ASSESSMENT OF TOTAL PHENOLICS, TOTAL FLAVONOIDS CONTENT AND ANTIOXIDANT CAPACITIES AND PHYTOCHEMICALS SCREENING OF SOME SELECTED HERBS.

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Roll No. 0119/21

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Session: 2019-2020

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Applied Human Nutrition and Dietetics

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects and that all revisions required by the thesis examination committee have been made

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DEDICATED TO MY BELOVED FAMILY & RESPECTED TEACHERS

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ABBREVIATION

DPPH : 2,2-diphenyl-1-picrylhydrazyl

ABTS : 2,2-Azinobis-(3-Ethylbenzthiazolin-6-sultonic Acid)

et al : Et alii/ et aliae/ et alia

Etc : Et cetera

G : Gram

GAE : Gallic acid equivalent

Kg : Kilogramme

Mg : Miligram

TE : Trolox equivalent

QE : Quercetin equivalents

GAE : Gallic acid equivalents

L. : Linn

PPM : Parts per Million

M : Meter

DNA : Deoxyribonucleic acid

spp. : Species

µg : Microgram

Abstract

The research study was conducted to comprehend the bioactivity and phytochemical screening of *Coriandrum sativum(Dhaniya), mentha spicata(Tulshi), ocimum sanctum(pudina)* and *centella asiatica (Thankuni)* using methanol and ethanol as solvent for extraction. Phytochemicals were examined by the standard test methods for different chemical group found in plants. The preliminary phytochemical analyses showed that the extracts contain Alkaloids, Flavanoids, Tannins, Saponin and Carbohydrates. Through using Folin–Ciocalteu assay the Total phenolics were measured and based on aluminium chloride method total flavonoids content estimation was carried out in the sample extract by spectrophotometrically. For the estimation fo the total antioxidant capacity, the DPPH (1,1-diphenyl-2-picrylhydrazyl radical) method was introduced. To find out the level of significance at $P < 0.05$,one way analysis of variance was performed. Total phenolic content of ethanolic extract of the plant was ranging from 72.83±0.001 to 34.37±0.005 mg gallic acid equivalents/ g dry weight. However, estimated phenolic content in methanolic extract ranges from 69.74±0.001 to 21.502±0.05 mg gallic acid equivalents/g dry weight. These findings reveal the ethanolic extract contains more potential antioxidant capacity. The leaves extract in ethanolic solvent was highly enriched with both phenolic and flavonoid contents compared to methanolic extract. A significant positive correlation was observed between TPC determined using Folin-Ciocalteu and the DPPH (1,1-diphenyl-2-picrylhydrazyl radical) method. The outcome suggests that phenolic chemicals are the main driver of these medicinal plants' antioxidant action. From the results, it is to conclude that these plants might be a reliable source of free radicals for food applications.

Keywords: *Coriandrum sativum, mentha spicata, ocimum sanctum , centella asiatica,* flavonoid contents, total phenolics ,antioxidant capacity, DPPH.

CHAPTER 1: INTRODUCTION

1.1 Background of the study

People nowadays are looking for alternative to incorporate more natural items into their diets and lifestyles and have become more fascinating to the recent world for their use as value addition of food products, replacement of different synthetic chemicals and nutraceuticals. Hrebs containing significant levels of active biological components, mainly flavonoids anthocyanins polyphenolic and vitamins might provide identifiable health benefits to humans rather than the standard nutrients they contain (Hasler, 2002; Falk, 2004). Non-nutritive plant compounds, phytochemicals play efficient role in plant defense mechanism. Plants of antimicrobial capability include a variety of bioactive secondary metabolites such as tannins, saponins alkaloids, and flavonoids, according to phytochemical study. Anticancer, antibacterial, and antiviral properties are found in flavonoids and alkaloids (Biswa et al., 2011).

Phenolic and polyphenolic substances are examples of secondary metabolites. These plant-derived chemical compounds have been found working as disease preventing agents (Kumar *et al*., 2014). Secondary metabolites are the biologically active compounds that are nonnutritive to the plants, but provide disease deterrent properties to them. To protect their selves from various diseases the plants produce these chemicals (Mazid *et al.,* 2011). These components are recognized as secondary metabolites of plants and have also antimicrobial, antiviral and anti-inflammatory properties along with the antioxidant capacity (Ignat et al., 2011; Santas et al., 2010).

At around 4500-1600 BC the use of medicinal plants in Rig-veda is noted down by Indo-Aryans (Tucakov, 1971). Looking back upon the last 2000 years of medical history, it is clear that the best source of medicine for humans has primarily been plants. 12,000 of the over 248,000 species of higher plants—of which there are known to be 128 thousand—have therapeutic qualities. Due to its enormous potential to advance the global health care industry, the importance of research on natural medicinal plants is growing (Guguloth *et al.*, 2011). The World Health Organization

reported that the majority of community on Earth rely on traditional medicine, which generally uses plant extracts or their active components, for their primary health care requirements. As shown in a common statistic, 25% of all drugs used today come from plants.(Johnsy *et al.,* 2012).

The information of therapeutic plants is closely protected and kept inside the family and passed on from generation to generation. In case of infectious diseases, one of the major uses of medicinal plants is to treat them or to reduce the symptoms (Pavel, 2007). They enhance the plant's color, flavor, and scent while defending it against disease and other harm. Phytochemicals are the broad term for plant compounds that shield plant cells from environmental dangers such stress, pollution,UV exposure, dehydration, and pathogenic attack (Valko, 2004). It is now widely acknowledged that they contribute to preserving human health when their dietary intake is substantial. A total of 4,000 phytochemicals have been cataloged and divided into categories based on their chemical makeup, physical properties, and protective functions. About 150 phytochemicals have undergone extensive research. Dietary phytochemicals are found in a wide range of foods, including fruits, vegetables, legumes, seeds, whole grains, nuts, herbs, and spices. Whole wheat bread, strawberries, grapes, cherries, raspberries, onions, tomatoes, legumes, garlic, beans and soy products are examples of common sources. (Braekke, 2006).

Phenolic compounds are that as it may, not dispersed equitably all through distinctive parts and areas of the plant. The exterior layers of plants regularly contain higher levels of phenolics than their insides parts (Brown *et al.,* 2019). Phytochemicals mount up in diverse parts of the plants, such as in the stems, roots, leaves, flowers, fruits or seeds. The outer layers of the various plant tissues are frequently where the majority of phytochemicals, especially the pigment molecules, are concentrated. (Harichandan, 2009). Depending upon the growing condition, variety, processing, cooking levels vary from plant to plant (Joseph, 2013).

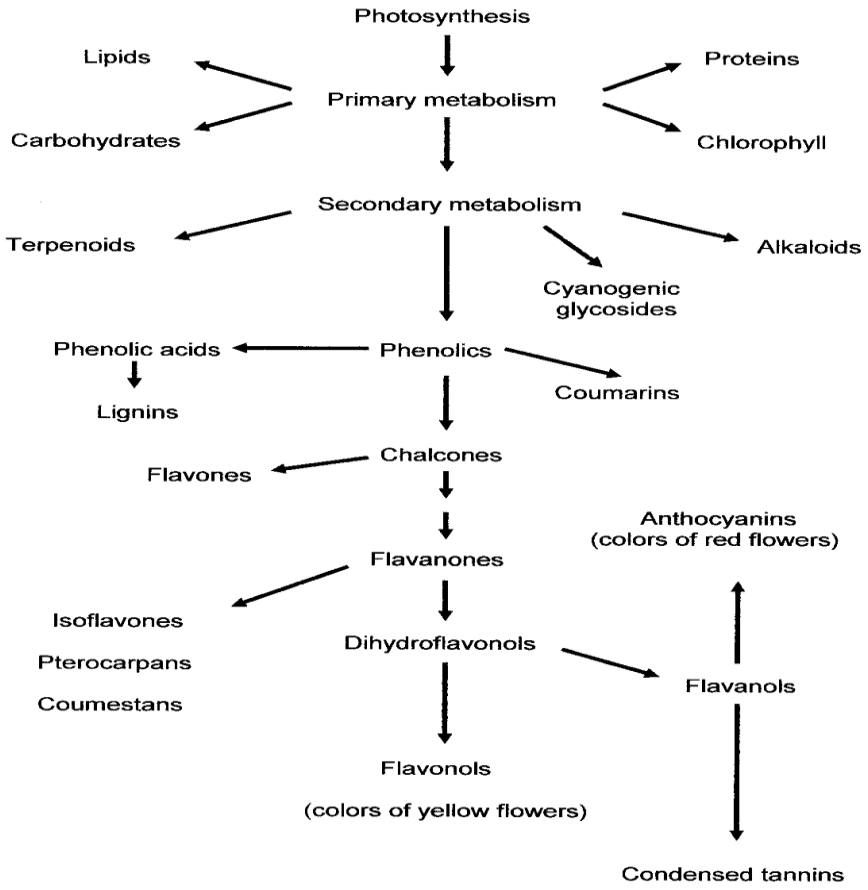


Figure 1.1: Inter-relationships between the primary and secondary metabolism in plants (Giada , 2013)

Coriander is enriched with various secondary metabolites (phytochemicals) which are exceptionally critical in coriander protection components and confers the stimulant, carminative, diuretic purgative and antipyretic distinctiveness to coriander. Moreover, it is being largely utilized in pharmaceutical businesses around the world due to its medicinal properties (Nathenial, *et al*.; 2019).

Ocimum sanctum, widely known as Tulsi, belongs to the laminaceae family. *Ocimum sanctum* is grown throughout India and Southeast Asia, with India being the world's greatest supplier of medicinal plants. Antimicrobial infection, anticancer, arthritis, antifungal, chronic fever, eye disease, hepato protective anti fertility, antispasmodic, analgesic and antiemetic are some of the diseases for which it is used (Panchal *et al.*, 2009). In Ayurvedic treatment, the rishis have also recognized Tulsi for thousands of

years as a prime herb. In the past few decades, scientists and researchers have conducted a number of studies to highlight the function of essential oils & eugenol in the therapeutic properties of Ocimum sanctum L (Sen et al.; 2013).

In South East Asia, besides being a valuable traditional medicine, Centella asiatica L. is a nutritionally significant plant. (Nathenial *et al*., 2019). The South Asian region, particularly Bangladesh, is habitat to a well-known traditional plant known locally as "thankuni" (Polash, et al., 2017). Its antioxidant activity was comparable to that of the synthetic antioxidant butylhydroxyanisole (BHA). The herb's significantly increased antioxidant activity is facilitated by the abundance of flavonoid, carotene, polyphenol, tannin, Vitamin C, and DPPH components in C. asiatica. (Chandrika *et al*., 2015). It act as a potent drug in ulcer, depression and venomous insufficiency. The use of C. asiatica in treating the aforementioned disorders will be justified as a result of phytochemical analysis of this plant, which will also reveal particular causal compounds with potent anti-disease effects. (Rahman *et al.,* 2012). The triterpenes of *Centella* contains many compounds including madecassic acid, madecassoside, asiaticosside, brahminoside, asiatic acid thankiniside, isothankunisode brahmoside,, madasiatic acid, brahmic acid, centelloside, cenellicacid and centic acid.(Zheng et al., 2007).

Mentha spicata L., often known as garden mint or spearmint, is mostly utilized in the fabrication of Indian food. It's commonly eaten as mint chutney or as a flavor enhancer in a variety of meat recipes. Mentha is a genus of roughly 25–30 species that belongs to the Lamiaceae (Labiatae) family (Kanatt *et al*., 2007). In addition, In clinical studies, mint's analgesic effects have been demonstrated to treat headaches. Additionally, it has been demonstrated to alleviate stomach pain and dyspepsia in patients undergoing upper and lower GI tract endoscopies as well as bothersome muscular spasms (Mckay and Blumberg, 2006).

Herbs are only treatments for a variety of illnesses and health issues. One alternate method of discovering therapeutic medicines is to do work on the phytochemical constituents found in medicinal plants (Mazid *et al.*, 2011). Supplementing with

dietary antioxidants shows promise in boosting the antioxidant defense and repair mechanisms. Many different types of natural polyphenols have been extracted and characterized because of their extraordinary range of biochemical and pharmacological activities. The plant has much enhanced antioxidant activity thanks to the presence of flavonoid, polyphenol, tannin, vitamin C, carotene, and DPPH (2,2-diphenyl-1-picrylhydrazyl) components. As byproducts of metabolic reactions, reactive oxygen species (ROS) such as hydroxyl radicals (-OH), superoxide anion and hydrogen peroxide are formed (Williams *et al.,* 2000). The remarkable ability of polyphenols to act as antioxidants is because the following properties of the compounds:

1) acting as chelate metal ions

2) ability to donate hydrogen;

3) capability to inhibit selectively enzymes responsible for the catalysis of various oxidation processes (Li *et al.,* 2004).

Polyphenols' effectiveness in all three processes is highly influenced by their structure. The deterioration of cell structures, such as proteins, carbohydrates, lipids, and nucleic acids, is brought on by an excess formation of reactive nitrogen species (RNS) and reactive oxygen species (ROS). Polyphenols are able to delay these effects (Rao *et al.,* 2011). Moreover, the excessively produced ROS or RNS is thought to have an influence on several pathologies, including inflammatory disorders, cancer, cardiovascular diseases, neurological degeneration like Alzheimer's diseases and Parkinson's diseases and premature aging (Perumal, et al.; 2011).

Plant phenolics encompass a favorable influence on health by preventing low density lipoproteins from oxidizing, limiting the augmentation of harmful bacteria, viruses, and fungus, promoting the escalation of good bacteria, and blocking or activating enzymes that fasten particular receptor (Papuc *et al.*, 2017; Shahidi, 2015). Radicals produced by antioxidants with molecular structures like phenols, which donate hydrogen atoms to lipid radical's antiradical antioxidants operate, are stable species and will subsequently end the oxidation chain reaction (Tirzitis and Bartosz., 2010).

In the present research, herbs such as *coriander sativum*, *mentha spicata*, *ocimum sanctum*, *centella asiatica*, were subjected to examine total phenolics and antioxidant activity of extracts and were compared to in respect of two different solvent. Although prior articles have already addressed the amount of phenolic compounds and their makeup in various botanical species (Al-Juhaimi , 2011; Bhatt et al., 2013; Tajner *et* al., 2020), they were often determined for individual organs of individual herbs. Traditional methods are employed to study the antioxidant activity of herbs in relation to their total phenolic content, and only one test is utilized to measure their capacity to scavenge free radicals. flavonoids, phenolic compounds, Alkaloids,saponins, tannins, terpenoids, and other chemicals with therapeutic and industrial importance can be found in novel sources according to phytochemical screening. (Panchal *et al.*, 2019). Aim of the research to find the phytochemicals existance in the methanolic and ethanolic extracts of selected samples.

Aims and objectives:

1. Assessment of the contents of Total phenolics , Total flavonoids and relative antioxidative capability in ethanolic and methanolic extracts of selected herbs

2. To elucidate the presence and absence of phytochemicals in selected herbs though various phytochemicals screening methods.

Chapter II : Literature Review

2.1 Herbs

A flowering plant whose stem above ground does not become woody and persistant and plant part utilized for its flavor, aroma, or therapeutic characteristics is known as a herb (Bent et al., 2004). Natural healing substances have been generated by nature for thousands years long, extending later to the dawn of time. The use of medicinal plants, particularly in traditional medicine, is now widely acknowledged and accepted as a legitimate profession (Pal and Shukla, 2003). The herbs are classified as five major categories: according to the active constituents present in them, Bitter (phenolic compounds, saponins, and alkaloids), Astringents (tannins), Aromatic (volatile oils), Mucilagnious (polysacharides), and Nutritive (food stuffs) (Dudek *et al*., 2014). A good number of research have been conducted thorought the centuries to explore more about herbs. Antioxidant molecules that are pharmacologically strong and have few or no adverse effects are currently being sought for application in preventative medicine and the food industry around the world (Ali *et al.,* 2008)

2.2 Origin and distribution

2.2.1 Mentha spicata L.

In the family Labiatae, there are around 210 genera and 3,500 species (Davidson, A., 2014). The Labiatae family name alludes to flowers with typical petals merged into an upper and lower lip. Locally it is known as Pudina. The Labiatae family name alludes to flowers with typical petals merged into an upper and lower lip. (Brickell *et al*., 1997). Mints grow to a height of 10 to 120 cm and can spread across an indeterminate area. In Botany, mint is the common name for any of the various herbaceous plants and perennial aromatic herbs that are cultivated for their essential oils and culinary purposes. The genus *Mentha* L. (Lamiaceae) produces secondary metabolites such as alkaloids, flavanoids, phenols, gummy polysaccharides. Terpens and quinines are used in food and pharmaceutical, cosmetics and pesticide industries (Khanuja, et al., 2000).

2.2.2 Scientific Classification

Kingdom	**Plantae**
Subkingdom	Viridiplantae
Super division	Embryophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Super order	Asteranae
Order	Lamiales
Family	Lamiaceae
Genus	Mentha L. – mint
Species	*Mentha spicata L.*

Figure 2.1: *Mentha spicata*

2.2. 3 Coriandrum sativum

2.2.3.1 Origin and distribution :

Coriander (*Coriandrum sativum* L.) a Mediterranean native spice, is an annual herb belonging to the carrot family (Apiaceae), is extensively disseminated and predominantly grown for seeds. It is a commonly utilized spice in cooking and also is an imperative therapeutic herb. In Bangladesh, it is also recognized as "Dhaniya. Although coriander's fresh leaves and seeds are used to garnish cuisine, the entire plant is edible. In fact, it is widely grown in, Central Europe, Russia, India, Turkey, Argentina, Morocco, and the United States (Sriti et al., 2012). Various species of coriander contain nutritional constituents as water, carbohydrates, protein, fat, sodium, calcium, iron, phosphorus, vitamin A and food energy. It also contains phytochemical constituents as tannins, flavonoids, saponins, alkaloids, sterol, terpenoids, and carbohydrates (Patel, et al., 2016). Besides the nutritional value, it has lots of benefits as an herbal medicine. It is a source of various drugs in local Pakistani pharmacology. Its fruit is an aphrodisiac stimulant, diuretic, laxative, antipyretic, stomachic and treats vomiting, indigestion, urethritis, urinary tract infections, rash, cystitis, sore throat, urticarial, cough, hay fever, diabetes, amoebic dysentery (Ahmed, et al., 2018).

2.2.3.2 Scientific Classification:

Kingdom	**Plantae**
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Apiales
Family	Apiaceae
Genus	Coriandrum
Species	*Coriandrum sativum.*

Figure2.2: *Coriandrum sativum.*

2.2.4 Centellla asiatica (L).

2.2.4 .1 Origin and distribution :

Centella asiatica is from the family of apiaceae is home-grown to the majority of the asian countries. *Centellla asiatica (L)* is a valuable medicinal herb that is a perennial creeper with a slight scent. (Brinkhaus, et al, 2000). It has long, filiform, prostrate stems with long internodes harboring roots, 1 to 5 reniform, deeply cordate, long-petioled, oval or orbicular-shaped leaves per node, and 3-6 small, umbel-shaped flowers that range in color from purple to white-green and appear from the axils of the leaves (Das, 2011). *Centella asiatica* is the most common species, creating a dense green carpet in gloomy, damp, swampy, wet areas like paddy fields and river banks (Das, 2011). Brahmi is the name given to the herb in Unani medicine, Mandookparni in Ayurveda, and Gotu kola in Western medicine. Generally, known as ‘Thankuni” in Bangladesh. Centella root, leaf, and other plant components have therapeutic characteristics that are widely known in traditional medicine.

2.2.4 .2 Scientific Classification:

Kingdom	Plantae
Divison	Tracheophyta
Subdivision	Spermatophtina
Class	Equisetopsida
Order	Apiales
Family	Apiaceae
Genus	Centella
Species	*Centellla asiatica* (L).

Figure 2.3: *Centellla asiatica*

2.2.5 Ocimum sanctum

2.2.5.1 Origin and distribution:

Ocimum sanctum L. (*Ocimum tenuiflorum*, Tulsi). The Lamiaceae family, which includes the fabled "Incomparable One," or "Queen of Herbs," is crucial for its therapeutic prospective. Plant extracts have been demonstrated to possess physiologically active ingredients that are nematicidal, insecticidal, fungistatic, or antibiotic and are utilized in traditional remedies (Grauso *et al* ; 2020). It is recognized that several components of O. sanctum L., including the flowers, leaves, roots, stems, seeds, and so forth, have therapeutic potential. *Ocimum sanctum* L. is a general vitalizer that also boosts stamina. The medicinal benefits of *ocimum sanctum* L. have been extensively studied, and it has been called an antiasthmatic and an antikaphic medication. (Sethi *et al.,* 2013). Several scientists have examined the pharmacological effects of Tulsi products obtained by different extraction methods, such as steam distillation, benzene extraction and petroleum extraction (Prakash, et al., 2005), reviewed all the scientific studies of the therapeutic significance of Tulsi and eugenol, a major component of Tulsi. These pharmacological studies may be helpful to establish a scientific basis for the therapeutic use of this plant, especially regarding the pharmacological effect on the central nervous system, immune system, cardiovascular system, reproductive system, and gastric and urinary systems.

2.2.5.2 Scientific Classification:

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Lamiaceae
Genus	Ocium
Species	*Ocimum sanctum*.

Figure 2.4: *Ocimum sanctum*

2.3 Nutritional properties:

C. asiatica possesses a high nutrient content beause of its high carotenoids, vitamin C, and vitamin B. C. asiatica is also widely available in the form of tea and soft beverages on the market(Singh, et al; 2010). Early studies reported that in 100 g C. asiatica, Protein 9.94 g/100g, Moisture 84.37g/100g, calcium 1.060 mg/100g, iron 32mg/100g, energy value 38.99calories/100g, fibre 18.33g/100g, vitamin C 9.73mg/100g, phosphorus 370mg/100g, carbohydrates 51.92g/100g, B-Carotene 1mg/100g (Prakasha, et al.; 2006 and Ong, et al.; 2011).

A study by Anbarasu, K. and Vijayalakshmi, G., (2007) revealed that Ocimum sanctum has the potential to be a source of vitamins C and A, minerals like Ca, zinc, and iron, in addition to phytonutrients like chlorophyll and others, which can improve the effective digestion, absorption, and utilization of nutrients from food and other herbs.. Nutrient constituents are as Carbohydrate 2.3g; Protein: 4.2g; Fat: 0.05g; Calcium: 25mg; Iron: 15.1mg, Phosphorus 287mg, edible portion 25mg and vitamin C 56mg.

The green leaves as well as dried fruits of coriander are the main sources of nourishment. Its leaves, like many other green leafy vegetables, are high in vitamins, minerals, and iron. Vitamin A (β-carotene) and vitamin C are abundant in its leaves. The green herbs provide up to 160mg/100g of vitamin C and 12 mg/100g of vitamin

A. It is a good reserve of thiamine, zinc, and dietary fiber and is low in saturated fat and cholesterol. Water makes up 84 percent of green coriander (Bhat, et al.; 2014)

Mint is one of the most well-known plants that is widely grown all over the world. The plant is extremely useful in the pharmaceutical and food industries. The important nutritional contents include proteins (0.6%), iron (0.262%), Calcium(0.158%), phytic acid(0.00092%), ascorbic acid (0.96±0.06%) vitaminE (9.89±0.15%), axerophthol (0.426±0.05%) and Na 7.2mg/100g (Hussain, et al.; 2021)

2.4 Phytochemical constituents & antioxidant capability:

The chemical makeup of herbs is quite complicated; they include a wide variety of nutrients and other biologically active substances, the amounts of which can vary greatly between strains and even between plants in the same field. The leaves of spearmint (Mentha spicata L.) were recently used to extract catechin, epicatechin, rutin, myricetin, luteolin, apigenin, and naringenin followed by supercritical carbon dioxide (SC-CO2) extraction and standard soxhlet extraction at various extraction schemes and parameters is revealed by Bimakr, M., et al; 2011). As revealed by the results, soxhlet extraction showed a higher crude extract yield (257.67 mg/g) in comparison with the SC-CO2 extraction (60.57 mg/g) and have seven bioactive flavonoids, the primary flavonoid components, in reduced concentration when compared to the ethanol (70%) soxhlet extraction (five bioactive flavonoids). As a result, it is recognized as a different method from CSE SC-CO2 extraction for extracting spearmint leaf bioactive flavonoid components with high concentrations.

Due to the increasing interest in the flavonoid content of foods and plants , Praveen Garg and Rajesh Garg (2019) have done the experiment to screen out the phytochemical content of the chloroform, ethanol, and methanol extracts of *Ocimum sativum,* including the detection of alkaloid, glycosides, flavonoid, tannin, polyphenols, saponin, and other compounds. The total flavonoids concentration was calculated using a spectrophotometric technique using aluminum chloride in the sample extract. When diterpines, saponins, proteins, flavonoids, amino acids,

carbohydrates, and alkaloids were extracted with methanolic and ethanolic solvents, phytochemical screening tests revealed the presence of flavonoids, saponins, proteins, carbohydrates, diterpines, and alkaloids in the stem parts and leaves.

Wangensteen, et al; 2004 have come to an conclusion that in both parts, the ethylacetate extract of coriander contributed to the greatest activity, with leaves exhibiting better antioxidant activity than seeds. The ethanol extracts from the leaves and seeds, respectively, demonstrated concentration-dependent DPPH scavenging action having 389.5 and 510.12 lg/ml of IC50 values.

2.4.1 Alkaloids:

Alkaloids are the most common type of secondary plant metabolite. They belong to a group of naturally produced organic molecules that are predominantly composed of basic nitrogen atoms. These nitrogen bases are produced when various radicals are used in place of some or all of the hydrogen atoms in the peptidic ring (Hao, 2009).

2.4.2 Glycosides:

Glycosides are solely bitter compounds found in the Genitiaceae family of plants. In the condensation process, the hemiacetal group of the carbohydrate plays only a minor role, and glycosides generated from sugars containing a range of organic hydroxyl or thiol groups as the condensation products. Anthracene glycosides (purgative and for the treatment of skin diseases), amarogentin, chalcone glycoside (anticancer), rographolide, gentiopicrin,ailanthone, and polygalin are examples of *C. asiatica* contains glycosides in inactive form (Das, 2011).

2.4.3 Tannins:

Tannins' phenolic group, which they include, gives them their antibacterial capabilities. Ayurvedic medicine have been utilized to treat enteric disorders such as diarrhea in Ayurveda were formulated based on tanin rich plant (Omojate G., *et al.*; 2014).

2.4.4 Polyphenols:

Phenolics, polyphenolics are the group of biologically active compounds that are available in abundance as natural color components in fruits. The backbones of derivatives of cinnamic acid and benzoic acid are C1-C6 and C3-C6, respectively. The two classes of phenolic acids are hydroxycinnamic and hydroxybenzoic acids. As a result, phenolic compounds share the existence of at least one aromatic ring that has been replaced with a hydroxyl group (Morton, L. W., et al.; 2000).

Some phenolic acids are found in free form in vegetables and fruits, whereas phenolic acids in bound form are found in the hull, bran, and seed. The existence of phenolic compounds in free form also occurs in plant tissues. (Hussain, et al.; 2004). Plant defense against pathogens and herbivore predators may play the most essential function, and hence they are used in the management of human pathogenic disorders (Tovar *et al*; 2002). The fundamental monomer in polyphenols is phenolic ring and generally these are classified as phenolic acids and phenolic alcohols (Abbas, M., et.; 2017). Polyphenol research took longer than expected because of their structural intricacy. Polyphenols are the most common antioxidants in our diet(O'Leary, K.A., et al.;2004). Polyphenols also have a pro-oxidant property, that works on the cell's mechanism. It could also entail inhibit cell propagation and apoptosis. Many other effects of polyphenols have been identified in studies, such as the decrease of enzymes like lipoxygenase and telomerase (D Archivio, et al.; 2007).

2.4.5 Flavonoids

Flavonoids are a kind of polyphenol found in a wide range of plant species. They have one benzene ring in their construction, and various studies have shown that they can act free radical scavengers (Kim and Lee, 2004). The chemicals are generated from flavans, which are parent compounds. There are around 4,000 flavonoids, with some of them working as pigments highly in plants. About 70% of plants include flavonoids like kaempferol, quercetin, and quercitrin. Other forms of flavonoids include flavones, dihydroflavons, flavans, flavonols, anthocyanidins, proanthocyanidins, calchones, catechin, and leucoanthocyanidins (Omojate *et al*; 2014).

According to Kumar, et al.; (2013) Major molecular mechanisms of action of flavonoids are enlisted as below:

- ✓ Decreasing the regulation of the mutant p53 protein,
- ✓ detain cell cycle,
- ✓ Inhibiting tyrosine kinase
- ✓ heat shock protein inhibition,
- ✓ estrogen receptor capability to bind, and
- ✓ Ras protein expression inhibition are some of the effects.

2.4.6 Saponins

Saponins are chemicals that behave like soap in water, producing foam when shaken. An aglycone named sapogenin is generated during hydrolysis. Saponins are chemicals that behave like soap in water, producing foam when shaken. An aglycone named sapogenin is generated during hydrolysis. Saponins are particularly dangerous because they promote blood heamolysis and have been linked to animal poisoning. They have a bitter and caustic flavor, as well as irritate mucosal membranes (Omojate Godstime *et al*; 2014).

2.4.6 Antioxidants:

Polyphenols' chemical activities in terms of their reducing properties as hydrogen or electron-donating agents indicate their potential for activity as antioxidants, scavengers of free radicals (antioxidants). The activity of an ant;oxidant is determined by:

- reacting as hydrogenor electron-donating agent
- depending on its capacity to delocalize and stabilize the unpaired electron, the destiny of the ensuing antioxidant-derived radical.
- reaction with other ant;oxidants.
- metal-chelating potential (Di Majo, D., et al.; 2006).

Globally, infectious diseases are the main factor in fatalities. Antibiotic resistance is already a global concern, but the rise of multidrug-resistant bacteria jeopardizes the clinical effectiveness of many of the antibiotics that are currently in use. (Westh, 2004).

Polyphenols have a good structural chemistry for scavenging free radicals, and has been proven to be more efficient, in vitro antioxidants are more effectivein comparison to vitamins C and E on a molar basis (Shah, et al.; 2014). The relative capacities of the compounds to scavenge free radicals are defined by a hierarchy of the antioxidant activities of flavonoid and isoflavonoid depends on structure. Pulse radiolysis has been used in studies to examine the interactions of the superoxide anion (O2"-), hydroxyl radical ('OH), lipid peroxyl radical (LO0"), azide radical (N3'), and model t-butyl alkoxyl radicals (tBuO') with polyphenols, as well as the stability of the antioxidant radicalsand the rate constants of the reactions (Hudson, B.J. et al.; 1983).

In ocimum sanctum Total phenolic and total flavonoid contents were measured by Folin-Ciocalteu colorimetric method and aluminum chloride colorimetric method respectively. By using radical scavenging assay of 1,1-diphenyl-2-picrylhydrazyl (DPPH) antioxidant activity of the extracts was evaluated. The extracts are mostly used to analyze flavonoids, tannins, terpenoids glycosides, alkaloids, polyphenols, and steroids. According to the results, the plant has a sizable quantity of total phenolic and total flavonoid content, with methanol extract having the highest levels of these compounds, followed by chloroform and hexane extract. Methanol extract had the highest antioxidant activity (IC50 value: 47.73 g/mL), followed by chloroform extract (IC50 value: 79.46 g/mL), and hexane extract (IC50 value: 94.68 g/mL). Ascorbic acid was used as the ascorbic acid standard. The overall phenolic and flavonoid content of a plant influences how much of an antioxidant effect it has. (Pathak, I., et al.; 2019).

Hashim *et al (2011)* revealed in their study, similar to grape seed extract (88%) and vitamin C (88%) in terms of antioxidant content is centella (84%).The extract of *Centella asiatica* whole plant in alcoholic solvent was evaluated , Results strongly suggested that the plant has cardioprotective properties that limit the myocardial injury caused by ischemia-reperfusion in rats. Cardioprotective effect against

ischemia-reperfusion produced myocardial infarction. (Pragada *et al.,* 2004). Zainol, M.K., *et al* (2003) reported that compared to other plant parts, the ethanol extract of C. asiatica's root had the maximum activity, while the plant's leaves had the highest antioxidant activity among its various components and the highest phenolic levels.

According to several investigations, Mentha species' methanolic extracts have a significant amount of polyphenols that could be employed as antioxidant source and a natural food preservative. Among predominant bioactive constituents of mint were rosmarinic acid, luteolin, eriocitrin, hesperidin, and trace concentrations of pebrellin, gardenin B, and apigenin. (Mata et al. (2007). The primary COX-2 (Cyclooxygenase-2) activity antagonists may be the polyphenols in mint infusions. Various researches have already shown that flavonoids reduce COX-2 activity. (Liang etal. 2001)

Coriandrum sativum L. fruits recommended as a new prospective source of natural antioxidant and could be alternstive to food additive by Msaada, K., et al.; (2007).In their study showed that there are variations in total polyphenols (0.94 ±0.05–1.09 ±0.02 mg GAE/g DW), total flavonoids (2.03 ±0.04–2.51 ±0.08 mg EC/g DW) significantly ($P < 0.05$) and remarkable DPPH radical scavenging activity in methanolic extracts exhibited having IC50 values ranged from 27.00± 6.57 to 36.00 ± 3.22 lg/mL. EC50 values of reducing power activity varied significantly ($P< 0.05$) from 54.20 ± 6.22 to 122.01 ± 13.25 lg/mL. By comparing methanolic and ethanolic extracts of coriander (Coriandrum sativum L.) seeds with the corresponding standards using the method of RP-HPLC, the flavonoids, quercetin, chlorogenic acid, rutin and caffeic acid are identified and quantified. The methanolic extract created sharp peaks in contrast to the ethanolic extract by Rajeshwari and Andallu (2011).

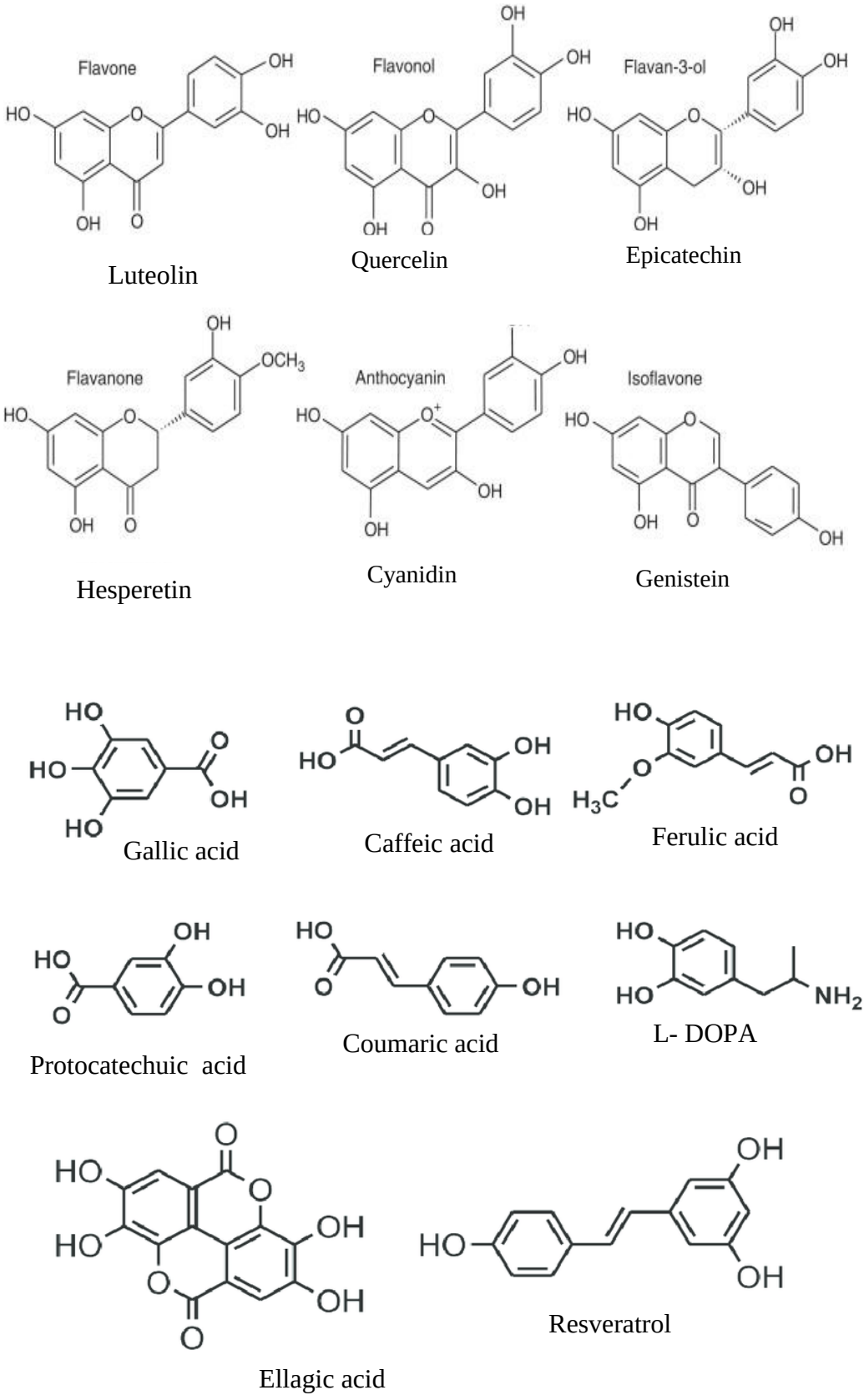


Figure 2.5: Chemical structures of phenolic compounds frequently found in herbs

2.5 Screening of phytochemicals

In this context, the term refers to the extraction, screening, and identification of the plants' medicinally potent compounds. Phytochemicals are non-nutritive substances that are made by plants and contain curative, disease-preventive elements. Currently, there are many more phytochemicals being discovered than the more than 900 that have already been identified as being present in food (Süntar, I., 2020). Flavonoids, alkaloids, tannins, carotenoids and some of the bioactive molecules, phenolic compounds, that can be obtained from plants. The term "phytochemical" refers to a group of substances that are regarded as important nutrients since they are found naturally in plants and are necessary for maintaining healthy physiological functioning in humans. Humans must therefore get these compounds through diet (Ibekwe, N.N. et.al.; 2014).

2.5.1 Phytochemistry and medicinal properties:

Phytochemicals have also made significant strides in the treatment and prevention of high blood pressure, diabetes, and macular degeneration. A molecule may have more than one organic function, acting as a specialist in both antioxidants and antibacterials, unlike phytochemicals which are categorized by their functions. Only a small number of phytochemicals have well-understood physiological characteristics, and more and more researchers are focusing on how they might help prevent or treat diseases like cancer and heart disease.(Joseph B., et al.; 2003).

Secondary metabolites, like alkaloids, flavanoids, phenols, and gummy polysaccharides are formed by the Mentha L. (Lamiaceae) genus. Terpenes and quinines are employed in the food and pharmaceutical industries, as well as cosmetics and pesticides. Due to its divergent aroma, certain species of this genus are also utilized in both fresh and dried form as herbal teas and sauces. (Khanuja *et al*., 2000).

The phenolic diterpenes, flavonoids, and phenolic compound are the attention of the preponderance of investigations on antioxidant elements in the Lamiaceae family (Kivilompolo *et al;* 2007). Flavonoids are natural antioxidants that are less hazardous comparing to synthetic antioxidants like BHT and BHA. As a result, these secondary plant phenolics have got the most interest and intensively investigated(Heim *et al.,*

2002). Polyphenols have grown to be a significant topic of medical research due to their essential capacity to function as antioxidants. Secondary metabolites produced by plants, polyphenols all include an aromatic ring (at least one hydroxyl group), which is a structural characteristic in common with other secondary metabolites. Additionally, due to the lower toxicological safety of their synthetic equivalents, naturally occurring antioxidants are preferred over their synthetic counterparts (Naczk, 2004).

The attributes and mode of action of the plant's bioactive constituents, namely triterpenic acid (asiatic acid madecassic acid), triterpenic saponin (madecassoside and asiaticoside), flavanoids, and other phenolic compounds in Centella asiatica, have been reported widely in numerous articles. These activities encompass potential antioxidant, antimicrobial, cytotoxic, neuroprotective, and other activities. (Seevaratnam, *et al.;* 2012).

Giving O. sanctum L. extract to normal rats fed fructose for 30 days reduced serum glucose levels considerably compared to the control group. The extract of O. sanctum L., on the other hand, has lesser effect on hyperinsulinemia (Gholap et al., 2004). The mechanism of O. sanctum L.'s glucose-lowering action in male mice. According to the findings, O. sanctum L. lowers cortisol and glucose levels in the blood, as well as having an antiperoxidant impact. As a result, O. sanctum L. could help to manage diabetes mellitus caused by corticosteroids.(Grover et al., 2005). Oils extracted from the leaves and inflorescence of Tulsi have been claimed to have numerous useful properties, including as expectorants, analgesics, anti-emetics, and antipyretics; stress reducers and inflammation relievers; and as anti-asthmatic, hypoglycemic, hepatoprotective, hypotensive, hypolipidemic, and immunomodulatory agents (Singh *et al.*, 2010). Several scientists have examined the pharmacological effects of Tulsi products obtained by different extraction methods, such as steam distillation, benzene extraction and petroleum extraction (Prakashe,et al., 2005), reviewed all the scientific studies of the therapeutic significance of Tulsi and eugenol, a major component of Tulsi.

Bioactive and Illness anticipating phytochemicals displayed in the plant are shown in Table 1.1.

Table 1.1 Bioactive phytochemicals in medicinal plants

Classification	Main Groups of compounds	Biological function	References
Antibacterial and antifungal	Terpenoids, phenolics alkaloids,	Inhibitors of microorganisms, the risk of fungal infection reduction	Othman, L et al; 2019
Antioxidants	Polyphenolic flavonoids, compounds, tocopherols, carotenoids, ascorbic acid.	Quenching Oxygen free radical, lipid peroxidation inhibition. Restrains COX-2 expression by inhibiting tyrosine kinases, imperative to induction of COX-2 gene expression	Chen, Y.C.,et al;2000
Anticancer	Carotenoids, curcumine, polyphenols, Flavonoids.	Inhibitions of tumor, repressed anti metastatic activity and development of lung cancer	Weng, C.J et al;2012
Detoxifying agent	Reductive acids, tocopherols, isothiocyanates, phenols, indoles, aromatic carotenoids, retinoids, cyanates, phytosterols	procarcinogen activation Inhibition, Inhibitors of drug binding of carcinogens i, tumourogenesis inhibition.	Shimada, T., 2006
NSA (Non- starch polysaccharides)	Cellulose, mucilages, hemicellulose, lignins, pectins,	Ability of Water holding, delaying in nutrient absorption, toxins and bile acids binding	Saxena, M et al 2013
Others	Alkaloids, volatile flavors, terpenoids,	Act as Neuro pharmacological agents, antioxidants,	Sheikh, I.,et al;2020

According to the literature cited in this chapter, it is apparent that plant has been one of the essential resources of medicines since the commencement of human civilization. Plants are used as antioxidants, antidiabetic, hepatoprotective, antitumor, antifungal, antibacterial, antiviral etc. A structured and systematic study is authentic to assess the appropriate efficacy of medicinal herbs. Bangladesh has a great number of medicinal plants, although many of these vital medicinal plants have yet to be discovered for their therapeutic potential. Furthermore, several of these elements' quantities are influenced by a variety of growing, harvesting, processing, and storage factors that are still unknown.

Chapter III: Materials and Method

3.1 Study area and Study period:

The research work was completed for a period of six months from August 2021 to January 2022. Experimental procedures were carried out in the laboratory of the Department of Food Processing and Engineering, Department of Applied Food Science and Nutrition, Department of Physiology, Biochemistry and Pharmacology at Chattogram Veterinary and Animal Sciences University, CVASU, Bangladesh.

3.2 Experimental design:

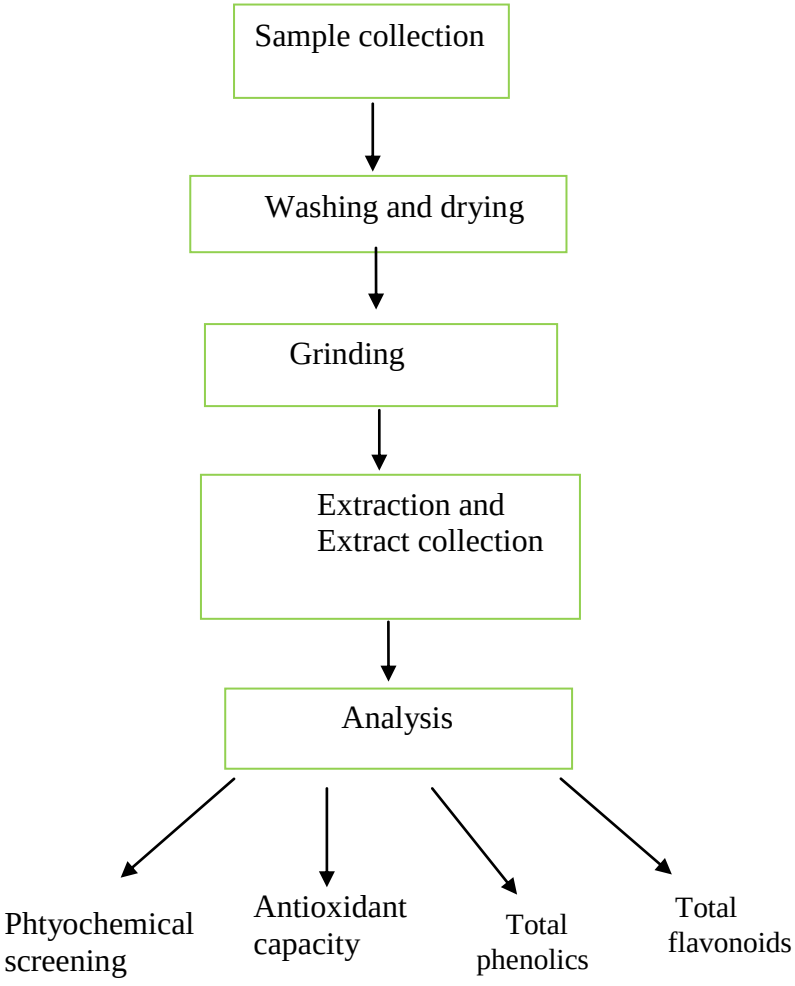


Figure 3.1: Stepwise design for the experiment

3.3 Collection of plant materials

Mature and disease free tulshi plants was collected from the medical plants garden of CVASU and dhaniya and pudina from collected from the local market of Chattogram. Thankuni was collected from local area of village named Kadal pur, situated in Rawzan,Chattogrm.

3.3.1 Sample Preparation :

The leaves were comprehensively washed to remove sand and other particles and drying in cabinet dryer at 60℃ around 24 hours. The samples were ground using a blending machine until powder obtained to confirm homogeneity. The powdered sample was passed through a fine (2mm mesh) sieve to remove any remaining residue. The finely powdered sample was then stored into labeled plastic containers before use.

Table 3.1: Herbs used in this study

Scientific Name	**Local Name**	**Family**	**Plant part used**
Ocimum sanctum	Tulshi	Lamiaceae	Leaves
Centella asiatica	Thankuni	Meliaceae	Leaves
Mentha spicata	Pudina	Lamiaceae	Leaves
Coriandrum sativum	Dhaniya	Meliaceae	Leaves

3.3.2 Preparation of extracts:

Preparation and identification of bioactive compounds were determined according to a slightly modified method described by (Ferreres, et al., 2008). Cabinet-dried samples were put into the appropriate beakers, adding methanol and 80% ethanol, and shaken gently for 72 hours at room temperature. After that, the solvent and residue

were separated by straining. The residue was twice more extracted, each time using a different solvent, while the filtrate was collected and kept at room temperature.

To obtain the crude extracts, the filtrates were mixed and evaporated using a rotary evaporator at decreased pressure and 60 °C. Prior to further examination, weighed the raw extracts and kept at 4 °C.

3.3.3 Chemicals reagents

The investigation was conducted using just standard-grade chemicals and reagents. Felhing's solutions A and B, Mayer's reagents, Iodine solution , Folin-Ciocalteu reagent, Ammonia solution, glacial acetic acid, sodium carbonate, Absorbic acid, Gallic acid, rutin, DPPH (1,1-Diphenyl-2-picrylhydrazyl), FeSO4, Nicotinamide Adenine Dinulotide.

3.4 Total flavonoid content (TFC)

TFC of all of the extracts will be evaluated using the aluminum chloride colorimetric method described by (Chang et al., 2002). Stock solution (1 mg/mL) of extracts was prepared. Quercetin was dissolved in 80% ethanol to make standard solutions (1.0, 3.0, 5.0, and 7.0 mg/mL) to plot a standard curve. Aliquots of 0.5 mL of diluted extract or standard solution then mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 mol/L potassium acetate, and 2.8 mL of distilled water in the cuvette. The mixture will be left at room temperature for 30 min. The absorbance will be read at wavelength 415 nm in UVvisible spectrophotometer. For the blank, 10% aluminum chloride will be substituted with distilled water of the same amount. TFC will be calculated and expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g).

3.5 Total phenolic content (TPC):

To analyse tannins, anthocyanins, monomeric phenolic compounds and polymeric pigments present in the sample the total phenolic content is will be determined.

TPC of all of the extracts will be determined consistent with the method described with slight alterations (Azizi et al., 2010). Stock solutions (1 mg/mL) of extracts and standard solutions of gallic acid (1.0, 2.0, 4.0, 6.0, 8.0 mg/mL) will be prepared. Extracts or gallic acid standard solution (0.3 mL) will be pipetted into a cuvette. Diluted FC reagent (1.5 mL) will be then added and mixed. The mixture will be left for 3 min before adding 1.5 mL of sodium carbonate (75 g/L) solution and left for 60 min. The absorbance was read at wavelength 765 nm using a UV spectrophotometer and ethanol will be used as the blank. TPC will be calculated and expressed as milligrams of gallic acid equivalents (GAE) per gram of extracts (mg GAE/g).

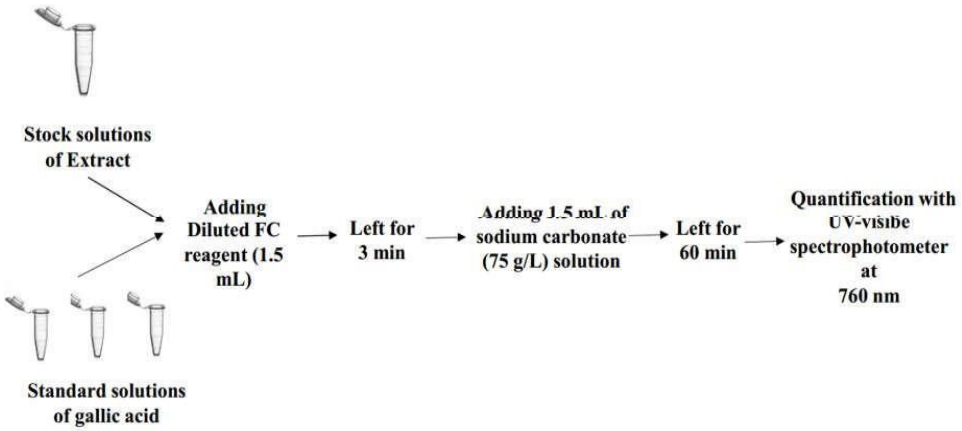


Figure 3.2: Determination of Total phenolic content (TPC)

3.6 Gallic acid calibration curve

The Gallic acid calibration curve was prepared by the Folin-Ciocalteu reagent method with modification. Gallic acid (10 mg) was dissolved in methanol (1 mL). It was a concentration of 10mg/mL and then diluted by adding methanol to prepare serial concentrations 10, 25, 50 and 100µg/mL. The above same procedure was followed for gallic acid standard. At a constant wavelength of 760 nm, the absorbance was measured for all standard solutions by using UV-spectrophotometer (UV professional double beam, Shimadzu made) (Hossain, M.A., et al.; 2013).

3.7 DPPH free radical scavenging assay :

Antioxidant capacity of the extracts was determined using DPPH(1,1-dipenyl-picryl hydrazyl) assay as described by (Azlim Almey et al., 2010) with slight modifications. By dissolving 6 mg of DPPH in 100 mL methanol and ethanol, methanolic and

ethanolic DPPH solution was prepared. The methanolic DPPH solution (2 mL) was added to 1 mL of each extract solution of different concentrations and the mixture was left for 30 min and the absorbance was read at wavelength 517 nm. By mixing 1 mL of methanol with 2 mL of DPPH solution the control was prepared. Methanol was used as a blank. Trolox was used as a standard. Antioxidant capacity based on the DPPH free radical scavenging ability of extracts was calculated and expressed as milligrams of Trolox equivalents (TE) per gram of extracts (mg TE/g).

The percentage of DPPH radical-scavenging potency of each plant extract was calculated as:

DPPH radical-scavenging activity (%I),

$$= \frac{\text{Blank absorbance} - \text{sample absorbance}}{\text{Blank absorbance}} \times 100 .$$

3.7 Extraction of plants for phtyochemical screening:

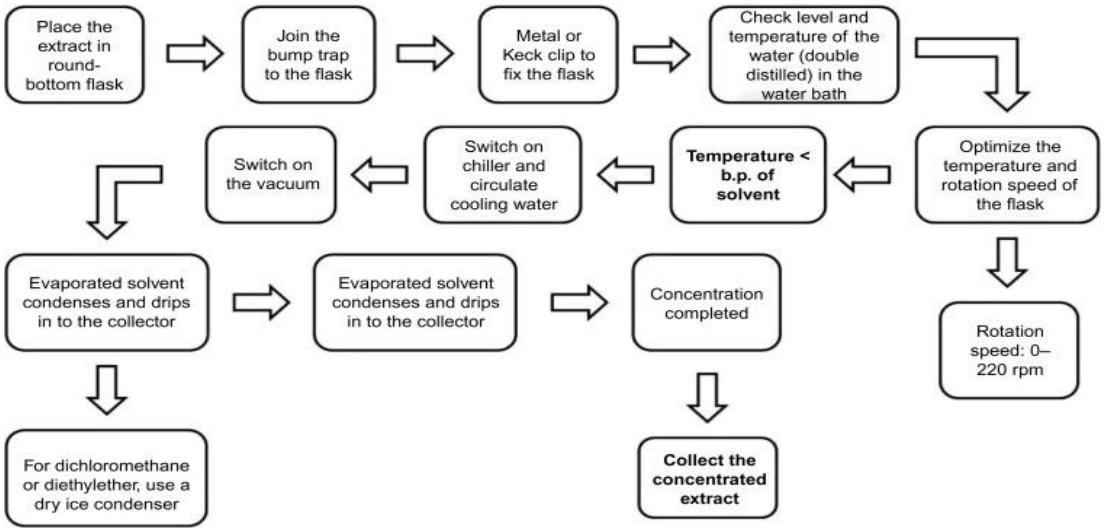


Figure 3.3: Stepwise Procedures for extraction of plants using solvent extraction method.

Figure 3.4: Rotary evaporator used for solvent extraction.

3.8 Phytochemical Screening :

Phytochemical screening of the methanolic and ethanolic extracts of samples were tested for the occurrence of tannins, saponins, alkaloids, cardiac glycosides, polyphenol, terpenoids, steroids,proteins, reducing sugar following the standard procedures described by Harichandan, et al.; 2019

3.8.1 Test for proteins:

Million's test: Crude extract was mixed with 2ml of Millon's reagent. Precipitation are found which turned red on gentle heating. That confirmed the presence of protein.

3.8.2 Test for carbohydrates:

Fehling's test: Fehling A and Fehling B reagents were mixed as equal amount and 2ml of it was added to the plant extract and then heated the sample gently. The presence of reducing sugars is confirmed by appearance of brick red precipitate.

3.8.2 Test for phenol:

2 ml of alcohol and 2-3 drops of ferric chloride solution was added to 1 ml of crude extract, bluish green or black coloration indicated the presence of phenols.

3.8.3 Test for Tanin:

1 ml of distilled water and 2-3 drops of ferric chloride solution was added to 0.5 ml of crude extract. A black coluration indicated the presence of tannin.

3.8.4 Test for flavonoids:

Shinoda test: Crude extract was mixed with small amount of magnesium and concentrated HCl was added drop wise. Appearance of pink scarlet colour after few minutes indicated the presence of flavonoids.

3.8.5 Alkaline reagent test:

0.5 ml of crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

3.8.6 Test for saponins:

1ml of crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

3.8.7 Test for glycosides:

Keller-kilani test:

0.5 ml of crude extract was mixed with 2ml of glacial acetic acid containing 2-3 drops of 2% solution of FeCl3. Then 2ml of concentrated H_2SO_4 was poured into the mixture. The presence of cardiac glycosides is confirmed by a brown ring at the interface.

3.8.8 Test for alkaloids:

2ml of 1% HCl was mixed with crude extract and heated gently. After heating, Mayer's And Wagner's reagents were added to the mixture. If precipitate was observed in the reaction mixture which indicated the presence of alkaloids.

Chapter VI: Results

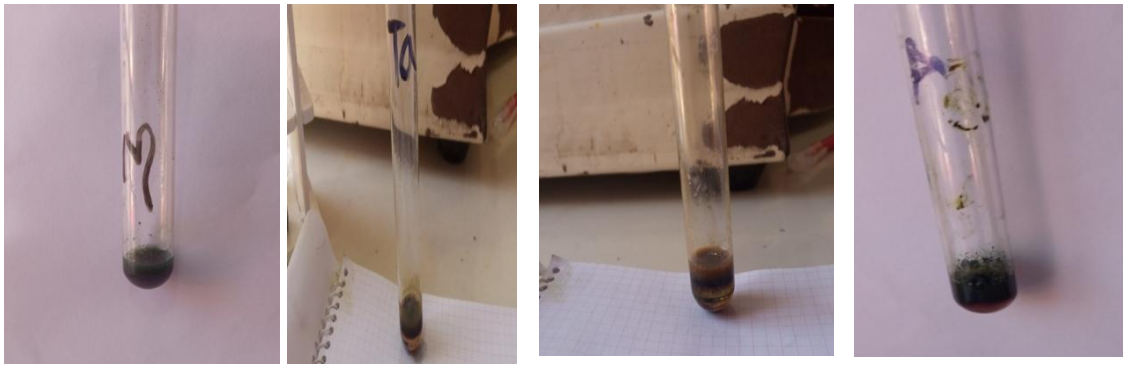
4.1 Phytochemicals screening

The phytochemical study exposed the presence of various phytocompounds in both ethanolic and methanolic solvent extracts. In the both Ethanolic solvent extract and methanolic solvent extract various phyto-compounds like Flavonoids, Alkaloids, Glycosides, Saponins, Phenols were present in most of the samples. Proteins presences is only found in *Centella asiatica* and *Coriendrum sativum* in methanolic extract.

Tests for phytochemical screening	Ethanolic extract				Methanolic extract			
	O. sanctum	*C. asiatica*	*C. sativum*	*Mentha spicata*	*O. sanctum*	*C. asiatica*	*C. sativum*	*Mentha spicata*
Carbohydrates	+	+	+	+	+	+	+	+
Proteins	-	-	-	-	-	+	+	-
Flavonoids	+	+	+	+	+	+	+	+
Tannin	+	+	+	+	+	-	+	+
Glycosides	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+
Phenolics	+	+	+	+	_	+	+	+
Saponin	+	+	+	+	+	+	+	+

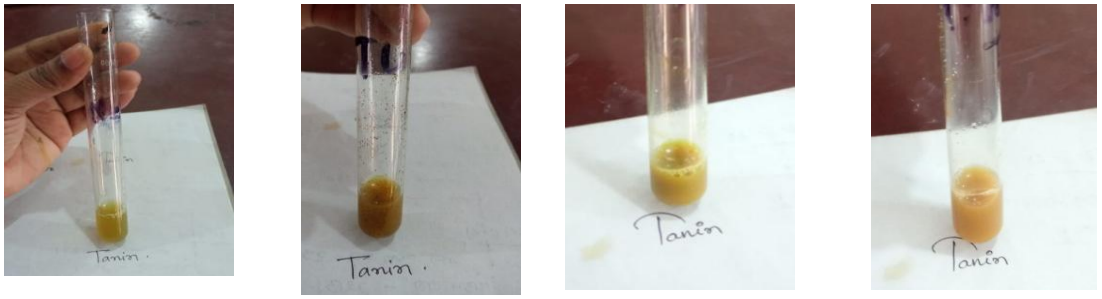
(+) = presence & (-) = absence A preliminary study has reported that the leaves extract contained large number of bioactive secondary molecules like phenols, alkaloids, tannins, glycosides, carbohydrates, flavonoids

Table 4.1: The presence of these components in these herbs is an indication that it may have some medicinal potential.



Mentha spicata *Ocimum sanctum* *Coriendrum sativum* *Centella asiatica*

Figure 4.1.1: Test for Carbohydrate



Mentha spicata Ocimum sanctum Coriendrum sativum Centella asiatica

Figure 4.1.2: Test for Tanin

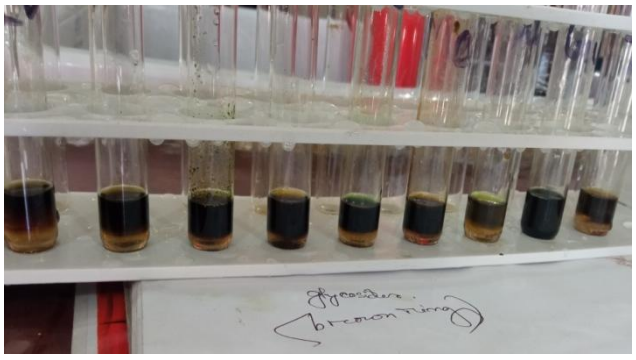
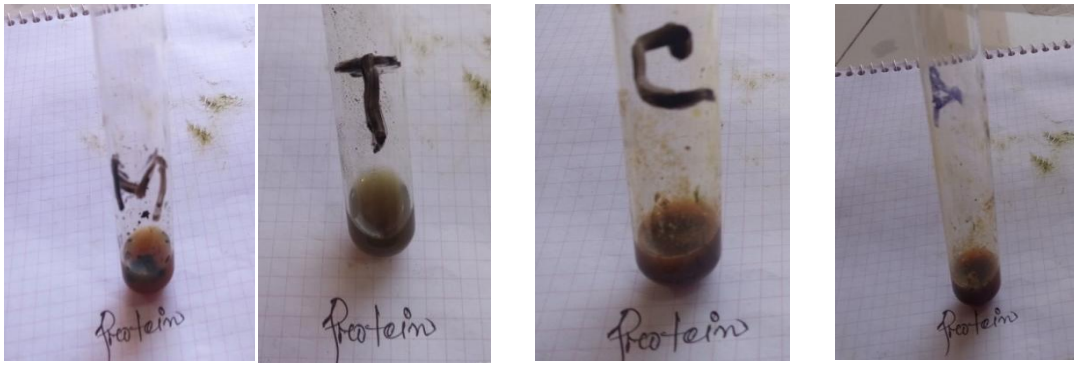


Figure 4.1.3: Glycosides Test

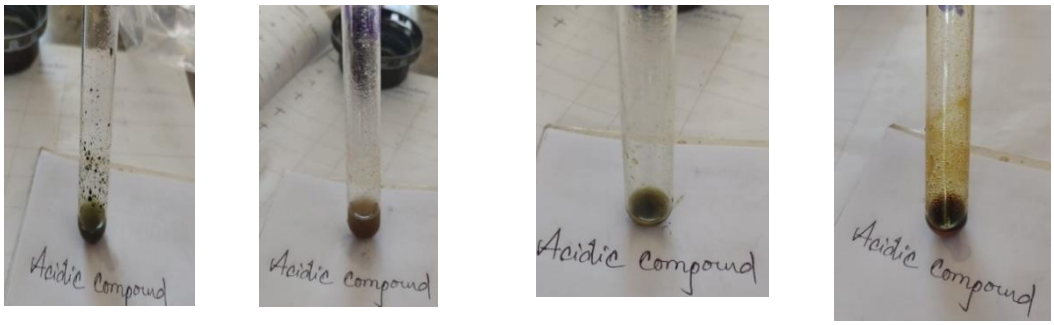


Mentha spicata *Ocimum sanctum* *Coriendrum sativum* *Centella asiatica*

Figure4.1.4: Test for protein

Mentha spicata *Ocimum sanctum* *Coriendrum sativum* *Centella asiatica*

Figure 4.1.5: Test for phenolic compound



Mentha spicata *Ocimum sanctum* *Coriendrum sativum* *Centella asiatica*

Figure 4.1.6: Test for acidic compound

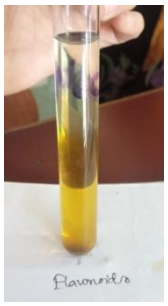
Mentha spicata

Ocimum sanctum

Coriendrum sativum

Centella asiatica

Figure 4.1.7: Test for saponin



Mentha spicata

Ocimum sanctum

Coriendrum sativum

Centella asiatica

Figure 4.1.8: Test for flavonoids

4.2 Bioactive compounds

The results of bioactive compounds (TFC and TPC) are presented in table . There have a significantly different values found among all samples. Table 4.2 showed (ME±SD) of bioactive compounds of four selected herbs samples. In Sample of

Cenetlla asiatica had the highest total phenolic content (TPC) 32.8±0.03 (mg/100 g) whereas in sample *Coriendrum sativum* had found the lowest value 14.7±0.04 (mg/100 g) in ethanolic extract. The highest value of total flavonoid content (TFC) in ethanolic extract 21.23±0.06 (mg QE/100 g) was found in the Sample *Ocimum sanctum* and the lowest value 19.0±0.01 (mg QE/100 g) was for Sample *Coriendrum sativum.* In case of methanolic extract the highest value of total phenolic content (TPC) is found in *Centella asiatica* as 29.0±0.02 (mg/100 g) and in *Mentha spicata* ,the lowest value 19.4±0.02 (mg/100 g) is found whereas the lowest value of Total flavonoid content (TFC) (mg GAE/100g) is found in *Centella asiatica* as 4.09±0.04 (mg GAE/100g). The result showed significant total phenolic and flavonoid content in plant extracts. Descriptive statistics in One-way ANOVA procedures were perform ed to study data statistically at 5% significance level.

Solvent	Samples	Plant parts used	Total phenolic content (TPC) (mg QE/100 g)	Total flavonoid content (TFC) (mg GAE/100g)
Ethanol	*Cenetlla asiatica*	Leaves	32.8±0.03	19.88 ±0.04
	Ocimum sanctum	Leaves	24.7±0.02	21.23±0.06
	Coriendrum sativum	Leaves	14.7±0.04	3.49±0.01
	Mentha spicata	Leaves	22.0±0.05	38.28±0.02
Methanol	*Cenetlla asiatica*	Leaves	29.0±0.02	4.09±0.04
	Ocimum sanctum	Leaves	28.0±0.03	17.08±0.01
	Coriendrum sativum	Leaves	22.7±0.04	4.074±.02
	Mentha spicata	Leaves	19.4±0.02	34.58±0.03

All values are expressed as Mean $\pm$ SD. The data were expressed as mean ± standard deviation. The mean differnce is significant ($p<0.05$). All the analyses were performed at least in triplicate.

Table 4.2 : Total phenolic content and Total flavonoid content in herbs

4.3 Antioxidant capacity:

Antioxidant activities of herbs extract were examined in two different solvent, ethanol and methanol. The highest scavenging effect as Total Anti-oxidant Capacity (TAC) (mg TE/100 g) is presented by centella asiatica in both methanolic and ethanolic extract as 72.83±0.001 (mg TE/100 g) and 69.74±0.001 (mg TE/100 g) . The lowest value found in *Coriendrum sativum* in both solvent ranging as 34.37±0.005 (mg TE/100 g) and 21.502±0.00057 (mg TE/100 g).

Solvent	Samples	Plant parts used	Total Anti-oxidant Capacity (TAC) (mg TE/100 g)
Ethanol	*Centella asiatica*	Leaves	72.83±0.001
	Ocimum sanctum	Leaves	41.962±0.001
	Coriendrum sativum	Leaves	34.37±0.005
	Mentha spicata	Leaves	51.43±0.001
Methanol	*Cenetlla asiatica*	Leaves	69.74±0.001
	Ocimum sanctum	Leaves	53.787±0.04
	Coriendrum sativum	Leaves	21.502±0.05
	Mentha spicata	Leaves	52.44±0.02

Table 4.3: Antioxidant capacity of herb samples using the DPPH method

Chapter V: Discussion

5.1 Phytochemicals

Phytochemical screening of the plant extract confirmed the occurrence of several bioactive compounds like flavonoids, tannins, alkaloids which could be responsible for the resourceful medicinal properties of this plant. By the preliminary phytochemical test, the methanol and ethanol extract shows the presence of flavonoids, alkaloids, tannins, saponins, carbohydrates and but the absence of proteins in most of the samples. The phytochemical screening of ethanolic extract indicates the presence of alkaloids, saponins, flavonoids, terpenoids, carbohydrates and phenolic compounds and the absence of tannins and quinines(Kumar, et al., 2014). The phytochemical constituents such as alkaloids, steroids, flavonoids, tannins, phenols and several other aromatic compounds of plants serve a defense mechanism against predation by many microorganisms, insects and other herbivore (Bonjar *et al*.,2004). Glycosides can act as cardio stimulants in cases of cardiac failure (Sood *et al*., 2005). Tannins have anti-diarrheal and haemostasis properties (Asquith *et al*., 1986). Flavanoids are responsible for antioxidant and immunostimulatory properties. According to Cragg *et al*., 1999 and Khanna *et al*., 2003 alkaloids, glycosides, flavonoids and saponins are antibiotic principles of plants. These antibiotic principles are the defensive mechanisms of the plants against pathogens.

Centella asiatica contains eminent levels of natural antioxidants compounds with a good antioxidant manifestation among the 43 edible plants like tannins and total phenolics are cultivated throughout the world 90 (Chanwitheesuk *et al.;* 2005) . Antibiotic principles of plants and these antibiotic principles are actually the defensive mechanisms of the plants, that hostile to pathogens are excerted by alkaloids, glycosides, flavanoids and saponin (Khanna *et al*., 2003). The presence of several phenolic constituents which possess an ultimate structure for the scavenging of free radicals play a part to the anti-oxidant activity of the plant extract such as flavonoids, tannins etc. (Jelodar, et al.; 20070. According to the Folin-Ciocalteu technique, the total phenolic content ranged from 3.23 to 11.7 g/100 g of dry sample and strongly correlated (r2=0.90) with antioxidative activity. According to certain

theories, C. asiatica's antioxidative properties are mostly attributed to phenolic components. (Zainol, et al.; 2003).

Phytochemical screening of ethanolic extract of Mentha spicata leaves had disclosed the presence tannins, flavonoids, alkaloids, glycosides, saponins, steroids and phenol by confirmatory reaction with the respective test reagent. The most important bioactive phytochemical constituents are Flavonoids, Tannin, Alkaloids, Saponins, Coumarins, Steroids, Anthraquinones, and Sterols and Terpenes are available in Mentha spicata extracts (Soni, et al.; 2013; Ullah, et al.; 2011).Using ferric chloride test the presence of tanins and phenols were validated and flavonoids, and saponins were examined using alkaline reagent test (Tiwari et al., 2011), and foam test, respectively, were estimated by Polash, that shows similar result to this study. Kellar Killani's test was used to evaluate the presence of the glycosides in ethanolic extracts (Nagaraja et al., 2014).

Considering the emerging applications of *Coriander Sativum* in the field of medicine; the aim of the study was to determine the presence of different phytochemicals and their concentration in alcoholic extract of coriander for its physical and morphological evaluation. In this study, methanol solvent extract and ethanol solvent extract was used for phytochemical screening of coriander and different tests were performed on this extract. The results of this analysis indicated that flavonoids (NaOH Test) were present in very high concentration, tannins (Ferric Solution Test), quinines (HCI Test), terpenoids (Salkowaski Test) and cardiac glycosides (Killer killiani test) were present in low concentration in extract, whereas alkaloids (Wagner's Test), phenols (FeCl2 Test), phlobatannins (Precipitate Test) and carbohydrates (Molisch's Test) were found to be present in least concentrations. Absence of oxalates (Ethanoic Acid Glacial Test), saponins (Foam test) and proteins (Ninhydrin Test) was observed in both extract. Due to the presence of these secondary metabolites, coriander stands as a prospective herb for anticancer, antibacterial, antiviral and antifungal therapy.

The presence of proteins, saponins, flavonoids, amino acids, carbohydrates, alkaloids in leaves and stem parts which were extracted with methanolic and ethanolic solvents in the study carried out by Garg, et al.; (2019) that al also figured out in Table 5.1 in ocimum sanctum leaves extract. The results in this study were in harmony with Ani

(2016), ethanolic extracts of three different labiatae species were found to contain alkaloids, saponins, flavonoids, phenols, cardioactive glycosides, terpenoids, tannins and carbohydrates. Osimum basilicum had the highest concentration of flavonoids and cardioactive glycosides, followed by Mentha spicata.

In this study, phytochemical analysis of the plant extracts showed positive results for several compounds. The plant extracts have been shown to contain alkaloids, flavonoids, saponins, phenols, tannins, terpenoids etc., in leaves extract. The results obtained in this study suggest that identified phytochemical compounds may be this plant's bioactive constituents, indicating that plants are valuable reservoirs of bioactive compounds of substantial medicinal advantage.

5.2 Total phenolic content (TPC) and Total flavonoid content (TFC)

The biological activity of plant phenolics and flavonoids is strong, demonstrating the need for their identification. The research's primary goal was to determine the total phenolics and flavonoids in samples of medicinal plants of various species, which was motivated by the compelling evidence of the biological actions of phenolic compounds.

In the present study, the total phenolic and flavonoid contents of *Coriandrum sativum, mentha spicata, ocimum sanctum , centella asiatica* leaves powder extracts were determined. TPC and TFC were calculated by extrapolation from the calibration curve prepared from gallic acid and quercetin concentrations, respectively. Table 4.2 displays the TPC and TFC of three different extracts of samples above mentioned. The adequate amount of total phenolic and total flavonoid content in *O. sanctum* was estimated *(* B. Piyali et al.; 2014); S. Guleria**,** et al.; 2013) ; S. Kaur, 2014).

The quantity and quality of phenolic components present in the extracts are not revealed by TPC as evaluated by the Folin Ciocalteu technique (Wu, X., et al.; 2004). Total phenolics found in methanolic extract coriander leaves in this study is 28.0±0.03 mg/g. The methanolic extract of coriander leaves is affluent in total phenolic contents, which contain 30.25 mg/g that is correspondence with the present study data (Shahwar et al.; 2012). Coriander fruits grown in Tunisia, Syria, and

Egypt were examined for their total polyphenol and flavonoid contents using methanolic extracts, and it was found that there were found significant differences in total polyphenols (0.94 0.05 to 1.09 0.02 mg GAE/g dwb) and total flavonoids (2.03 0.04 to 2.51 0.08 mg CE/g dwb) (Msaada *et al*.; 2017). After screening the methanolic crude extracts of seven commonly used spices, including Coriandrum sativum in Bangladesh with the IC50 value of coriander extract was determined 58.36 g/mL (Sultana *et al*.; 2010).

Total phenolics contents in plants extract have found as highest value than in methanolic extract in this study. But due of methodological inconsistencies, statistics on the phenolic content of Mentha species are usually dispersed throughout the publications and impossible to compare (Fatiha, B., et al.; 2015). According to the quantitative investigation, Tulsi leaf has a significant amount of phenols, ranging from 1.6 to 7.6 percentages (Borah, R. et al.; 2018). The amount ranging from 0.91 to 1.28 and 1.56 to 2.24 percentages respectively of alkaloid and flavonoids were estimated. It is documented that the contents of ascorbic acid were 156.55mg/100g, phenolics 53.75mg/100g and flavonoids 93.42 mg/100g fwb in coriander leaf and in coriander stem were 60.61, 1.45 and 10.67 mg/100g fwb, respectively by Ji et al. (2011)

The total amount of phenolic compounds of the evaluated spearmint extract figured out 22mg/g which was in concurrence with Dorman et al. (2003) who reported a total phenolic content forMentha spicataL. (spearmint) extract of 24 mg/g, expressed as gallic acid equivalents.

Total phenolic content (TPC) of the extract of O. sanctum anticipated by Folin-Ciocalteu reagent mottled significantly. TPC measured as Gallic acid equivalent (GAE) varied extensively ranging from 24.8±0.03 mg/g in ethanolic extract to 28.0±0.03 mg/g in methanolic extract. The flavonoid content expressed as quercetin equivalents, varied from 21.23±0.06 in ethanolic extract to 17.08±0.01 mg QE/g in methanolic extract that shows a correlation with TPC measured as Gallic acid equivalent (GAE) varied widely ranging from 212.26 ± 6.3 mg/g in OsB to 52.68 ± 1.8 mg/g in OsH by Chaudhary,et al (2020). Ocimum sanctum leaf methanolic

extract has higher flavonoid content than ethanolic solvent extract (Garg, et al.; 2019).

5.3 Antioxidant capacity:

The antioxidant capacity among the all samples showed in table 4.4 have a significant difference. DPPH was a widely utilized substrate to assess antioxidant activity, particularly when examining the free radical scavenging capabilities of both biological and chemical molecules. Antioxidant studies with ethanol extracts have already been reported for the herbs samples in Table4.2 in higher value than the methanolic extract. Antioxidant activity of the extracts in ethanolic extract ranging from 72.83±0.001 (mg TE/100 g) to 34.37±0.005, where *Centella asiatica* > *Mentha spicata* > *Ocimum sanctum* > *Coriendrum sativum* represent this order.

The free radical scavenging activity of coriander leaves extract was reported to be 26.82 percent at concentration of 500 g/mL in the bioactivity of the coriander extracts. The seeds and leaves of coriander in methanol, both demonstrated significant radical scavenging activity respectively (64.40 0.81 percent) and (72.19 0.64 percent) (Al-Juhaimi et al.; 2011), in comparison with the n-hexane extracts, which were respectively (52.67 ± 2.05%) and (60.80 ± 1.01%) was estimated by Pandey et al. (2012).

The methanol extract had the highest level of antioxidant activity (IC50 value: 41.34 g/mL) when compared to the ascorbic acid standard (Perumal, et al.; 2011). The findings may shed light on the probable use of C. sativum L. as a source of phenolic component raw resources for industrial use.

The plant extract showed substantial potential antioxidant action. It confirms a prior observation that the existence of compounds with free hydroxyls in the chemical makeup of the plant is what gives C. asiatica's ethanolic extract of its antioxidant action (Mensor, et al.; 2011). Because of the reduction of hydroperoxides, inactivation of free radicals, chelation of metal ions or combinations different parts of C. asiatica may be the potential source of the antioxidative activity. The maximum

activity was seen in the ethanol extract of C. asiatica's root. However, it was not statistically different from the leaves. (Abdul-Hamid, et al.; 2002))

The leaf and stem of the plants under study were found to be particularly rich in flavonoids and phenolics after phytochemical screening and quantitative measurement of chemical constituents. The typical antioxidants found in a variety of medicinal plants are called flavonoids (Abbas, M., et al.; 2017, Ali, et al.;2008). The present study was executed to analyse the phenolics and flavonoids component in medicinal plant *Ocimum sanctum, Centella asiatica, Coriendrum sativum* and *Mentha spicata*. This investigation results showed more flavonoid component present in leaves of *Ocimum sanctum and Centella asiatica* . However, flavonoid component was lesser in both methanol extract of *Coriendrum sativum* leaves. It is widely known that there is a strong association between the levels of phenols in plant extracts and antioxidant activity. The quality of polyphenols is at least as significant as their quantity, as no such association between flavonoids concentration and DPPH scavenging was seen.

Chapter VI Conclusion

In the present investigation, extractions from *Coriandrum sativum, mentha spicata, ocimum sanctum , centella asiatica* leaves dried powder have been evaluated in methanol and ethanol solvents. Alkaloids, flavonoids, saponins, tannins and glycosides were found in the extracts after a phytochemical screening. Therfore, phenolics are the main contributor to the antioxidant activity of these examined species of the chattogram region. The highest antioxidant capabilities and might be indispensable sources of natural antioxidants for additional antioxidant molecule separation and purification. This research backs up the notion that certain of the medicinal plants investigated may be a reliable supply of organic antioxidants for the food industry. The effect of the use of these natural antioxidants on food sensory properties (such as taste and odour) should be focused in future research. The herbs reviewed in these studies can be prospective herbal plants in many medical applications due to their extremely low toxicity, which is proved by their long-standing widespread use as a natural product. Utilization of this conception is essential since it is not only morally just but also economically feasible, sustainable, and grounds few risks and formalities. Due to their well-known positive health effects, it is generally advised to enhance the intake of foods high in antioxidant components. Additionally, antioxidants are employed in industry to increase the stability of foods and cosmetics. synthetic antioxidants such as propyl gallate, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are particularly widespread. Due to their toxicity and potential health hazards, the usage of these antioxidants has been called into question (Wong,et al.; 2006; Li, 2008). The hunt for antioxidants from natural sources, such as fragrant spice plants, is therefore receiving a lot of interest right now. This is due to the fact that these substances have scavenging properties and are also recognized by customers for being natural, non-synthetic products .

Chapter VII: Recommendations and Future Perspectives

A general recommendation to the public is to increase the intake of foods rich in antioxidant compounds due to their well-known healthy effects. On the basis of present investigation, the following suggestions and prospects are made for the further research work.

I. Further experiment can be carried out for the analysis of essential oil of these herbs.
II. The effect of the use of these natural antioxidants on food sensory properties (such as taste and odour) would be addressed in future research.
III. To identify and quantify major bioactive flavonoid compounds profile further analyze can be effective by high performance liquid chromatography (HPLC).
IV. Further research is needed (specially on those plants found with very high antioxidants with high DPPH values) to investigate what chemical compounds are present in these medicinal plants and their potential to be used as possible natural substitutes for artificial antioxidants currently used in food processing.
V. Food products development such as rosogolla and ice-cream can be very effective using the essence or dried powder of tulsi and development of noodles enriched with Centella asiatica powder may a source of nutritious food.

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Appendices

Sample weighing

Sample Filtering

Rotary evaporator

Adding reagents

Sample testing

Hot water bath

Sample placing

Spectrometric analysis

Brief Biography

Shahajadi Pinkey passed the Secondary School Certificate Examination in 2010 from Chittagong Engineering university School and College, Chittagong, and then Higher Secondary Certificate Examination in 2012 from Chittagong Engineering university School and College, Chittagong. She obtained her B.Sc. (Honors) in Food Science and Technology from the Faculty of Food Science and Technology at Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. Now, she is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition, Chattogram Veterinary and Animal Sciences University (CVASU). She has an immense interest to work in improving the health status of people through proper guidance and suggestions and to create awareness among people about food safety and nutrition.