry wt) and TFC value of ethanol extract of stem bark was (118.0±1.41 mg QE/g dry wt) (Chhetri and Khatri, 2017). TPC values of ethanolic extract of bark of sil timur was (76.42±1.08 mg GAE/g) and fruit was (70.21± 2.43 mg GAE/g), TFC value of sil timur (fruit) was (19.23± 2.21 mg QE/g) and (96.56± 1.12 mg QE/g) and tannin content of sil timur fruit was (36.43±1.09 mg TAE/g) (Namsa *et al.*, 2011).

2.7 Antioxidant capacity of Timur

Antioxidants, which are compounds that inhibit and/or reduce the effects of free radicals (Soares *et al.*, 2005),can be defined as compounds that protect the cells against the harmful effects of oxygen and nitrogen free radicals that are formed in oxidative processes. High free radical levels generate an imbalance, triggering oxidative stress, the metabolic process responsible for the onset of several types of chronic degenerative diseases. Antioxidants can be obtained by eating food containing vitamins E and C, carotenoids, phenolic compounds, and other compounds (Ali *et al.*, 2008). Phenolic compounds are responsible for most of the antioxidant activity in fruits, making them a natural source of antioxidants (Heim *et al.*, 2002). The phenolic content in food and plants depends on a number of intrinsic factors such as the genus, species and cultivar, and on extrinsic factors such as agronomic and environmental factors, handling and storage (Tomás-Barberán and Espín, 2001).

Antioxidant property can be inferred on the basis of % radical scavenging activity (RSA) and IC_{50} value. Antioxidant activity DPPH inhibition of the plant extract is expressed as % inhibition of stable radical or inhibition concentration fifty (IC_{50}) in reference to a standard compound. The plant with higher % RSA has the lower IC_{50}. The plant extract with lowest IC_{50} value is considered having better antioxidant properties (İ. Gülçin *et al.*, 2012).

For *Zanthoxylum armatum*, the higher IC_{50} value indicates lower radical scavenging activity or lower antioxidant potential. The fruits extracts had the highest antioxidant capacity compared to the seeds and bark extracts. The IC_{50} value of the fruits extracts was close to that of the standard, i.e 45.62 μg/mL for wild fruits. And the bark showed moderate antioxidant capacity with the IC_{50} value of 67.82 μg/mL for wild extracts (Phuyal *et al.*, 2020). The free radical scavenging activity of methanolic fruits extracts ranged from 59.56 to 64.85% (Nooreen *et al.*, 2017). The IC_{50} value of *Zanthoxylum armatum* bark in methanolic extracts was (149.26±3.00 μg/mL). Antioxidant activity was found high in fruits of timur than bark (Irshad *et al.*, 2021). IC_{50} value of boke timur fruit was (46.38 μg/mL) and bark of boke timur was (65.23 μg/m) (M. Singh and Shikha, 2017). IC_{50} value of bark of boke timur was 149.26 µg/mL (Mukhija and Kalia, 2014). IC_{50} values of boke timur fruit was (52.54 μg/mL) and boke timur bark was (75.21 μg/mL) Brijwal *et al.* (2013). IC_{50} values of boke timur fruit was (56.12 μg/mL) and bark of boke timur was (83.33 μg/mL) in ethanolic extract Mukhtar and Kalsi

(2018). IC_{50} value of boke timur (fruit) (69.21 μg/mL), was found to be high in selected herbs Skotti *et al.* (2014). IC_{50} value of boke timur fruit was (66.20 μg/mL) and bark of boke timur was (78.65 μg/mL)in petroleum ether extract (Karmakar *et al.*, 2015). The highest antioxidant activity was found in boke timur fruit (71.67 μg/mL) (Sepahpour *et al.*, 2018).

For *Lindera neesiana*, thus, antioxidant activity of the different methanolic plant extracts were determined using the solution of DPPH (0.2 mM) and taking ascorbic acid as the pure antioxidant reference compound. IC_{50} value of standard (ascorbic acid) found for Lindera neesiana fruit for methanol extract was 42.29 μg/mL (Adhikari, 2016). DPPH radical scavenging activity of ethanolic samples of *lindera neesiana* stem bark expressed in term of IC_{50} value was 175.32 μg/mL (Chhetri and Khatri, 2017). Sil timur fruit have high antioxidant activity with compared to bark of it (Candy *et al.*, 2013). IC_{50} value of sil timur fruit was (60.21 μg/mL) in ethanolic extract Khalaf *et al.* (2008). IC_{50} value of sil timur fruit was (76.04 μg/mL) and bark of sil timur was (204.34 μg/mL) in petroleum ether extract (Karmakar *et al.*, 2015).

2.8 Factor affecting Phytochemical and Antioxidant Activity:

2.8.1 Cultivar effect:

Genetic composition is the main determining factor which can directly influence the phytochemicals of vegetables, since it has been shown that the differences in phytochemical compounds between cultivars are greater than those between plants of the same cultivar grown under different conditions (Hu, 2012). The strong effect of genetic materials on the phenolic profile has been demonstrated in several horticultural crops such as potato, fava bean, tomato, garlic, globe artichoke and cardoon (Rouphael *et al.*, 2016).

2.8.2 Extraction method and solvent used

Solvent type and extract preparation methods affect phytochemical concentration and different activities of plants. Polar solvent enables extraction of significant amounts of phenolics and flavonoids. The study done by (Vidic *et al.*, 2014) shows that there is significant difference in the phytochemical contents due to the difference in the method of extraction used where Soxhlet extraction was better than ultrasound extraction of the sample.

Methanolic extract induced the best extraction yield and more complex composition of phenolics. Moreover, scientists have discovered that highly polar solvents, such as methanol, have a high effectiveness as antioxidants (Altemimi *et al.*, 2017). Another study reveals that solvent used in extraction affects the yield, total phenolic, total flavonoid, flavonols, condensed tannins, carotenoids contents and their antioxidant and antimicrobial properties in various degrees. The highest contents of phytochemical contents were obtained with methanol extracts (Felhi *et al.*, 2017) . Similar result was given by the study of (Widyawati *et al.*, 2014) where methanolic extract showed better phytochemical content than ethanol, aquadest, ethyl acetate and hexanes.

2.8.3 Environmental factors

Environmental factors including sunshine radiation, temperature variation and climatic conditions within a geographical location may influence the level of phytochemicals (Oh *et al.*, 2009). Mineral composition, soil type, temperature, light and water content are among the frequently reported factors that affect the total phytochemical contents in plants (Dolara *et al.*, 2005). Optimum fertilization is important to ensure favourable levels of phytochemicals. High

levels of fertilizer (nitrogen, phosphorus and 21 potassium) application may result in increased vegetative growth and yield with a decrease in the level of phytochemicals (Tiwari and Cummins, 2013).

2.8.4 Post-harvest factors:

Phytochemicals are very susceptible to deterioration during postharvest, due to fungal decay, physiological disorders, pests, mechanical injury, over-ripeness, and inadequate temperature and relative humidity during storage or transport, which may result in great losses in quality components including phytochemical (Carillo-López and Yahia, 2017).

Food-processing operations also have a major influence on the stability of phytochemicals in fruit and vegetables and their products. Conventional (thermal), modern or non-thermal (e.g. high-pressure processing pulsed electric field, ultrasound/sonication, ozone, ultraviolet), domestic (e.g. washing, peeling, cutting) and industrial (e.g. canning, drying) processing are widely reported to degrade the level of phytochemicals in processed food products (Tiwari and Cummins, 2013).

2.8.5 Post-harvest storage conditions:

Storage temperature, atmosphere gas composition and use of chemicals are major factors that influence the quantity and quality of phytochemicals (Li *et al.*, 2012). Lower temperature can slow the deterioration of phytochemicals. Opposed to lower temperature, high temperature also brings a significant change in total phenolic, flavonoid, tannin content and antioxidant activity compared with its fresh form. However, their concentration may vary according to the drying methods used and the duration of exposure to hot air (Michalczyk *et al.*, 2009). Exposure to high temperature leads to discharge of phenolic compounds through disintegration of cellular constituents which then results in the migration of components, leading to losses by leakage or breakdown by various chemical reactions involving enzymes, light and oxygen as well as (Davey *et al.*, 2000).

2.8.6 Others

An appropriate extraction of phenolic compounds depends on multiple factors, such as their chemical nature, raw material, storage time and conditions. Not least it depends on the

extraction and quantification methods, choice of standards, and presence of interference (Dimcheva and Karsheva, 2018).

2.9 Different method of extraction of phytochemical constituents

The extraction methods mostly used has been discussed below:

2.9.1 Cold extraction method

The different parts of plants dried in an artificial environment at low temperature (50-60°C) and dried powder then further used for extraction purpose using various solvents. Weigh the dried powder and added into conical flask with respective solvents and allow keeping at room temperature for thirty minute shaking after each twenty four hours for seven days. Finally filter the extract using whatman filter paper under vacuum and dry it at room temperature in watch glass dish. Note down the weight of each dish prior to drying of the extracts and after drying too. Calculate the weight of the extract from the difference (J. Harborne, 1973).

2.9.2 Solvent extraction method

Universal Extraction System (Buchi) is recently used for solvent extraction. The dried powder of various plant parts placed in glass thimble for extraction purpose using various solvents. The procedure is carried out for 10 cycles for each extract and adjusts the temperature just below the boiling point of the respective solvents. The resulting solvent extract is filtered, concentrated in vacuum concentrator and used to determine the presence of phytoconstituents (J. Harborne, 1973).

2.9.3 Supercritical fluid extraction (SFE)

Supercritical Fluid Extraction (SFE) involves use of gases, usually CO_2, and compressing them into a dense liquid. This liquid is then pumped through a cylinder containing the material to be extracted. From there, the extract-laden liquid is pumped into a separation chamber where the extract is separated from the gas and the gas is recovered for re-use. Solvent properties of CO_2 can be manipulated and adjusted by varying the pressure and temperature. The advantages of SFE are, no solvent residues left in it as CO_2 evaporates completely (Patil and Shettigar, 2010).

2.9.4 Microwave-assisted extraction (MAE)

It simply termed as microwave extraction, that combines microwave and traditional solvent extraction. Heating the solvents and plant tissue using microwave increases the kinetic of extraction, is called microwave-assisted extraction (Delazar *et al.*, 2012). The target for heating in dried plant material is the minute microscopic traces of moisture that occurs in plant cells. The heating up of this moisture inside the plant cell due to microwave effect, results in evaporation and generates tremendous pressure on the cell wall. The cell wall is pushed from inside due to the pressure and the cell wall ruptures. Thus the exudation of active constituents from the ruptured cells occurs, hence increasing the yield of phyto constituents (Dell'Ova *et al.*, 1974; Gordy *et al.*, 1966).

2.10 Different methods of phytochemical and antioxidant determination

2.10.1 Different methods of antioxidant determination

- DPPH radical scavenging activity
- Superoxide radical scavenging effect
- Reducing power
- Chelating effect on ferrous ions

Antioxidant activity assays employed the inhibition of free radical DPPH test/method which is one of the oldest and most frequently used methods for total antioxidant potential/capacity of food and biological extracts. The radical scavenging activity values were expressed as the ratio percentage of sample absorbance decrease and the absorbance of DPPH solution in the absence of extract at 517 nm (İ. Gülçin, 2009).

2.10.2 Different methods of phytochemical determination

- Gas Chromatography
- High Performance Liquid Chromatography (HPLC)
- High Performance Thin Layer Chromatography (HPTLC)
- Optimum Performance Laminar Chromatography (OPLC)

Qualitative and quantitative analysis of phytochemicals can be done using Gas Chromatography Mass Spectroscopy (GCMS). GCMS can be applied to solid, liquid and gaseous samples. First the samples are converted into gaseous state then analysis is carried out

on the basis of mass to charge ratio. High Performance Liquid Chromatography is applicable for compounds soluble in solvents. High performance thin layer chromatography is applicable for the separation, detection, qualitative and quantitative analysis of phytochemicals.

Part III

Materials and Methods

3.1 Materials

3.1.1 Raw materials

Boke timur (fruit), boke timur (bark), sil timur (fruit) and sil timur (bark) were collected in village of Dhankuta, Nepal.

3.1.2 Equipment and chemicals

The list of chemicals and equipment used for the analysis is shown in appendix B.

3.2 Method

3.2.1 Outline of experimental procedure

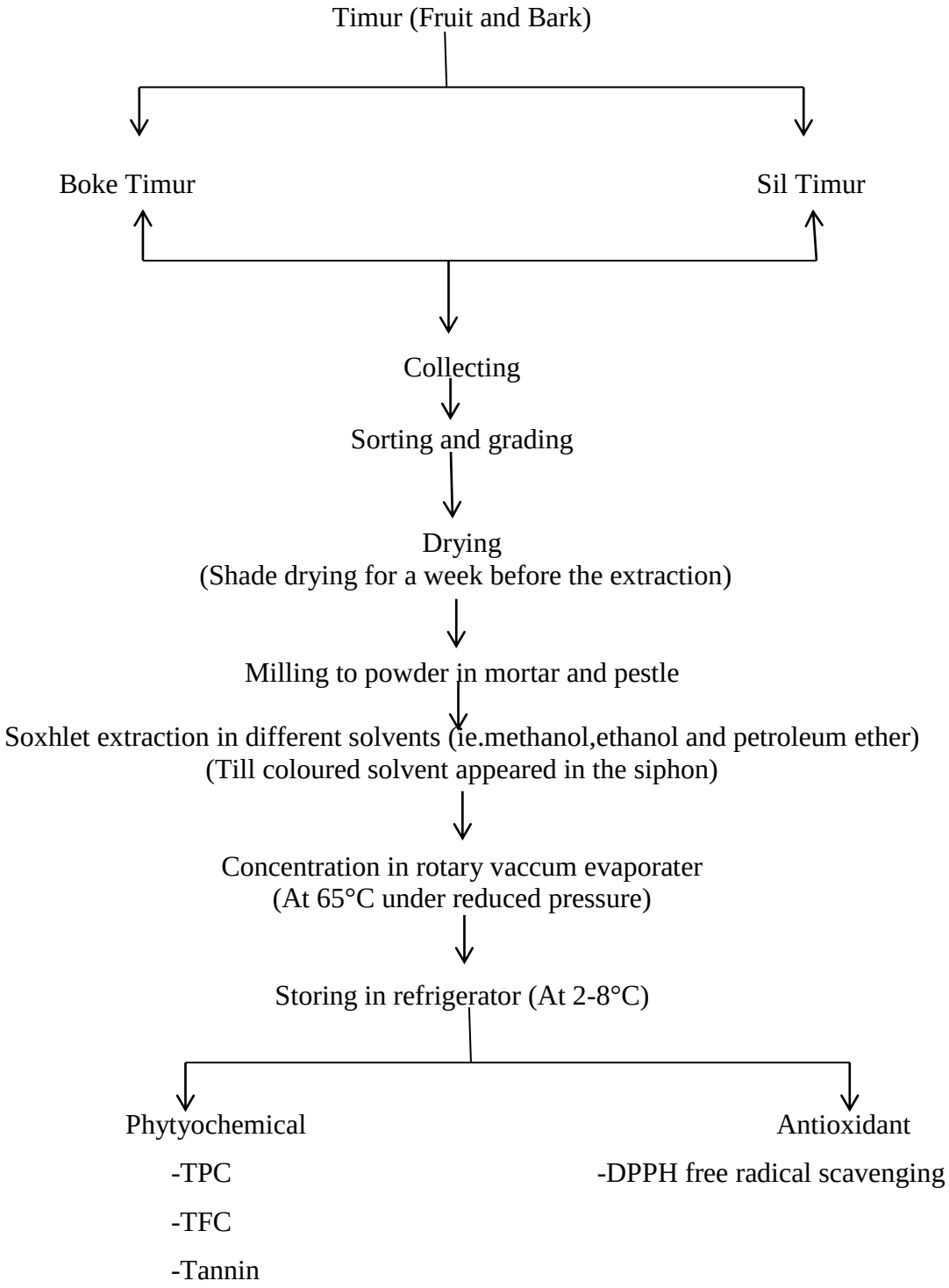

Fig.3.1 Flow diagram of methodology

3.3 Experimental procedure

3.3.1 Chemical analysis

3.3.1.1 Proximate analysis

3.3.1.1.1 Determination of moisture content

Moisture content involves the reflux distillation of the food with an immiscible solvent having a higher boiling point and a lower specific gravity than water, e.g. toluene, heptane or xylene. The refluxed water settles as the solvent floats in a graduated tube, in which it can be measured by volume. The volatile oils which also distil over remain mixed with the solvent and are not measured. The equipment used for this method of moisture determination is called Dean and Stark apparatus as (Ranganna, 1986).This results were expressed in terms of percentage.

$$\text{Moisture content, \%} = \frac{\text{Volume of water (ml)}}{\text{Wt of sample taken}} \times 100$$

3.3.1.1.2 Determination of crude protein

Crude protein was determined by the Kjeldahl method, total protein was calculated by multiplying the nitrogen content by a factor of 6.25 (Ranganna, 1986). The calculated data were presented per 100 g on dry basis.

$$\text{Nitrogen \%} = \frac{(\text{sample titre-black titre}) \times \text{Normality of HCl} \times 14 \times 100}{\text{Wt of sample} \times 100}$$

3.3.1.1.3 Determination of ash content:

The ash content was determined by incinerating the seeds (5 g) in a muffle furnace at 525ºC for 4-6 hours (Ranganna, 1986). The calculated data were presented as g/100 g on dry basis.

$$\text{\% of toal ash} = \frac{\text{wt of the ash}}{\text{Wt of sample taken}} \times 100$$

3.3.1.1.4 Determination of crude fat

The fat content of the samples was determined as described in (Ranganna, 1986). The calculated data were presented as gram per 100 g on dry basis.

$$\% \text{ crude fat} = \frac{\text{Wt of ether soluble materials} \times 100}{\text{Wt of sample}}$$

3.3.1.1.5 Determination of crude fibre

Crude fiber was determined by using chemical process, the sample was treated with boiling dilute Sulphuric acid, boiling sodium hydroxide and then with alcohol as standard method of (Ranganna, 1986). The calculated data were presented as g/100 g on dry basis.

$$\% \text{ Crude fiber} = \frac{(\text{loss in wt noted})}{\text{Wt of sample taken}} \times 100$$

3.3.1.1.6 Determination of carbohydrate

Total carbohydrate content of the samples were determined by difference method.

Carbohydrate % =100 - [sum of protein, total ash, fiber and fat]

Procedure for extraction

Universal Extraction System (Buchi) is recently used for solvent extraction. The dried powder of various plant parts placed in glass thimble for extraction purpose using various solvents. The procedure is carried out for 10 cycles for each extract and adjusts the temperature just below the boiling point of the respective solvents. The resulting solvent extract is filtered, concentrated in vacuum concentrator and used to determine the presence of phyto constituents (J. Harborne, 1973).

3.3.1.2 Qualitative Analysis for Phytochemicals

The plant extracts were screened for the presence of the phytochemical classes by using the standard following methods (Jaradat *et al.*, 2015).

a) Test for Proteins

- Ninhydrin test: Boil 2 ml of 0.2% Ninhydrin solution with the entire plant crude extract, appeared violet color indicate the presence of proteins and amino acids.

b) Test for Carbohydrates

- Iodine test: 2 ml of iodine solution mixed with crude plant extract. Purple or dark blue colors prove the presence of the carbohydrate.

c) Test for Phenols and Tannins

- Two milliliters of 2% solution of $FeCl_3$ mixed with crude extract Black or blue-green color indicated the presence of tannins and phenols.

d) Tests for Flavonoids

- Alkaline reagent test: 2 ml of 2% NaOH solution was mixed with plant crude extract, intensive yellow color was formed, which turned into colorless when added 2 drops of diluted acid to solution, this result indicated the presence of flavonoids.

e) Test for Saponins

- Five milliliters of distilled water was added to crude plant extract in a test tube and it was shaken vigorously. The foam formation indicated the presence of saponins.

f) Test for glycosides

- Salkowski's test: H_2SO_4 concentrated (about 2 ml) was added to the entire plant crude extract. A reddish-brown color produced indicated the entity of steroidal aglycone part of the glycoside.

g) Test for Steroid

- Two milliliter of chloroform and concentrated H_2SO_4 were mixed with the entire plant crude extract. In the lower chloroform layer produced red color that indicated the presence of steroids. Another test was performed by mixing 2 ml of each of acetic acid with H_2SO_4

concentrated and crude extract with 2 ml of chloroform. Green color indicated the entity of steroids.

3.3.1.3 Phytochemicals Quantitative Analysis

3.3.1.3.1 Total phenolic content

TPC was determined using the Folin–Ciocalteu method (Singleton *et al.,* 1999) with slight modifications. The reaction mixture was prepared by mixing 0.5 ml of plant extract solution, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of Na_2CO_3 aqueous solution. The samples were thereafter incubated in a thermostat at 45°C for 45 min. The absorbance was determined using spectrophotometer at wave length=765nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of gallic acid equivalent expressed in terms of (mg of GAE/g of extract).

3.3.1.3.2 Total flavonoid content

Total flavonoid content was determined using a modified aluminum chloride assay method as described by Barek and Hasmadi (Barek *et al.,* 2015). 2 ml of solution was pipette out in a test tube in which 0.2 ml of 5% Sodium Nitrate ($NaNO_3$) was mixed and stand for 5 minutes. 0.2 ml of 5% Aluminum Chloride ($AlCl_3$) was pipetted out, mixed in the tube and allowed to stand for 5 minutes. This followed addition of 2 ml of 1N Sodium Hydroxide (NaOH) in the tube and finally volume was made up to 5ml. The absorbance was measured after 15 minutes at 510nm against a reagent blank. The test result was correlated with standard curve of Quercetin (20, 40, 60, 80, 100µg/ml) and the total flavonoid content is expressed as mg QE/g of dry weight.

3.3.1.3.3 Total tannin content

The tannins were determined by Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% Na_2CO_3 solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and100µg/ml) were prepared in

the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/ Visible spectrophotometer. The tannin content was expressed in terms of mg of TAE /g of extract (Marinova *et al.*, 2005; Miean and Mohamed, 2001; Rajeev Singh *et al.*, 2012).

3.3.1.3.4 Free Radical Scavenging Activity Using (DPPH)

Extracts (100µL) were dissolved in 3.9 mL freshly prepared methanolic solution of DPPH (1 mM, 0.5 mL). The mixture was vortexed for 15 seconds and then left to stand at room temperature for 30 min in the dark. The absorbance of the resulting solution was read spectrophotometrically (UV/VIS spectrometer) at 517 nm. The percentage inhibition of the radicals due to the antioxidant activity of leaf extracts was calculated using the following formula (Hatano *et al.*, 1988).

% inhibition = ($A_{control} - A_{sample}$)/ $A_{control} \times 100$

$A_{control}$ is the absorbance of the DPPH solution with nothing added (control).

I% = percentage of inhibition.

The radical scavenging activities of the extracts are expressed in terms of their IC_{50} values. The data were presented as mean values ± standard deviation ($n = 3$). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity (Zhao *et al.*, 2008).

3.3.2 Statistical Analysis

Analysis was carried out in triplicate. Data on analysis of TPC, TFC, DPPH radical scavenging activity, and tannin, in different part of sample and different solvents were tabulated for comparison and using Microsoft excel-2010. Data were statistically processed by Gene stat version 12.1.0.3338 for analysis of variance (ANOVA). Means of data were compared by post hoc method at 5 % level of significance.

Part IV

Result and discussion

Fruit and bark of two varieties of Timur were collected from village of Dhankuta, district. The part of Timur was first seperated and dried. The dried sample were grinded to fine powder and extracted by using different solvents such as methanol, ethanol and petroleum ether .These extracts were used for further quantitative analysis of total phenolic content, total flavonoid content , tannin and DPPH free radical scavenging activity.

4.1 Proximate composition of sample:

Proximate composition of fruit and stem bark of boke timur and sil timur are presented in table 4.1.

Table 4.1 Proximate composition of Timur (on dry basis %)

Constituents	Boke-timur (fruit)	Boke-timur (bark)	Sil-timur (fruit)	Sil-timur (bark)
Ash content	$10.05^{c}\pm0.05$	$13.02^{b}\pm0.02$	$3.8^{d}\pm0.08$	$18.02^{a}\pm0.10$
Crude protein	$7.23^{b}\pm0.03$	$9.56^{a}\pm0.06$	$6.86^{c}\pm0.05$	$4.43^{d}\pm0.05$
Crude fat	$7.04^{b}\pm0.04$	$1.05^{c}\pm1.11$	$43.5^{a}\pm2.06$	$1.35^{c}\pm0.06$
Crude fiber	$12.03^{c}\pm0.08$	$33.36^{b}\pm0.14$	$12.07^{c}\pm0.05$	$36.36^{a}\pm0.88$
Carbohydrate	$60.76^{a}\pm0.26$	$37.3^{b}\pm0.14$	$27.37^{d}\pm0.65$	$33.93^{c}\pm0.05$

*Note: Values are the mean ±SD of three determination . All the values are expressed on dry basis.

The mean value of ash content in fruit and stem bark of boke and sil timur was 10.05, 13.02, 3.08 and 18.02% respectively. The analysis of variance (Appendix B) showed that there was significant difference between ash content in different samples ($p< 0.05$).

The mean value of crude protein in fruit and stem bark of boke and sil timur was 7.23, 9.56, 6.86 and 4.43% respectively. The analysis of variance (Appendix B) showed that there was significant difference between protein content in different samples ($p< 0.05$).

The mean value of crude fat in fruit and stem bark of boke and sil timur was 7.04, 1.05, 43.05 and 1.35% respectively. The analysis of variance (Appendix B) showed that there was significant difference between crude fat in different samples ($p< 0.05$).

The mean value of crude fiber in fruit and stem bark of boke and sil timur was 12.03, 33.36, 12.07 and 36.36% respectively. The analysis of variance (Appendix B) showed that there was significant difference between crude fiber in different samples ($p< 0.05$).

The mean value of carbohydrate in fruit and stem bark of boke and sil timur was 60.76, 37.03, 27.37 and 33.93% respectively. The analysis of variance (Appendix B) showed that there was significant difference between carbohydrate in different samples ($p< 0.05$).

Similar results were reported by (Barkatullah *et al.*, 2015) and DFTQC food composition table 2017, who showed the mean value of ash content, protein, fat, crude fiber and carbohydrate were 11.90, 7.74, 8.90, 59.59 and 10.50% respectively for boke timur (fruit), 13.49, 10.98, 1.22, 38.64 and 30.83% respectively for boke timur (bark), protein 7.1, fat 42.15, carbohydrate 26.3 and crude fiber 14.4% respectively for sil timur (fruit). Similar results was followed by (Nugroho *et al.*, 2021).

4.2 Preliminary phytochemical qualitative analysis of timur

The methanolic, ethanolic and petroleum ether extract of fruit and stem bark of boke and sil timur was prepared to conduct preliminary phytochemical analysis and shown in table 4.2.

Table 4.2 Preliminary phytochemical qualitative analysis

	Boke T (fruit)			Boke T (bark)			Sil T (fruit)			Sil T (bark)		
Test	M	E	PE	M	E	PE	M	E	PE	M	E	PE
Protein	+	+	+	+	+	+	+	+	+	+	+	+
Carbohydrate	+	+	+	+	+	+	+	+	+	+	+	+
Phenol	+	+	+	+	+	+	+	+	+	+	+	+
Tannin	+	+	+	+	+	+	+	+	+	+	-	-
Flavonoid	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+	+	+	-
Steroids	+	+	+	-	-	-	+	+	+	-	-	-

*Note: Positive= +, Negative= -

The phytochemical screening of methanolic, ethanolic and petroleum ether extract of fruit and stem bark of boke and sil timur showed presence of protein, carbohydrate, phenol, flavonoid, saponins and steroids was present in fruit of boke and sil timur. In ethanoli c and petroleum ether extract of stem bark of sil timur, tannin was absent. Similar result were reported by (Chhetri and Khatri, 2017). Similar result was followed by (Kheroda Devi *et al.*, 2019) and (Adebola *et al.*, 2017).

4.3 Effect of solvent on phyto constituents of different part of timur

4.3.1 Total phenolic content of different parts of timur

The total phenol content of fruit and stem bark of timur extracted by methanol, ethanol and petroleum ether are given in table 4.3.

Table 4.3 Total phenol content of methanolic, ethanolic and petroleum ether extracts of different parts of timur

Sample	Total Phenol Content (mg GAE/g)		
	Methanolic Extract	Ethanolic Extract	Petroleum ether Extract
Boke timur (fruit)	175.06^{c}±4.40	106.44^{d}±1.30	69.33^{d}±1.03
Boke timur (bark)	169.27^{c}±1.76	90.79^{c}±1.08	46.0^{b}±1.55
Sil timur (fruit)	79.05^{b}±0.52	64.35^{b}±0.57	59.82^{c}±0.40
Sil timur (bark)	54.23^{a}±1.17	60.39^{a}±0.15	19.33^{a}±0.96

*Note: The values in Table 4.3 are the mean of the triplicates ± standard deviation. Values in the column having the same superscript are not significantly different at 5% level of significance.

Among methanolic extracts, mean value of total phenol content was found highest in fruit of boke timur (175.06 mg GAE/g), followed by bark of boke timur (169.27 mg GAE/g), fruit of sil timur (79.05 mg GAE/g) and bark of sil timur (54.23 mg GAE/g) respectively. Statistical analysis at 5% level of significance (Appendix B) showed that there was significantly difference with each samples in total phenol content, whereas fruits of boke timur and bark of boke timur was not significantly different.

Among ethanolic extracts, mean value of total phenol content was found highest in fruits of boke timur (106.44 mg GAE/g), followed by bark of boke timur (90.79 mg GAE/g), fruits of sil timur (79.05 mg GAE/g) and bark of sil timur (54.23 mg GAE/g) respectively. Statistical analysis at 5% level of significance (Appendix B) showed that there was significantly difference with each samples in total phenol content.

Among petroleum ether extracts, mean value of total phenolic content was found highest in fruit of boke timur (69.33 mg GAE/g), followed by fruit of sil timur (59.82 mg GAE/g), bark of boke timur (46.0 mg GAE/.g) and bark of sil timur (19.33 mg GAE/g) respectively.

Statistical analysis at 5% level of significance (Appendix B) showed that there was significantly difference with each samples in total phenol content.

Phuyal *et al.* (2020) reported that total phenolic content in crude extract (in 80% methanol) of wild timur (fruit) was found to be (185.02 mg GAE/g) and (185.15 mg GAE/g) for wild bark. Adhikari (2016) reported that the total phenol content of sil timur (fruit) was found to be (83.95 mg GAE/g) and similar results was reported by Joshi *et al.* (2016). This results was accordance with Chhetri and Khatri (2017), Similar result was followed by Ibrar *et al.* (2012) and Namsa *et al.* (2011) in ethanolic extract. Similar results was reported by Mukhija and Kalia (2014) and followed by Cao *et al.* (2016) and Sepahpour *et al.* (2018) in petroleum ether extract.

4.3.2 Total flavonoid content of different part of timur

The total flavonoid content of fruit and stem bark of timur extracted by methanol, ethanol and petroleum ether are given in table 4.4.

Table 4.4 Total flavonoid content of methanolic, ethanolic and petroleum ether extracts of different parts of timur

Sample	Total Flavonoid Content (mg QE/g)		
	Methanolic Extract	Ethanolic Extract	Petroleum ether Extract
Boke timur (fruit)	91.42^{c}±0.98	28.78^{b}±1.41	18.23^{b}±0.98
Boke timur (bark)	88.47^{c}±1.82	19.14^{a}±0.69	10.29^{a}±1.006
Sil timur (fruit)	23.40^{a}±4.26	17.01^{a}±1.48	14.41^{b}±1.77
Sil timur (bark)	80.76^{b}±1.40	98.47^{c}±1.02	23.52^{c}±2.16

*Note: The values in Table 4.4 are the mean of the triplicates ± standard deviation. Values in the column having the same superscript are not significantly different at 5% level of significance.

Among methanolic extracts, mean value of total flavonoid content was found highest in fruit of boke timur (91.42 mg QE/g), followed by bark of boke timur (88.47 mg QE/g), bark of sil timur (80.76 mg QE/g) and fruit of sil timur (23.40 mg QE/g) respectively. Statistical analysis of 5% level of significance (Appendix B) showed that there was significantly difference with each samples in total flavonoid content, whereas fruit of boke timur and bark of boke timur was not significantly different.

Among ethanolic extracts, mean value of total flavonoid content was found highest in bark of sil timur (98.47 mg QE/g), followed by fruit of boke timur (28.78 mg QE/g), bark of boke timur (19.14 mg QE/g) and fruit of sil timur (17.01 mg QE/g) respectively. The statistical analysis at 5% level of significance (Appendix B) showed that there was significantly difference with rach samples in total flavonoid content, whereas bark of boke timur and fruit of sil timur was not significantly different.

Among petroleum ether extracts, mean value of total phenolic content was found highest in bark of sil timur (23.52 mg QE/g), followed by fruit of boke timur (18.23 mg QE/g), fruit of sil timur (14.41 mg QE/.g) and bark of boke timur (10.29 mg QE/g) respectively. The statistical analysis at 5% level of significance (Appendix B) showed that there was significantly difference with each samples in total flavonoid content, whereas fruit of boke timur and fruit of sil timur was not significantly different.

Boke timur (fruit) value was lower than Phuyal *et al.* (2020) reported that total flavonoid content in wild boke timur (fruit) was found to be (103.7 mg QE/g) and boke timur wild bark was found to be(91.27 mg QE/g). Adhikari (2016) reported that TFC of sil timur (fruit) was found to be (23.89 mg QE/g) and sil timur (bark) was found to be (84.21 mg QE/g) and similar results was followed by Alam and Ashraf (2019) and Joshi *et al.* (2016) in methanolic extract. This results was in accordance with Chhetri and Khatri (2017). Similar result was followed by (Ibrar *et al.*, 2017) and Namsa *et al.* (2011) in ethanolic extract. The results was in accordance with Mukhija and Kalia (2014) and same results was followed by Cao *et al.* (2016) and Sepahpour *et al.* (2018) in petroleum ether extract.

4.3.3 Total tannin content of different parts if timur

The total tannin content of fruit and stem bark of timur extracted by methanol, ethanol and petroleum ether are given in table 4.5.

Table 4.5 Total tannin content of methanolic, ethanolic and petroleum ether extracts of different parts of timur

Sample	Total Tannin Content (mg TAE/g)		
	Methanolic Extract	Ethanolic Extract	Petroleum ether Extract
Boke timur (fruit)	$28.78^{c}\pm0.95$	$17.19^{b}\pm0.85$	$19.17^{b}\pm1.04$
Boke timur (bark)	$10.57^{b}\pm1.43$	$6.15^{a}\pm0.82$	$7.61^{a}\pm0.58$
Sil timur (fruit)	$44.04^{d}\pm1.70$	$33.31^{c}\pm2.39$	$28.31^{c}\pm1.38$
Sil timur (bark)	$6.46^{a}\pm0.78$	ND*	ND*

*Note- *ND=Not detected, the values in Table 4.5 are the mean of the triplicates ± standard deviation. Values in the column having the different superscript are significantly different at 5% level of significance.

Among methanolic extracts, mean value of total tannin content was found highest in fruit of sil timur (44.04 mg TAE/g), followed by fruit of boke timur (28.78 mg TAE/g), bark of boke timur (10.57 mg TAE/g) and bark of sil timur (6.46 mg TAE/g) respectively. Statistical analysis at 5% level of significance (Appendix B) showed that there was significantly difference with each samples in total tannin content.

Among ethanolic extracts, mean value of total tannin content was found highest in fruit of sil timur (33.31 mg TAE/g), followed by fruit of boke timur (17.19 mg TAE/g), bark of boke timur (6.15 mg TAE/g) and tannin was not detected in bark of sil timur. Statistical analysis at 5% level of significance (Appendix B) showed that there was significantly difference with each samples in total tannin content.

Among petroleum ether extracts, mean value of total tannin content was found highest in fruit of sil timur (28.31 mg TAE/g), followed by fruit of boke timur (19.17 mg TAE/g), bark of boke timur (7.61 mg TAE/g) and tannin was not detected in bark of sil timur. Statistical analysis at 5% level of significance (Appendix B) showed that there was significantly difference with total tannin content.

Cai *et al.* (2004) reported that total tannin content in boke timur (fruit) was found to be (29.21 mg TAE/g) and boke timur (bark) was found to be (9.45 mg TAE/g). Agbo *et al.* (2015) reported that Total tannin content in sil timur (fruit) was found to be (45.66 mg TAE/g) and sil timur (bark) was found to be (9.43 mg TAE/g). Similar results was followed by Batubara *et al.* (2012) and Khan *et al.* (2011) in methanolic extract. Similar results was followed by (Ibrar *et al.*, 2017), Namsa *et al.* (2011), (Soni and Sosa, 2013).and also followed by Chhetri and Khatri (2017). Kheroda Devi *et al.* (2019) and Adebola *et al.* (2017) in ethanolic extract. Chhetri and Khatri (2017), (Kheroda Devi *et al.*, 2019) and (Adebola *et al.*, 2017).also reported that tannin content in sil timur (bark) was not present in petroleum ether extract. This may be because of drying of raw timur and its bark as Obeta (2015) reported that the drying of Vernonia amygdalina and Gongronema latifolium leaves increases it's tannin content. The results was in accordance with Mukhija and Kalia (2014) and same results was followed by Cao *et al.* (2016).

4.3.4 DPPH radical scavenging activity of different parts of timur

The DPPH radical scavenging activity of fruit and stem bark of timur extracted by methanol, ethanol and petroleum ether are given in table 4.6.

Table 4.6 Total DPPH radical scavenging activity of methanolic, ethanolic and petroleum ether extracts of different parts of timur

Sample	DPPH radical scavenging activity (IC_{50})		
	Methanolic Extract	Ethanolic Extract	Petroleum ether Extract
Boke timur (fruit)	47.64^{a}±1.05	55.27^{a}±1.47	70.03^{a}±1.68
Boke timur (bark)	70.36^{b}±2.24	78.84^{c}±2.57	81.09^{b}±0.68
Sil timur (fruit)	49.82^{a}±2.93	64.44^{b}±1.13	72.04^{a}±3.08
Sil timur (bark)	145.11^{c}±0.86	196.06^{d}±2.03	210.06^{c}±4.04

*Note: The values in Table 4.6 are the mean of the triplicates ± standard deviation. Values in the column having the same superscript are not significantly different at 5% level of significance.

Ascorbic acid used as the standard and the different extracts showed variable antioxidant properties. The IC_{50} value of ascorbic acid was 39.22μg/mL, which is shown in (appendix B). The higher IC_{50} value indicates lower radical scavenging activity or lower antioxidant potential (Phuyal *et al.*, 2020). Among methanolic extracts, mean value of DPPH radical scavenging activity was found highest in fruit of boke timur (47.64 μg/mL), followed by fruit of sil timur (49.82 μg/mL), bark of boke timur (70.36 μg/mL) and bark of sil timur (145.11 μg/mL) respectively. Statistical analysis at 5% level of significance (Appendix B) showed that there was significantly difference with each samples in DPPH free radical scavenging activity, whereas fruit of boke timur and fruit of sil timur was not significantly different.

Among ethanolic extracts, mean value of DPPH radical scavenging activity was found highest in fruit of boke timur (55.27 μg/mL), followed by fruit of sil timur (64.44 μg/mL), bark of boke timur (78.84 μg/mL) and bark of sil timur (196.06 μg/mL) respectively. The statistical analysis at 5% level of significance (Appendix B) showed that there was significantly difference with each samples in DPPH free radical scavenging activity.

Among petroleum ether extracts, mean value of DPPH radical scavenging activity was found highest in fruit of boke timur (70.30 μg/mL), followed by fruit of sil timur (72.40 μg/mL), bark of boke timur (81.90 μg/mL) and bark of sil timur (210.6 μg/mL) respectively.

The statistical analysis at 5% level of significance (Appendix B) showed that there was significant difference with each samples in DPPH free radical scavenging activity, whereas fruit of boke timur and fruit of sil timur was not significantly different.

The result was in accordance with Phuyal *et al.* (2020), IC_{50} values of fruit of boke timur and bark of boke timur was **(**45.62 µg/mL**)** and (67.82 µg/mL**)** respectively. The IC_{50} value of the boke timur and fruit of sil timur extracts was close to that of ascorbic acid standard. Adhikari (2016).reported that IC_{50} value for fruit of sil timur was 42.29 µg/mL and Mukhija and Kalia (2014) also reported that IC_{50} values of bark of boke timur was 149.26 µg/mL which is similar. Similar results was followed by (Irshad *et al.*, 2021), (M. Singh and Shikha, 2017) and (Candy *et al.*, 2013) in methanolic extract. The results was in accordance with Brijwal *et al.* (2013), Mukhtar and Kalsi (2018) Chhetri and Khatri (2017) and Khalaf *et al.* (2008) in ethanolic extract. The results was in accordance with Skotti *et al.* (2014) , in which IC_{50} value of boke timur (fruit) (69.21 µg/mL), was found to be high in selected herb. Similar results was followed by Karmakar *et al.* (2015) and Sepahpour *et al.* (2018) in petroleum ether extract.

Part V

Conclusions and recommendations

5.1 Conclusions

The crude methanolic, ethanolic and petroleum ether extracts of fruit and bark of boke timur and sil timur were analysed for total phenol content, total flavonoid content, total tannin content and antioxidant activities. Based on the results following conclusions were drawn:

1. The total phenol content was found highest in boke timur (fruit) in methanolic extract (175.06 mg GAE/g), whereas lowest was found in sil timur (bark) in petroleum ether extract (19.33 mg GAE/g).
2. The total flavonoid content was found highest in boke timur (fruit) in methanolic extract (91.42 mg QE/g), whereas lowest was found in boke timur (bark) in petroleum ether extract (10.29 mg QE/g).
3. The total tannin content was found highest in sil timur (fruit) in methanolic extract (44.04 mg TAE/g), whereas lowest was found in sil timur (bark) in methanolic extract (6.46 mg TAE/g) and tannin was not present in sil timur (bark) in ethanolic and petroleum ether extract.
4. The DPPH radical scavenging activity was found highest in boke timur (fruit) in methanolic extract (IC_{50} =47.64 μg/mL), whereas lowest was found in sil timur (bark) in petroleum ether extract (IC_{50} =210.06 μg/mL).
5. Hence, extraction of phytochemical and antioxidant can be seen high in methanol and in boke timur (fruit).

5.2 Recommendations

1. Phytochemical constituents of other parts of timur plants and their antioxidant activity can also be studied.
2. Others solvents can also be used for extraction of phyto constituents.
3. Other phyto constituents can also be studied.

Part VI

Summary

Phytochemicals in foods and their effects on disease risk is limited, but there's enough evidence mostly from looking at the association between foods rich in phytochemicals and disease risk to strongly suggest that consuming foods and beverages rich in these compounds may help prevent disease. However, it isn't known whether the health benefits are the result of individual phytochemicals, the interaction of various phytochemicals, the fiber content of plant foods, or the interaction of phytochemicals and the vitamins and minerals found in the same foods. Phytochemicals like phenols, flavonoids, tannin and antioxidant are naturally present in the plants. There is evidence from laboratory studies that phytochemicals and antioxidant may reduce the risk of cardiovascular disease, cancer, typ-2 DM, neurogeneration etc.

Thus, in the current study medicinal herb like fruit and stem bark of boke timur and sil timur were bought from village of Dhankuta district. These sample were collected, sorted, milled to fine powder. This powder sample were put in thimble and extracted using methanol, ethanol and petroleum ether solvents in soxhlet extraction. This extract concentrated in rotary vacuum evaporator at 65°C under reduced pressure and stored at 2-8°C. The analysis was made for total phenol content, total flavonoid content, total tannin content DPPH radical scavenging activity.

The proximate analysis of fruit and stem bark of boke and sil timur was done. The qualitative preliminary phytochemical analysis of the parts of medicinal herb was performed using methanol, ethanol and petroleum ether to detect the presence of phyto-constituents. All three solvent showed the presence of phenol, flavonoid tannin and antioxidant activity but tannin was absent in ethanol and petroleum ether extract of bark of sil timur.

The total phenol content was found highest in boke timur (fruit) in methanolic extract (175.06 mg GAE/g), whereas lowest was found in sil timur (bark) in petroleum ether extract (19.33 mg GAE/g) The total flavonoid content was found highest in boke timur (fruit) in methanolic extract (91.42 mg QE/g), whereas lowest was found in boke timur (bark) in

petroleum ether extract (10.29 mg QE/g). The total tannin content was found highest in sil timur (fruit) in methanolic extract (44.04 mg TAE/g), whereas lowest was found in sil timur (bark) in methanolic extract (6.46 mg TAE/g) and tannin was not detected in sil timur (bark) in ethanolic and petroleum ether extract. The DPPH radical scavenging activity was found highest in boke timur (fruit) in methanolic extract (IC_{50} =47.64 μg/mL), whereas lowest was found in sil timur (bark) in petroleum ether extract (IC_{50} =210.06 μg/mL. So, extraction of phytochemical and antioxidant can be seen high in methanol and in boke timur (fruit).

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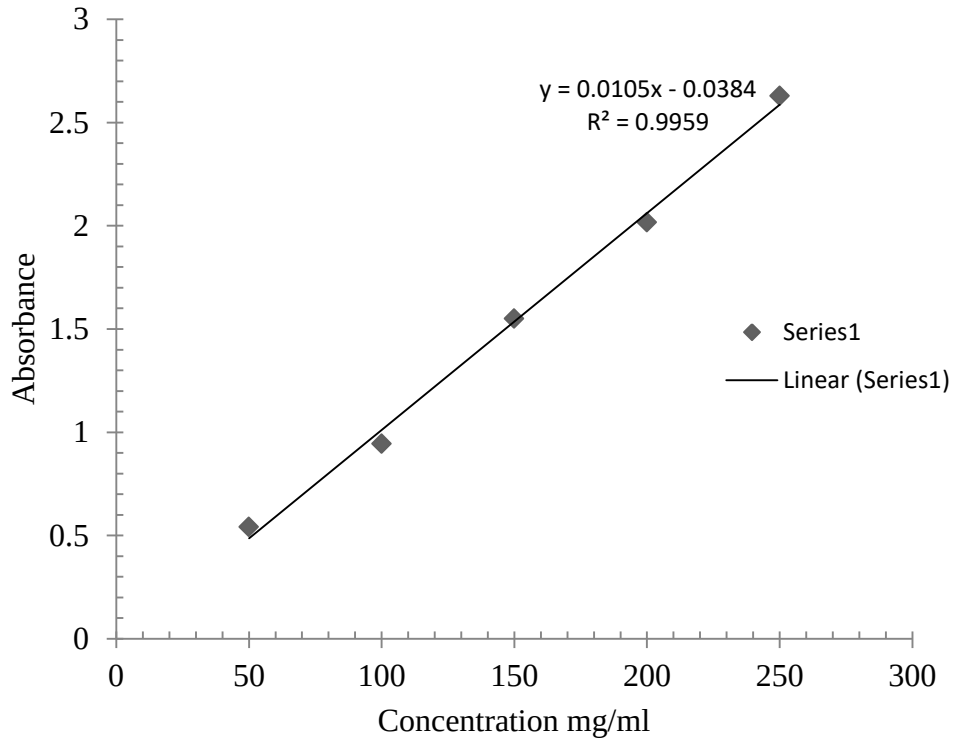
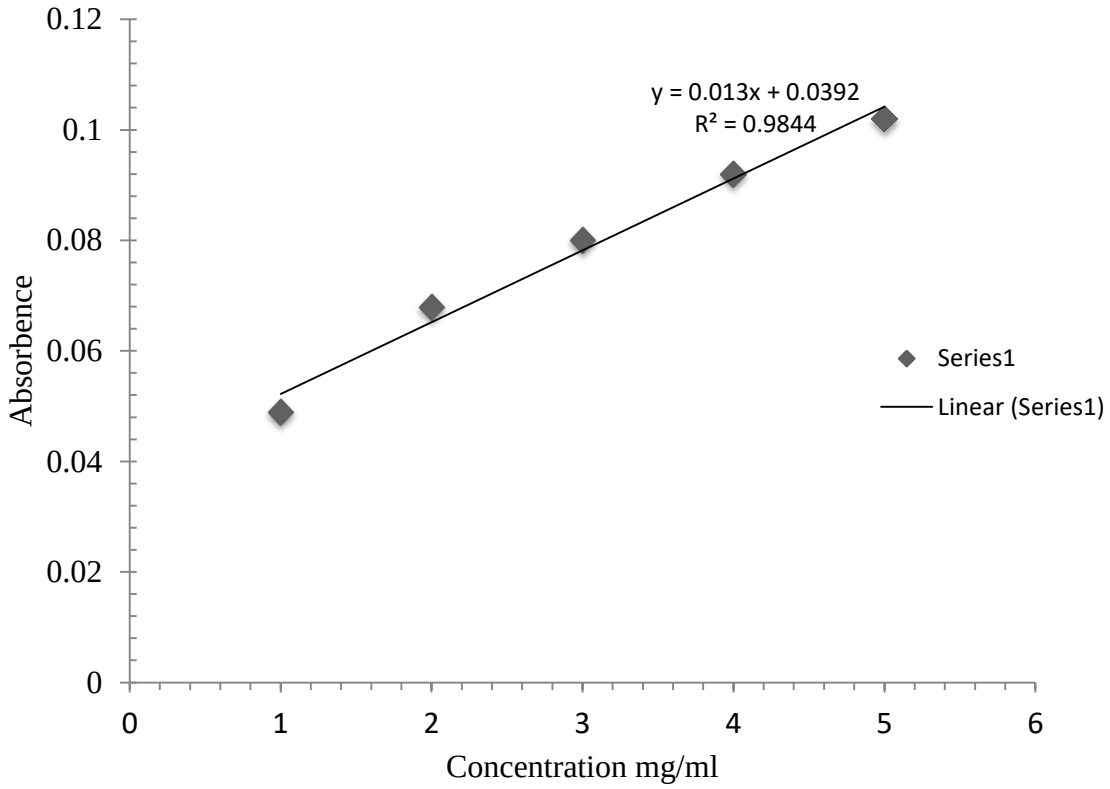
Appendices

Appendix A

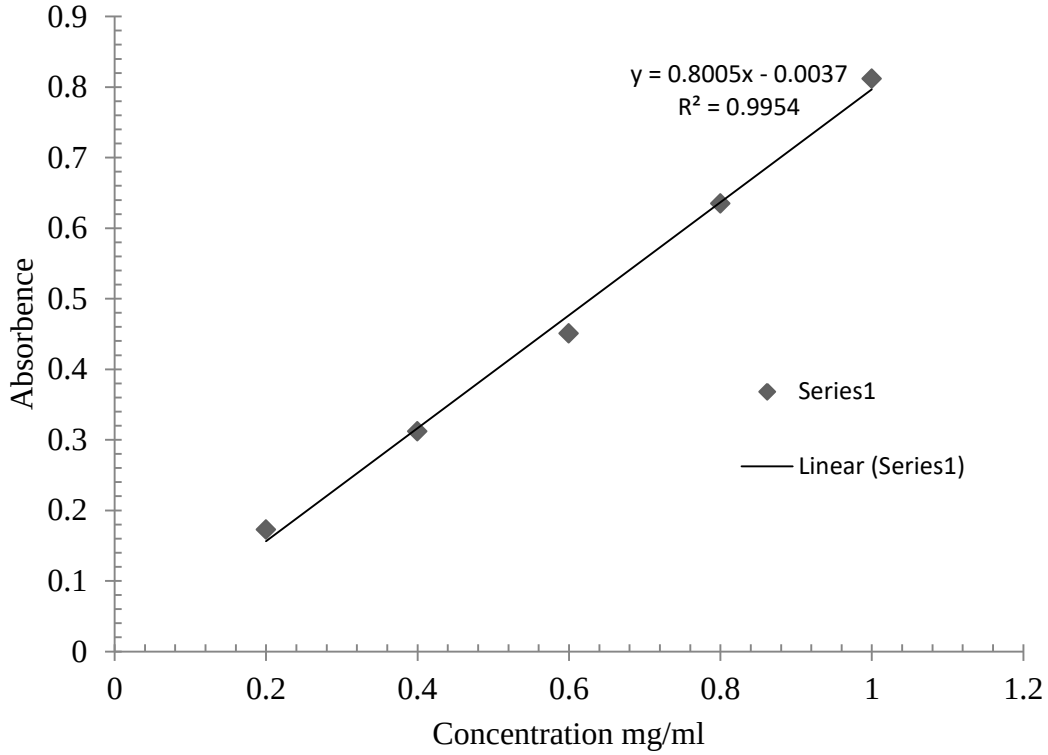
1. Standard curve gallicacid for total phenol content



2 Calibration Curves of Quercetin for flavonoid



3. Calibration curves of tannic acid for tannin



Appendix B

Table B 1.1 List of chemical used

Chemical	Supplier/Manufacturer	Other Specifications
Sodium hydroxide (NaOH)	Thermo Fisher Scientific India Pvt. Ltd.	Pellets, AR grade, 98%
Hydrochloric acid (HCL)	Thermo Fisher Scientific India Pvt. Ltd.	36%, LR grade
Sulphuric acid (H_2SO_4)	Thermo Fisher Scientific India Pvt. Ltd.	97%, LR grade
Boric acid	Merck (India) Limited	Amorphous
Potassium Permanganate	Avantor Performance Materials ltd.	99% Assay
Potassium thiocyanate	Thermo Fisher Scientific India Pvt. Ltd.	97% Assay
Tannic acid	Avarice LaboratoriesPvt. Ltd.	Analytical Reagent
Nitric acid	Fisher Scientific India Pvt. Ltd.	68-75% Assay
Sulphuric acid	Fisher Scientific India Pvt. Ltd.	97% Assay
DPPH	HiMedia Laboratories Pvt. Ltd.	
Hydrochloric acid	Fisher Scientific India Pvt. Ltd.	35-37% Assay
Quarcetin	Avarice Laboratories Pvt. Ltd	Analytical reagent
Gallic acid	Avarice Laboratories Pvt. Ltd	Analytical reagent
L-ascorbic acid	S.D. fine chemicals Ltd.	99% Assay

Table B 1.2 List of equipment used

Physical apparatus	Specification
Electric balance	Phoneix instruments, India
Spectrophotometer	Labtronics, India
Soxhlet apparatus	Y.P. scientific glass work
Hot air oven	Victolab, India
Incubator	Y.P. scientific glass work
Muffle furnace	Accumax, India
Rotary vacuum evaporator	OEM manufacturer, India
Colorimeter	Jenway Ltd., UK
Heating mantle	Y.P. scientific glass work

ANOVA tables

Table B 1.3 One way ANOVA for ash content

Source of variation	df	s.s.	m.s.	v.r.	F pr.
Sample	3	339.50916	113.1697	1344.19	<.001
Residual	8	0.67353	0.08419		
Total	11	340.18269			

Table B 1.4 One way ANOVA for moisture content

Source of variation	df	s.s.	m.s.	v.r.	F pr.
Sample	3	39.7919	13.26397	5253.06	<.001
Residual	8	0.0202	0.002525		
Total	11	39.8121			

Table B 1.5 one way ANOVA for crude protein content

Source of variation	df	s.s.	m.s.	v.r.	F pr.
Sample	3	39.7919	13.26397	5253.06	<.001
Residual	8	0.0202	0.002525		
Total	11	39.8121			

Table B 1.6 One way ANOVA for crude fat content

Source of variation	df	s.s.	m.s.	v.r.	F pr.
Sample	3	3731.017	1243.672	1169.77	<.001
Residual	8	8.505	1.063		
Total	11	3739.522			

Table B 1.7 One way ANOVA for crude fiber content

Source of variation	df	s.s.	m.s.	v.r.	F pr.
Sample	3	1573.707	524.569	2570.58	<.001
Residual	8	1.6325	0.2041		
Total	11	1575.339			

Table B 1.8 One way ANOVA for carbohydrate content

Source of variation	df	s.s.	m.s.	v.r.	F pr.
Sample	3	1903.999	634.6665	2739.96	<.001
Residual	8	1.8531	0.2316		
Total	11	1905.853			

Table B. 1.9 Two way ANOVA for TPC

a. Methanol:

Tukey HSDa,b

Samples		Subset		
	N	1	2	3
Sil T (bark)	3	54.2367		
Sil T (fruit)	3		79.0533	
Boke T (bark)	3			169.2767
Boke T (fruit)	3			175.0667
Sig.		1.000	1.000	0.078

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square (Error) = 6.038. a. Uses Harmonic Mean Sample Size = 3.000,b. Alpha = .05.

b.Ethanol

Tukey HSDa,b

Sample		Subset			
	N	1	2	3	4
Sil T (bark)	3	60.3900			
Sil T (fruit)	3		64.1567		
Boke T (bark)	3			90.79	
Boke T (fruit)	3				106.4467
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square (Error) = 1.234. a. Uses Harmonic Mean Sample Size = 3.000. b. Alpha = .05.

c. Petroleum ether

Tukey HSDa,b

Sample	N	Subset			
		1	2	3	4
Sil T (bark)	3	19.3333			
Boke T (bark)	3		46.3933		
Sil T (fruit)	3			59.5400	
Boke T (fruit)	3				69.3300
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square(Error) = .854. a. Uses Harmonic Mean Sample Size = 3.000. b. Alpha = .05.

Table B 1.10 Two way ANOVA for TFC

a. Methanol

Tukey HSDa,b

Sample	N	Subset		
		1	2	3
Sil T (fruit)	3	23.4067		
Sil T (bark)	3		79.9633	
Boke T (bark)	3			88.4733
Boke T (fruit)	3			91.4267
Sig.		1.000	1.000	0.508

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square (Error) = 6.254. a. Uses Harmonic Mean Sample Size = 3.000. b. Alpha = .05.

b.Ethanol

Tukey HSDa,b

Sample		Subset		
	N	1	2	3
Sil T (fruit)	3	17.0167		
Boke T (bark)	3	19.1413		
Boke T (fruit)	3		26.8867	
Sil T (bark)	3			98.4733
Sig.		0.192	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square (Error) = 1.353.a. Uses Harmonic Mean Sample Size = 3.000. b. Alpha = .05.

c. Petroleum ether

Tukey HSDa,b

Sample		Subset		
	N	1	2	3
Boke T (bark)	3	10.2967		
Sil T (fruit)	3		14.4100	
Boke T (fruit)	3		18.2467	
Sil T (bark)	3			23.5200
Sig.		1.000	0.063	1.000

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square (Error) = 2.398.a. Uses Harmonic Mean Sample Size = 3.000. b. Alpha = .05.

Table B 1.11 Two way ANOVA for total tannin content

a. Methanol

Tukey HSDa,b

Sample		Subset			
	N	1	2	3	4
Sil T (bark)	3	6.4600			
Boke T (bark)	3		10.5733		
Boke T (fruit)	3			28.7867	
Sil T (fruit)	3				44.0400
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square(Error) = 1.622. a. Uses Harmonic Mean Sample Size = 3.000. b. Alpha = .05.

b. Ethanol

Tukey HSDa,b

Sample		Subset			
	N	1	2	3	4
Sil T (bark)	3	0.0000			
Boke T (bark)	3		6.1533		
Boke T (fruit)	3			17.1933	
Sil T (fruit)	3				33.3167
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square(Error) = 1.787. a. Uses Harmonic Mean Sample Size = 3.000.b. Alpha = .05.

c. Petroleum ether

Tukey HSDa,b

Sample	N	Subset			
		1	2	3	4
Sil T (bark)	3	0.0000			
Boke T (bark)	3		7.6133		
Boke T (fruit)	3			19.7167	
Sil T (fruit)	3				27.3100
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square(Error) = .552.a. Uses Harmonic Mean Sample Size = 3.000. b. Alpha = .05.

Table B 1.12 Two way ANOVA for DPPH free radical scavenging activity

a. Methanol

Tukey HSDa,b

Sample	N	Subset		
		1	2	3
Boke T (fruit)	3	47.6367		
Sil T (fruit)	3	49.8233		
Boke T (bark)	3		70.3567	
Sil T (bark)	3			145.1133
Sig.		0.542	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square(Error) = 3.750. a. Uses Harmonic Mean Sample Size = 3.000. b. Alpha = .05.

b. Ethanol

Tukey HSDa,b

Sample	N	Subset			
		1	2	3	4
Boke T (fruit)	3	55.2667			
Sil T (fruit)	3		64.4367		
Boke T (bark)	3			78.8367	
Sil T (bark)	3				196.0600
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square(Error) = 3.555. a. Uses Harmonic Mean Sample Size = 3.000. b. Alpha = .05.

c. Petroleum ether

Tukey HSDa,b

Sample	N	Subset		
		1	2	3
Boke T (fruit)	3	70.0900		
Sil T (fruit)	3	72.4400		
Boke T (bark)	3		81.8767	
Sil T (bark)	3			210.0600
Sig.		0.380	1.000	1.000

Means for groups in homogeneous subsets are displayed. Based on observed means. The error term is Mean Square(Error) = 3.982.a. Uses Harmonic Mean Sample Size = 3.000.b. Alpha = .05.

Table B 1.13 DPPH free radical scavenging activity for boke timur fruit in methanolic extract

% inhibition (Scavenging activity**)**

Concentration	Ascorbic acid	Boke-timur (Fruit)	Boke-timur (Bark)	Sil-timur (Fruit)	Sil-timur (Bark)
100	88.29	75.93	61.2	70.3	35.12
75	75.4	71.54	51.02	66.23	30.23
50	67.34	59.65	43.21	59.65	27.29
25	60.29	51.03	40.09	54.34	25.96
0	0	0	0	0	0
IC_{50}	39.22	47.64	70.36	49.82	145.11

Table B 1.14 DPPH free radical scavenging activity for boke timur fruit in ethanolic extract

% inhibition (Scavenging activity)

Concentration	Boke-timur (Fruit)	Boke-timur (Bark)	Sil-timur (Fruit)	Sil-timur (Bark)
100	65.03	59.03	59.34	25.67
75	61.02	46.3	55.23	22.07
50	57.51	39.46	51.45	17.45
25	51.78	36.7	47.43	14
0	0	0	0	0
IC_{50}	55.27	78.84	64.44	196.06

Table B 1.15 DPPH free radical scavenging activity for boke timur fruit in ethanolic extract % inhibition (Scavenging activity)

Concentration	Boke-timur (Fruit)	Boke-timur (Bark)	Sil-timur (Fruit)	Sil-timur (Bark)
100	56.6	56.41	56.25	23.07
75	54.1	44.54	52.12	21.52
50	49.21	36.63	46.23	12.3915.45
25	40.26	33.32	39.04	12.39
0	0	0	0	0
IC_{50}	70.3	81.9	72.4	210.6

List of plates

P1 Using Kjeldahl apparatus

P2 Measuring absorbance in Spectrophotometer