EVALUATION OF NUTRITIONAL COMPOSITION, PHYTOCHEMICALS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *CENTELLA ASIATICA*

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Roll No. 0118/13

Registration No. 554

Session: 2018-2019

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

Department of Applied Food Science and Nutrition

Faculty of Food Science and Technology

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Tapas Dey

June, 2020

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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 & TEACHERS

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Abbreviations

%	:	Percentage
&	:	And
ANOVA	:	Analysis of variance
AOAC	:	Association of Official Analytical Chemists
℃	:	Degree Celsius
CA	:	*Centella asiatica*
CHO	:	Carbohydrate
dl	:	Deciliter
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
et al	:	Et alii/ et aliae / et alia
etc.	:	Et cetera
g	:	Gram
GAE	:	Gallic acid equivalent
Kg	:	Kilogram
mg	:	Milligram
NS	:	Not significant
NC	:	Normal control
PPM	:	Parts per Million
QE	:	Quercetin Equivalent
SD	:	Standard Drugs
SPSS	:	Statistical Package for Social Science
SDM	:	Standard Deviation of Mean

Abstract

In Bangladesh, *Centella asiatica* is widely known as Thankuni and is used as folk medicine. As it is said to have medicinal purposes such as diarrhoea, dysentery, constipation, wound healing, fever and kidney diseases, it is eaten raw by local people. This study aimed to evaluate nutritional composition, phytochemicals, antioxidants and antimicrobial activity to priorities the edibility and the medicinal uses of *Centella asiatica*. The presence of tannin, saponin, flavonoid, phenol, steroid, trapezoid and reducing sugar were revealed by phytochemical analysis of the methanolic extract. It has a decent amount of nutrient content, such as carbohydrates (42.96%), minerals (16.44%) and crude fiber (17.02%). DPPH free radical scavenging assay, total phenol content and total flavonoid content have determined the existence of antioxidant capacity of *Centella asiatica*. In terms of the values of the extracts, the DPPH free radical was found at 33.44 µg/ml where the IC_{50} value for standard ascorbic acid was 9.22 µg/ml. The quantitative analysis of total phenol content was 7.68 mg GAE/g and the total flavonoid content was 12.90 mg QE/g. Those results indicated that the plant has high antioxidant properties and has great potential to be used in the treatment of various diseases. *Centella asiatica* was extracted with methanol, ethanol and water. The extracts were then tested by the disc diffusion method for efficacy against *Staphylococcus aureus* and *E. Coli*. The *Centella asiatica* had a higher yield of 11-16 mm with ethanol as a solvent, followed by methanol and water. The growth of *Staphylococcus aureus* and *E. coli* was significantly inhibited by *Centella asiatica* extracts, which can be furthered established as an alternative to synthetic antibiotics. From the above biochemical quantification, it may be concluded that the plants can be incorporated as a good source of nutrients in our diet.

Keywords: *Centella asiatica*, Phytochemicals, Antioxidant, DPPH, Antibacterial activity

Chapter 1: Introduction

In Bangladesh, around 500 medicinal plants have been confirmed to be growing. Nearly 80% of rural people depend on medicinal plants for their primary health care. Today, the emphasis on plant science has increased worldwide and a vast amount of data has been collected to demonstrate the tremendous potential of medicinal plants used in different traditional medicine systems. *Centella asiatica* (CA) is an essential medicinal herb in the Ayurvedic tradition of India that has been used for thousands of years in the Orient as medicine. Indian pennywort (English), Thankuni (Bangladesh), Brahmi-buti (Hindi), Jal Brahmi or Mandookaparni are commonly recognized. The inhabitants of Java and other Indonesian islands also use the herb. It is one of the recorded 'miracle elixirs of life' known over 2000 years ago in China, known as gotu kola (Brinkhaus et al., 2000).

There are whole, crenate, orbicular and reniform leaves of *Centella asiatica*. The leaves are 1.5-6.5 cm in diameter, 7.5-15 cm long petioles, stipules are short sheathing base forming. The umbel inflorescence bears 3-4 pink sessile flowers. The stems show long internodes and are red. This plant is found up to an altitude of 650 m in marshy areas in Bangladesh, India, Sri Lanka and Madagascar (Brinkhaus et al., 2000).

In the nineteenth century, CA extracts were introduced into Indian pharmacopoeia, where, in addition to wound healing, various skin diseases such as leprosy, varicose ulcers, eczema, diarrhoea, fever, amenorrhea, and female genitourinary tract diseases were prescribed for the treatment of mental and neurological disorders. As a vegetable, it is also used (Brinkhaus et al., 2000). The analysis is being conducted to determine the antioxidant properties of *Centella asiatica*.

Free radicals are considered diseases associated with aging and age (Harman, 2009). Cancer, arthritis, Alzheimer's disease, diabetes, and so on can occur when free radicals destroy cells (Clancy and Birdsal, 2013 and Karthikeyan et al., 2011). Because of free radicals, extreme necrosis might occur. Targeted antioxidants can achieve better medicinal effects. It is found that antioxidants may decrease side effects and increase survival times, according to some reviewers (Block et al., 2008).

Chemical compounds that prevent growth or destroy microbes are antimicrobial agents. These compounds are also used as additives in food preparation. In order to enhance the quality of life of human beings, different antibiotics and antimicrobial drugs have been used over the years. Unnecessary use of antibiotics, however, makes the microbes resistant (Clardy et al., 2006) and thus needs more effective drugs to counteract the microbes that could cost more. Unwanted side effects of some antibiotics facilitate the use of plant extract as antimicrobial agents in addition to drug resistance (Dash et al., 2011). The growing issue of increasing bacterial antibiotic resistance and increasing interest in the use of natural medicine has led to the search for new antimicrobial agents, primarily from plant extracts (Dash et al., 2011). Medical herbs are natural treatments that are preferred for human and animal health.

Knowing the advantages of herbs, this plant has been introduced into the health care system by many. Triterpenes consisting of asiaticoside, asiatic acid, madecassoside and madecassic acid have medicinal value in different fields, but studies of their antibacterial agents are still missing in the most bioactive constituents in *Centella asiatica* (Norzaharaini et al., 2011). This research was therefore conducted to isolate and investigate the antimicrobial activities of extracts of *Centella asiatica*.

For the treatment of many human infectious diseases, medicinal plants with therapeutic properties are used because they contain many bioactive phytochemical components with curative effects. *Centella asiatica*'s therapeutic properties are primarily attributed to the presence of secondary metabolites such as alkaloids, cardiac glycosides, tannins, flavonoids, saponins, compounds, minerals and vitamins, amino acids, protein, 3-glucosylquercetin, 3-glucosyl and 7-glucosyl-kaempferols, polyacetylenees, protein, 3-glucosylquercetin, and 7-glucosyl-kaempferols (Vinoth et al., 2011). Antioxidants extracted from plants have many biological functions, such as anti-inflammatory, anti-cancer and antimicrobial phenolic compounds. It enhances memory, used especially in childhood bowel symptoms, used both externally and internally as poultice and powder in the treatment of leprosy and syphilitic ulcers respectively (Gambhire et al., 2009 and Mirzaei et al., 2013). By scavenging free radicals and inhibiting peroxidation and other radical mediated processes, plants can also protect the body from oxidative harm (Gyekyel et al., 2012). There is a need for isolation of newer biological compounds from plants that can function as novel drugs due to the profitable productivity of

medicinal plants in biological activities. In Southeast Asian countries such as India, Sri Lanka, China, Indonesia, and Malaysia, as well as South Africa and Madagascar, *Centella asiatica* is a tropical medicinal plant from the Apiaceae family (Jamil et al., 2007). The research was conducted to determine the phytochemicals, the function of antioxidants and the nutrient content of the *Centella asiatica* leaves.

However, there are only limited studies focuses on nutritional composition, antioxidant and phytochemical activity of *Centella asiatica*. Raw and cooked Thankuni is consumed in many areas of Bangladesh. Therefore, the present study was conducted to determine the nutritional composition, phytochemical, antioxidant and antimicrobial activity of *Centella asiatica*.

Aims and Objectives:

Even though, people in Bangladesh eat Thankuni as a vegetable without knowing its nutritional and medicinal value. That's why the main aim of this study is to inform the people about the nutritional and medicinal importance of the plant.

a) To evaluate nutritional composition of *Centella asiatica*.
b) To determine the presence of bioactive compounds in the plant.
c) To determine the antioxidant potential of the plant.
d) To determine antimicrobial effect of the plant.

Chapter 2: Review of Literature

2.1 Overview of *Centella asiatica*

Centella in many parts of the world grows in temperate and tropical swampy areas. Slender, creeping stolons are the roots, green to reddish-green in color, linking plants to one another. It has long-stalked, green, rounded apices with palmately netted veins that have a smooth texture. The leaves, approximately 2 cm, are borne on pericladial petioles (0.79 inch). The rootstock is made of rhizomes, which expand vertically downwards. They are creamy in color and are coated with root fur (Brinkhaus et al., 2000).

The flowers, born in small rounded bunches (umbels) near the soil surface, are white or crimson in color. Every flower is enclosed in two green bracts in half. Hermaphrodite flowers are thin, less than 0.12 inch (3 mm) in height, with five to six corolla lobes per flower. Five stamens and two styles carry every flower. The fruit is densely reticulated, separating it from hydrocotyle species that have smooth, ribbed or warty fruit. In three months, the crop matures, and the whole plant is harvested manually, including the roots. In its areas of distribution, Centella has several common names (Devkota and Promod, 2009).

Figure 2.1: *Centella asiatica*

This herb is used by the Chinese, Indians and Malays for various diseases ranging from mental illness care, impairment of the immune system, circulatory problems, skin problems, epilepsy of liver foods, asthma, hair loss and tetanus. It's used as a brain tonic, too. *Centella asiatica* is the use of ethnic medicinal plants by various ancient cultures and tribal groups on various continents. It is typically defined in the Ayurvedic system of medicine in India under the name Mandukaparni (Singh et al., 2010).

2.2 Morphology

Centella asiatica is a mildly aromatic, prostrate, stoloniferous, perennial, creeper herb with a height of up to 15 cm (6 inches). The stem is glabrous, striated and embedded in the nodes. *Centella asiatica* thrives widely in shady, marshy, damp and wet areas such as paddy fields, river banks that form a thick green carpet and the sandy loam (60 percent sand) is considered to be the most fertile soil for its regeneration rather than artificial soil (Devkota and Pramod, 2009). Leaves, 1-3 from each stem node, long petioles, 2-6 cm long and 1.5-5 cm thick, orbicular-renniform, leaf base sheathing, margins crenate, glabrous on both sides. Flowers are in fascicled umbels, each umbel consisting of 3-4 white to purple or pink flowers, in the month of April-June, flowering takes place. During the growing season, fruits are born at approx. 2 inches long, oblong, globular, strongly thickened pericarp in shape. Seeds have pedulous embryos compressed laterally (Singh et al., 2010).

Centella asiatica is located up to an altitude of 600 m in the tropical and sub-tropical regions of India. The edible leaves are yellowish-green, small, alternate with long petioles, and very distinctive in reniform, orbicular, or oblong-elliptic forms with seven veins. The plant grows horizontally through its green to red stolones which mix with each other and roots in underground (Chopra et al., 1956).

Taxonomy of *Centella asiatica* (https://en.wikipedia.org/wiki/Centella_asiatica)

Kingdim: Plantae

Subkingdom: Tracheobionta

Division: Magnoliphyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Apiales

Family: Apiaceae

Genus: Centella

Species: *Centella asiatica*

2.3 Chemical Constituents

Scientific studies have shown that a number of biochemical components have been found in *Centella asiatica* i.e., secondary metabolites. In medicinal and nutraceutical applications, the chemical constituents of Centella plants play a very important role and are assumed to be attributable to the biologically active components of triterpenes saponins. Centella triterpenes are composed of several compounds, including Asiatic acid, Madecassic acid, Asiaticoside, Madecassoside, Brahmic acid, Brahminoside, Thankiniside, Isothankunisode, Centelloside, Madasiatic acid, Centic acid, and Cenellicacid. The most important biologically active compounds among these triterpenes are: asiatic acid, madecassic acid, asiaticosside, madecassoside (Inamdar et al., 1996). They have been used as the biomarker components for Centella quality assessment because of their significance. The composition of the triterpene components of Centella, however, can be influenced by the location and different environmental conditions (James et al., 2009).

In addition to terpenoids, flavonoids such as quercetin, kaempherol, catechin, rutin, apigenin and naringin, as well as volatile oils such as caryophyllene, farnesol and elemene, also contain a high total phenolic content (Zainol et al., 2003). According to Zainol et al., (2003) the leaves were found to have the highest concentration of phytochemicals compared to the petioles and the roots.

Vitamin C, vitamin B1, vitamin B2, niacin, carotene, and vitamin A are also rich in Centella. Chloride, sulfate, phosphorus, iron, calcium, magnesium, sodium and potassium are found in the overall ash (Bhavana and Joyti, 2011).

2.4 Pharmacological uses of the Plant

2.4.1 Antioxidant properties

The wide spectrum of amphipathic molecules usually called "polyphenolic compounds" is due to antioxidant properties in medicinal plants. Phenolic products function primarily because of their redox properties that make them act as donors of hydrogen, reducing agents and quenchers of singlet oxygen. They act as metal chelators as well (Riceevans et al., 1995). In addition to the pharmaceutical industry, these phenolic compounds are also of interest in the food industry because they delay oxidative lipid degradation and thus increase the consistency and nutritional value of food. Scientists,

food producers, and consumers are interested in natural phenolic compounds because of their beneficial health effects (Huda-Foujan et al, 2009).

An antioxidant is a molecule that prevents the oxidation of other molecules and retains complex structures of overlapping antioxidants, such as glutathione and internally developed enzymes or vitamins A, C and E obtained by ingestion, for solidity, oxidative condition, livestock, and plants. Phytochemicals can scavenge free radicals, such as phenolic acids, polyphenols and flavonoids, contributing to the inhibition of oxidative processes that are responsible for several human diseases. In dietary supplements, antioxidants are commonly used and have been investigated to prevent diseases such as cancer or coronary heart disease (Tosun et al., 2012).

A polyphenol is a form of antioxidant containing a polyphenolic substructure with over 4,000 separate species, many of which have in vitro antioxidant function, but are unlikely to have in vitro antioxidant roles (Wasagu et al., 2015).

2.4.2 Antibacterial Activity

In human beings, various microorganisms are known to cause food spoilage and food-borne diseases. Contaminated water, milk and other foods cause approximately 200 diseases. *Cornebacterium diptheriae*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most commonly known causative species (Yoosook et al., 2000).

One of the significant plants exhibiting antibacterial activity against a large range of bacteria is *Centella asiatica*. In developing countries, diarrhea is a major public health problem. Multiple drug resistance among enteropathogens poses a major threat to diarrhea control in different geographic regions. The broad-spectrum behavior of *Centella asiatica* against a wide variety of enteric pathogens was observed by Mamtha et al., (2004). The alcoholic plant extract showed bactericidal activity within 2 hours in the case of *Vibrio cholerae*, Shigella and *Staphylococcus aureus*.

2.4.3 Phytochemicals Properties

Phytochemicals present in plants have a beneficial health effect or play an important role in disease healing. Plants have the potential to synthesize many secondary metabolites that are known by just 10 % of them. These metabolites include treatments for various diseases such as antimicrobial, anti-inflammatory, anti-diabetic, antihypertensive, etc. (Ayoola et al., 2008 and Mallikarjun et al., 2007). Since these

constituents have a beneficial effect on human health, the study of the constituents is crucial in terms of pharmaceutical and health care products.

2.4.4 Cytotoxic and Anti-tumor

Oral administration of the crude extract of C. asiatica and its partially refined fractions induced apoptosis in solid and Ehrlich Ascites tumor and enhanced the life span of these tumors bearing mice. It was found that asiatic acid has an anticancer effect on skin cancer (Bunpo et al., 2004).

2.4.5 Memory Enhancing

Important effects on learning and memory were demonstrated by aqueous herb extract and reduced levels of norepinephrine, dopamine and 5-HT and their brain metabolites (Bunpo et al., 2004). *Centella asiatica* contains brahmicacid, brahminoside, isobrahmic acid and brahmoside. It has properties that are psychotropic, sedative and anticonvulsant. It is also helpful for dementia, anxiety and mental illnesses.

2.4.6 Antidiabetic activity

Important safety was demonstrated by ethanolic and methanolic extracts of *Centella asiatica* and blood glucose levels were lowered to normal in the glucose tolerance test carried out in diabetic rats induced by alloxan. The wounds of diabetic-induced male Spraque-Dawley rats with Centella plant extract have been treated by Nganlasom et al., (2008). In contrast to control, the wounds of the plant extract treated wounds were found to be epithilialized faster than control (Dutta and Basu, 1968).

2.4.7 Wound Healing

Madecassol, an extract of this plant containing Madecassic acid, Asiatic acid and Asiaticoside, accelerates wound healing and grafting (Gopal Rao and Mastan, 2007). In wound healing, Asiaticoside facilitates the proliferation of fibroblasts and extracellular matrix synthesis (Lee et al., 2002).

2.4.8 Cardioprotective Activity

During Adriamycin-induced heart damage in rats, *Centella asiatica* demonstrated a cardioprotective effect on the antioxidant tissue defense system (Pointel et al., 1987). The whole plant alcoholic extract of *Centella asiatica* was evaluated by Pragada et al., (2004) for cardioprotective activity against myocardial infarction induced by ischemia-reperfusion in rats and their findings clearly indicate the plant's cardioprotective activity

in reducing myocardial injury induced by ischemia-reperfusion (Dutta et al., 1968).

2.4.9 Skin Protective Activity

Skin aging tends to be primarily associated with a decline in the amount of collagen type I, the main component of the dermis of the skin (Singh et al., 1969). Asiaticoside, a saponin component isolated from *Centella asiatica*, has been shown in human dermal fibroblast cells to induce type I collagen synthesis.

2.4.10 Immunomodulatory Effect

Immunomodulatory effects were demonstrated by Triterpenoid saponins of Centella. Immuno-stimulating activities were demonstrated by pectin isolated from *Centella asiatica* and tentative immunomodulatory activities were shown by methanol extracts (Singh et al., 1969). *Centella asiatica* ethanol extract activates the cell-mediated immune system by increasing the phagocytic activity of neutrophils (Cesarone et al., 2001).

Chapter 3: Materials and Methods

3.1 Study period and study area

The study period was six months from October, 2019 to March, 2020. Fresh Thankuni from local market of Chattogram metropolitan area was collected by me. All tasks were conducted in Dept. of Applied Food Science and Nutrition, Dept. of Food Processing and Engineering, Department of Applied Chemistry and Chemical Technology and Poultry Research and Training Center (PRTC) at Chattogram Veterinary and Animal Sciences University, Bangladesh.

3.2 Sample Collection

Fresh Thankuni (*Centella asiatica*) was collected from local market i.e., Jhawtola market, Chattogram, Bangladesh. Thankuni was then washed and stored in a cool and dry place until they were used.

3.3 Extract Preparation

Various sections (stems, leaves, petioles) of *Centella asiatica* were randomly collected and transported in sterile bags to the laboratory. It has been aseptically processed. The specimens were thoroughly washed and sun dried for 8-10 days. Methanol (80%) was macerated in the dried samples and permitted to stand for 48 hours (10 gm per 100 ml of methanol) and filtered. Then the filtrate was evaporated and processed using methanol in a hot air oven. The extracts were processed and used for further study in a refrigerator at 5°C (Brinkhaus et al., 2000 and Ayoola et al., 2008).

3.4 Nutritional composition of the sample

3.4.1 Determination of Protein

Principle: The Kjeldahl technique is used in organic and inorganic samples to determine the nitrogen content. The Kjeldahl method has been used in a wide variety of samples for nitrogen determination. For the measurement of protein content, the determination of Kjeldahl nitrogen is carried out in foods and beverages, meat, feed, cereals and forages. The Kjeldahl process is also used for the determination of nitrogen in wastewater, soil and other samples. It is an official method and it is described in different normative such as (AOAC, 2005), USEPA, ISO, DIN, Pharmacopeias.

Calculations: The percent nitrogen or percent protein calculations must take into account the type of receiving solution was used and any dilution factors used during the distillation process. N" represents normality in the equations below." 'ml blank' refers to the milliliters of base necessary to re-titrate a reagent blank if the receiving solution is standard acid, or refers to the milliliters of standard acid required to titrate a reagent blank if the receiving solution is boric acid. When the receiving solution is boric acid then the equation is

$$\% \text{ Nitrogen} = \frac{(\text{ml standard acid - ml blank}) \times \text{N of acid} \times 1.4007}{\text{Weight of sample in gram}}$$

3.4.2 Determination of Moisture content

Principle: Moisture determination is one of the most important and most widely used measurements in the processing and testing of foods. Since the amount of dry matter in a portion of food is inversely related to the amount of moisture it contains, the moisture content is of direct economic importance to the processor and the consumer. However, the influence of moisture on the stability and consistency of food is of much greater significance. Moisture content was determined by using the standard procedure of the Association of Official Analytical Chemists (AOAC, 2005).

Calculation: The percent of moisture was calculated as follow

$$\text{Moisture } \% = \frac{\text{Initial weight - Final weight}}{\text{Sample weight}} \times 100$$

3.4.3 Determination of ash content

Principle: The ash fraction contains all the mineral elements jumbled together. This method performs oxidization of all organic matter by incineration and determines the weight of remaining ash. 5g sample was placed in muffle furnace at 300°C for 1 hour (AOAC, 2005).

Calculation: The ash content was calculated by the following expression.

$$\text{Ash } \% = \frac{\text{The amount of the ash supplied sample}}{\text{weight of the sample}} \times 100$$

3.4.4 Determination of Crude fat

Principle: Fat is estimated by dissolving food samples into organic solvents (chloroform: methanol) separating the filtrate by filtration. Placing the filtrate into separating funnels and then separated mixture is then dried to measure the extract and finally, the percentage of fat is estimated. AOAC (2005) methods using a Soxhlet apparatus were used to determine the crude fat content of the samples.

Calculation: The percent of crude fat was expressed as follows expression.

$$\text{Fat \%} = \frac{\text{Weight of the extract}}{\text{Weight of the sample}} \times 100$$

3.4.5 Determination of Crude Fiber

Principle: Crude fiber is the water-insoluble fraction of carbohydrate consists mainly of cellulose, hemicellulose, and lignin. It is estimated through digestion of fat-free known amount of food sample by boiling it in a weak solution of acid (1.25% H_2SO_4) for 30 minutes followed by boiling in a weak solution of alkali (1.25% NaOH) for 30 minutes at constant volume and then deducting ash from the residue obtained. The crude fiber was determined according to the AOAC method (2005).

Calculation: The loss in weight represents the crude fiber

$$\text{Crude Fiber \%} = \frac{\text{Weight of residue with crucible - Weight of ash with crucible}}{\text{Weight of the sample (moisture and fat free)}}$$

3.4.6 Determination of total carbohydrate

The carbohydrate content was determined by calculating the difference between the Nitrogen Free Extractive (NFE). It was given as the difference between 100 and a total of the other proximate components.

Calculation: Hence it was calculated using the formula below

$$\text{\% CHO} = 100\% - \text{\% (Protein + Fat + Fiber + Ash + Moisture content).}$$

3.5 Detection of Phytochemicals

Phytochemicals were analyzed using standard methods (Aiyelagabe et al., 2001 and Astuti et al., 2011 and Ayoola et al., 2008 and Buzzini et al., 2008).

Tannin test: i) Applied 1 ml of distilled water and 1-2 drops of ferric chloride solution to 0.5 ml of extract solution and observed for blue-black coloration suggesting tannin presence.
(ii) 10% lead acetate solution was applied to 0.5 ml extract solution and noted for white precipitation suggesting tannin presence.

Saponin test: 0.2 g of saponin extract was shaken with 5 ml of distilled water and heated to boil. The existence of saponin is shown by frothing.

Flavonoid test: 0.5ml of extract was dissolved in 0.5ml of 10% NaOH solution; yellow coloration suggests flavonoid presence.

Phenol test: Applied 2ml of alcohol and a few drops of ferric chloride solution to 2ml of extract solution and observed for coloration.

Steroid test: 5ml of the extract solution was combined with 2ml of chloroform and 3ml of concentrated sulphuric acid was added to form a layer. Steroid presence is marked by the red color on the lower surface.

Reducing sugar test: 1ml of water and 5-8 drops of Fehling solution were applied to 0.5 ml of extract solution at hot temperature and observed for brick-red precipitation.

3.6 Determination of Phytochemicals

3.6.1 Total flavonoid content determination

Flavonoid content in samples was measured by the aluminum chloride colorimetric method as described by Shah and Hossain, (2014).

Preparation of standard quercetin solution: About 10 mg of quercetin was dissolved into 10 ml of distilled water. So, the concentration of the solution was 1mg/ml. This is called a stock solution. Then serial dilution was performed to prepare a different concentrated solution (6 ppm, 12ppm, 24ppm, 48ppm, 96ppm). **Figure 3.1** shows the standard curve of quercetin.

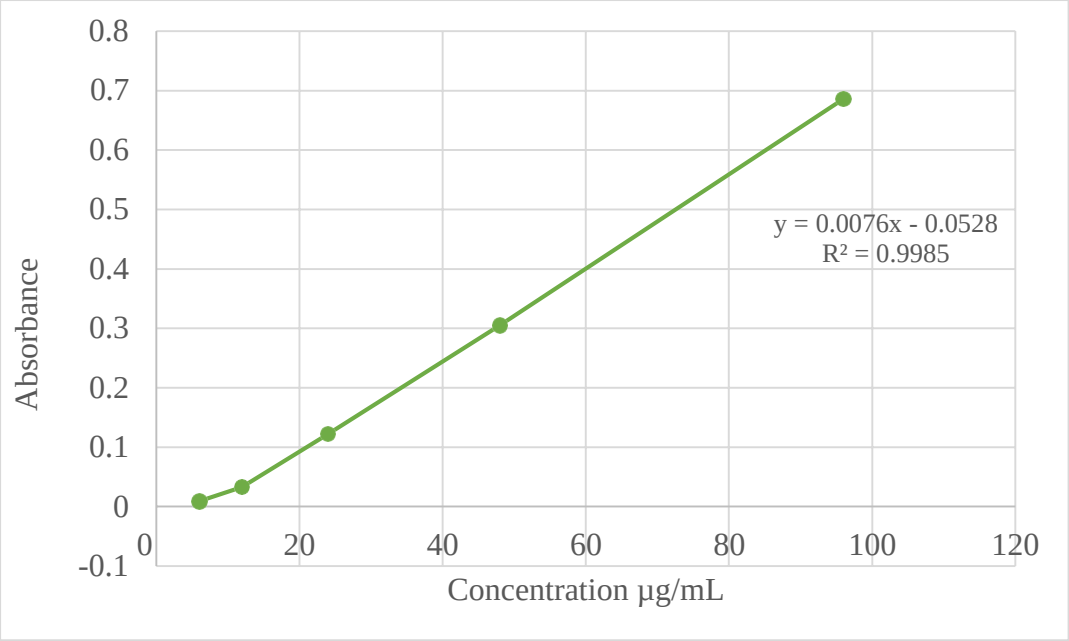

Figure 3.1: Standard curve of quercetin.

Procedure

At first 0.9815g of potassium acetate was dissolved in 10 ml water to prepare a 1M potassium acetate solution. 1g $AlCl_3$ was dissolved in 10 ml water to prepare a 10% $AlCl_3$ solution. About 1ml of sample or standard at different concentration solution was taken in a test tube. After that, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1M potassium acetate, and 8.6 ml of distilled water were added. To complete the reaction, the reaction mixture was then incubated for 30 min at room temperature. At 420 nm, the absorbance of the mixture was measured. To generate the calibration curve, Quercetin was used. The measurement of the total content of flavonoids in the extracts was triplicated and the results were combined. The final result was expressed as mg of the dry weight equivalent of quercetin (QE) per gram. All determinations were carried out three times(n=3).

3.6.2 Determination of Total Phenol content

Preparation of standard gallic acid solution

The total phenol content of extracts was evaluated by the Folin-ciocalteu method as described by Wojdylo et al., (2007). About 10 mg of gallic acid was dissolved into 10 ml of distilled water. So, the concentration of the solution was 1 mg/ml. This is called a stock solution. Then serial dilution was performed to prepare a different concentrated solution (2 ppm, 4 ppm, 8 ppm, 16 ppm, 32 ppm). **Figure 3.2** shows the standard curve of gallic acid.

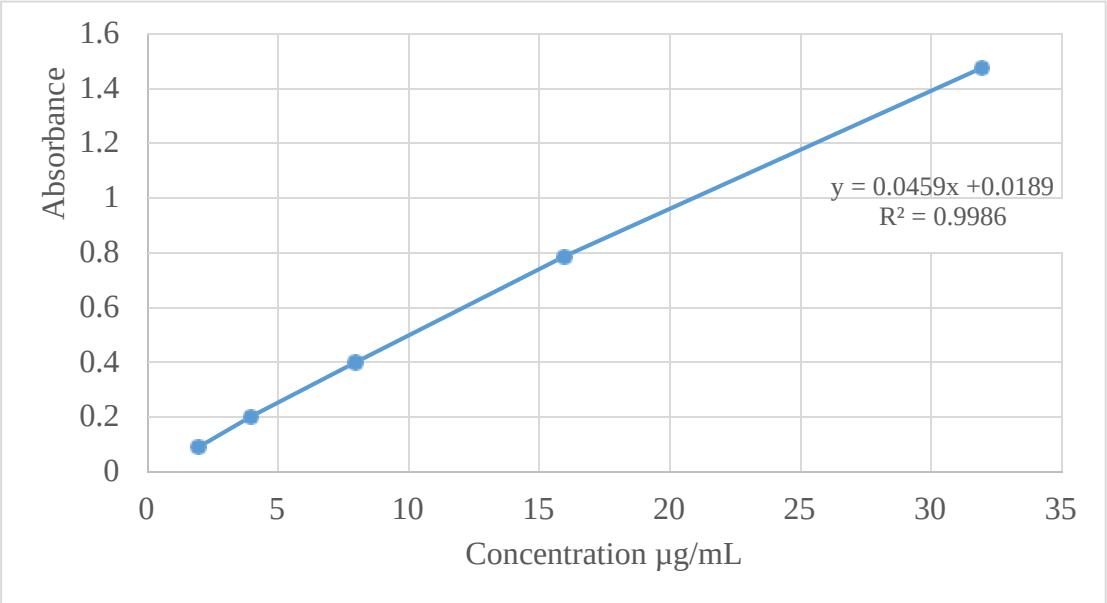

Figure 3.2: Standard curve of Gallic acid

Procedure

About 1ml of sample extracts or standard at different concentrations were mixed with 2 ml of Folin–Ciocalteu reagent (10 times diluted), and incubated at room temperature for 3 min. After that, 10 ml of 20% sodium carbonate was added to the mixture and left for incubation at room temperature for an hour. The absorbance of the mixture was measured at 765 nm with a Shimadzu UV– VIS-2600 spectrophotometer against a blank solution. The blank solution contained all the reagent mixture without extract or standard sample. Gallic acid standard curve was used to quantify total phenolic contents and the results were expressed as mg of gallic acid equivalent (GAE) per gram of dried weight. All determinations were performed in triplicate.

3.7 Determination of Antioxidant activity

DPPH radical scavenging abilities of the test samples were determined by following the method as described by Nariya et al., (2013).

At first 4 mg of DPPH was dissolved in 100 ml of methanol (95%) in a dark condition. 10 mg of ascorbic acid was dissolved in 10 ml of distilled water. So, the concentration of the solution became 1mg/ml. This is called a stock solution. Then serial dilution was performed to prepare a different concentrated solution (2 ppm, 4 ppm, 8 ppm, 16 ppm, and 32 ppm). **Figure 3.3** shows the standard curve of ascorbic acid.

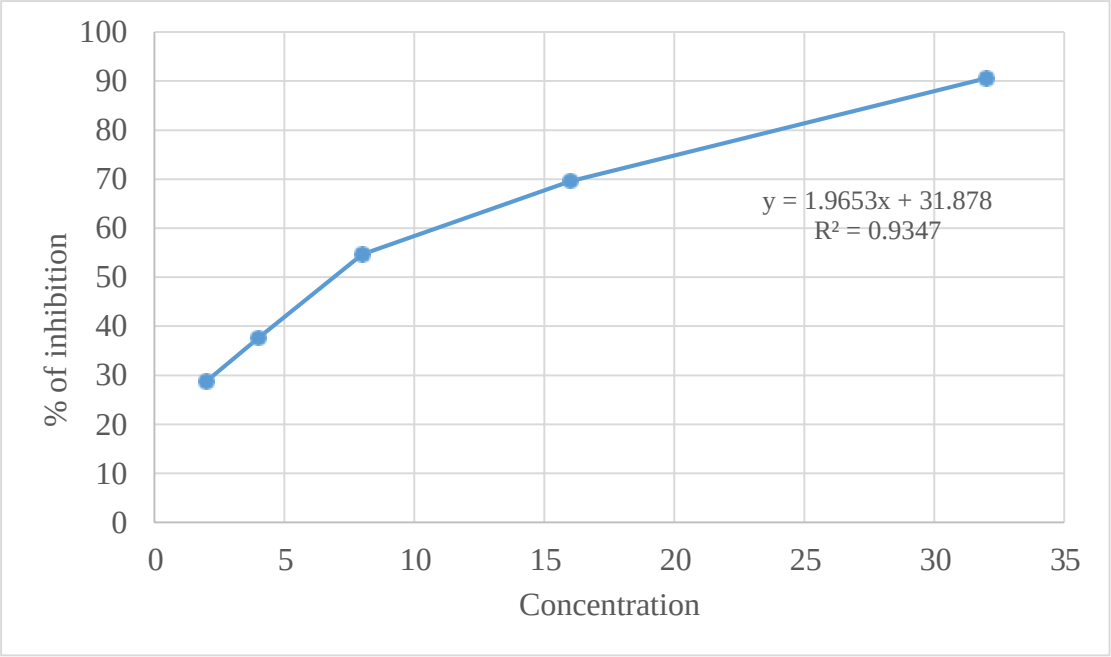

Figure 3.3: Standard curve of ascorbic acid.

Procedure

Serial dilution was performed to prepare a different concentrated solution (2 ppm, 4 ppm, 8 ppm, 16 ppm, 32 ppm). About 4 ml of DPPH solution was added to 1 ml of sample extracts or standards at different concentrations. The mixture was vigorously shaken and allowed to stand for 30 minutes in the dark at room temperature. The absorption of the solution was then measured by a UV-Vis spectrophotometer against the blank at 517 nm. The control sample was prepared to contain the same volume without any extract and reference ascorbic acid. Methanol was used as blank. IC_{50} was calculated from % inhibition. Scavenging of the DPPH free radical was measured using the following equation:

$$\% \text{ of Inhibition} = \frac{\text{Blank absorbance - Sample absorbance}}{\text{Blank absorbance}} \times 100$$

3.8 Antimicrobial Analysis

3.8.1 Preparation of agar plate

In this analysis, Nutrient Agar and Mannitol Salt Agar (MSA) were used as a medium for bacterial growth. By adding 28 g of nutrient agar powder to 1L of distilled water, nutrient agar was prepared. By adding 111 g of Mannitol salt Agar in 1L of distilled water, Mannitol Salt Agar was prepared. The blend was dissolved and subsequently autoclaved at 121°C for 15 minutes. In the Petri dish, the cooling media was poured and left hardened for 24 hr. prior to use (Arora DS and Kaur GJ, 2007).

3.8.2 Preparation of culture

E. Coli and *S. aureus* were obtained from the Microbiology Laboratory of CVASU. The bacteria cultures were prepared by adding a loop of bacteria to the sterilized Nutrient Agar and Mannitol Salt Agar and holding for 24 hr. in an incubator at 37°C.

3.8.3 Antimicrobial activity assay

The disc diffusion method suggested by the National Committee for Clinical Laboratory Standard (NCCLS, 2000) was susceptibly checked with minor adjustment. For the test, which matches the turbidity of a McFarland 0.5 standard solution. After pressing tightly against the inside wall of the tube to extract excess liquid, the bacterial culture was then swabbed uniformly around the entire surface of the media with a sterile swab. For around 5 minutes before putting the *Centella asiatica* extracts on the agar, the cultures were allowed to soak in the medium. As the control filter paper discs 6 mm in diameter were mounted on the agar, sterile distilled water was used. The filter paper discs were pipetted with 1 ml of extracts. Three replications were performed for each microbe. In order to estimate the radial growth of bacteria, the plates were incubated for 24 hours at 37°C and the inhibition zone was assessed.

3.9. Statistical analysis

All statistical analysis was done by using statistical package for social sciences (SPSS) version 25. The data was analyzed by using T-test. Data are presented as the mean± SDM (Standard Deviation Mean). P values <0.05 were considered significant.

Chapter 4: Results

4.1 Nutrient Content of *Centella asiatica*

Nutritive value of *Centella asiatica* (CA) is shown in **Table 4.1**. The result showed that CA contain highest amount of Carbohydrate (42.96%) followed by crude fiber (17.02%) and ash content (16.44%).

Table 4.1: Nutritional composition of *Centella asiatica*

Sample	Moisture %	Ash %	Protein %	Crude fiber %	Crude fat %	CHO %
CA	14.17±1.09	16.44±0.06	8.27±0.03	17.02±0.1	1.14±0.01	42.96±0.04

Legends: The value represented (ME±SD)

4.2 Phytochemicals screening of *Centella asiatica*

The phytochemical screening of methanolic extract of CA is shown in **Table 4.2**. CA was given a positive result throughout the screening of phytochemicals. The methanolic extracts of the plant indicates the presence of steroids, phenols, flavonoids, saponin, tannin and reducing sugars.

Table 4.2: Phytochemical screening of the methanolic extract of *Centella asiatica*

Sample	Steroids	Reducing Sugars	Saponin	Tannin	Phenol	Flavonoids
CA	+ve	+ve	+ve	+ve	+ve	+ve

Legends: (+ve) indicates presence of the phytochemicals.

4.3 Phytochemical composition of *Centella asiatica*

4.3.1 Total Phenol Content

Table 4.3 shows the total phenol content of methanolic extract of *Centella asiatica* was found 7.68 mg Gallic acid equivalent per gram.

Table 4.3: Total Phenol content of methanolic extract of *Centella asiatica*

Sample solution	Weight of the dry extract per ml(g.)	Absorbance	GAE Conc. C µg/ml	GAE conc. C mg/ml	TPC as QAE A= (c.v)/m	Mean	P value
1000	0.001	0.3709	7.68	0.00768	7.68	7.68±0.04	NS

Legends: GAE= Gallic Acid Equivalent, TPC= Total Phenol Content.

The value represented (ME±SD), and ***=significant (P< 0.05), NS=Not significant (P> 0.05).

4.3.2 Total Flavonoid Content

Table 4.4 shows the total flavonoid content of methanolic extract of *Centella asiatica* was found 12.90±0.04 mg Quercetin equivalent per gram.

Table 4.4: Total flavonoid content of methanolic extract of *Centella asiatica*

Sample solution	Weight of the dry extract per ml(g.)	Absorbance	QE conc. C µg/ml	QE conc. C mg/ml	TFC as QE A= (c.v)/m	Mean	P value
1000	0.001	0.0453	12.90	0.01290	12.90	12.90±0.04	NS

Legends: QE=Quercetin Equivalent, TFC= Total Flavonoid Content

The value represented (ME±SD), and ***=significant (P< 0.05), NS=Not significant (P> 0.05).

4.4 Antioxidant activity of *Centella asiatica*

4.4.1. DPPH activity of CA

Results for the DPPH free radical scavenging activity of methanolic extracts of CA is shown in **Table 4.5**. The half inhibition concentration (IC_{50}) value of ascorbic acid was 9.22 µg/ml. In contrast, the IC_{50} value of free radicals obtained was 33.44 µg/ml for *Centella asiatica*.

Table 4.5: DPPH scavenging radical activity of *Centella asiatica* and ascorbic acid

Concentration(µg/ml)	% Inhibition of Ascorbic acid	% Inhibition of *Centella asiatica*
2	28.78	23.36
4	37.63	26.89
8	54.67	35.24
16	69.59	41.68
32	90.57	46.27
IC_{50} (µg/ml)	9.22	33.44

Figure 4.1 depicted y=0.7276x+25.666 where y indicates % inhibition of CA, x indicates concentration and R^2= 0.846 describes the strength of a correlation between two variables.

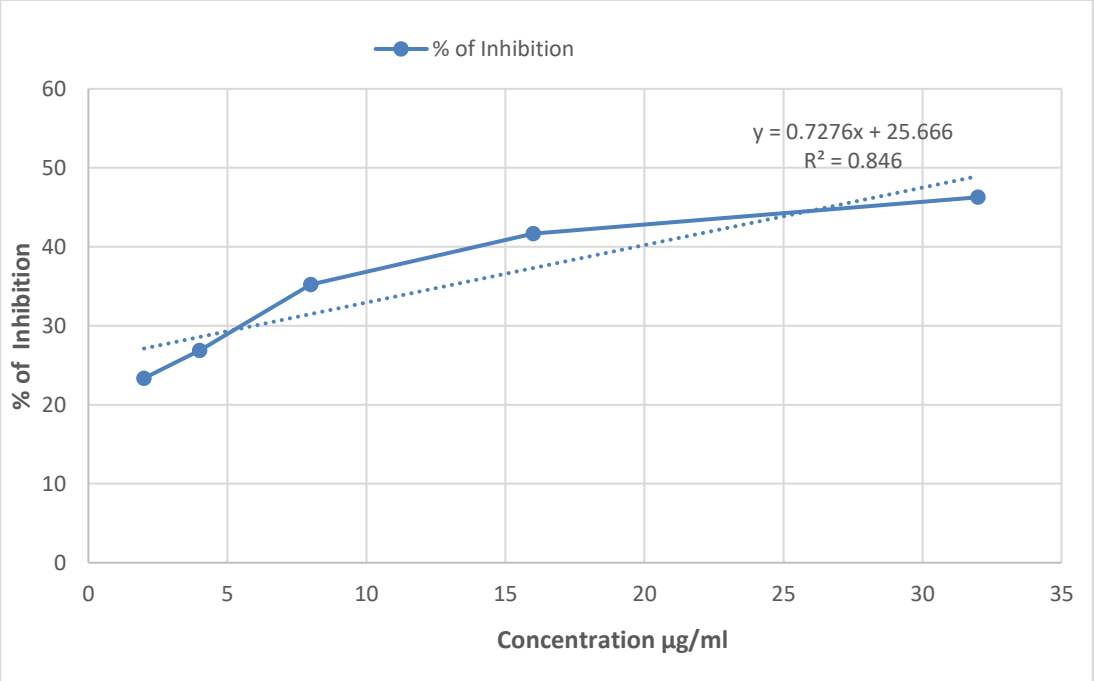

Figure 4.1: DPPH test curve of CA

Figure 4.2 showed the comparison between the standard ascorbic acid and *Centella asiatica* DPPH scavenging activity.

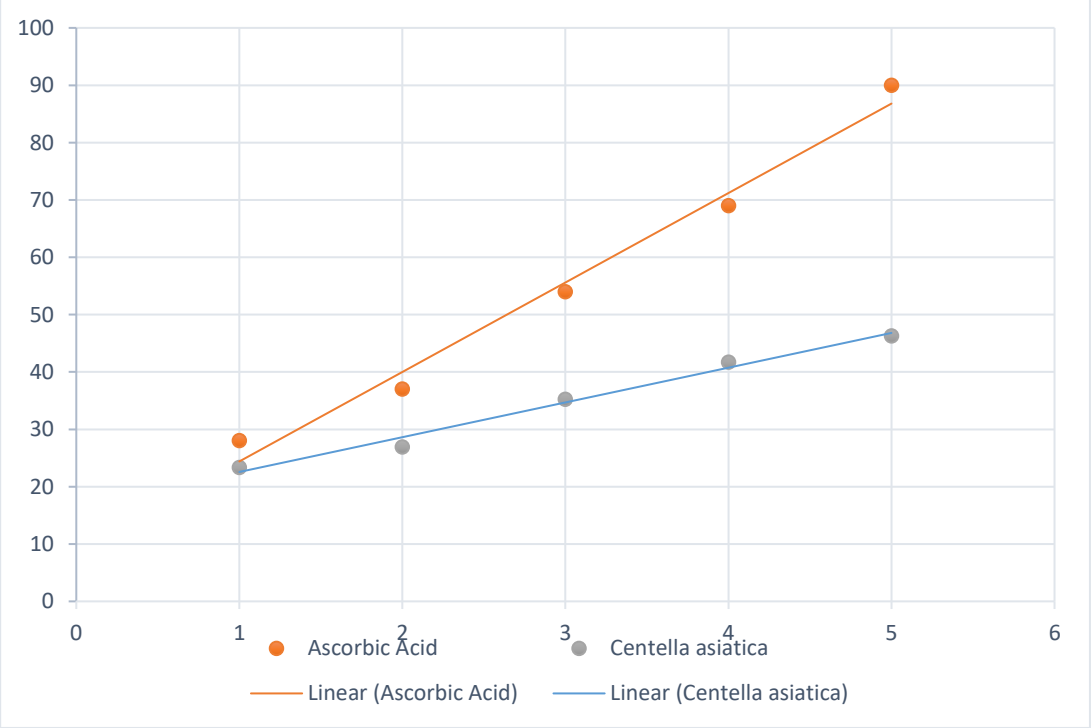

Figure 4.2: DPPH scavenging radical activity of *Centella asiatica*

4.5 Antimicrobial Activity of *Centella asiatica*

The result of antimicrobial activity of CA extracts against *E. coli* and *Staphylococcus aureus* are shown in **Table 4.6**. The largest zone of inhibition was shown by ethanol extract against both *S. aureus* (16mm) and *E. coli* (14mm). The diameter of the methanol extract inhibition zone was slightly smaller than ethanol extract against *S. aureus* (13mm) and *E. coli* (11mm). Water extracts was found less effective to inhibit the growth of both bacteria.

Table 4.6: Inhibition zone of *S. aureus* and *E. coli* against *Centella asiatica* extracts

Sample	*Staphylococcus aureus*	*E. coli*
Ethanol Extract	16 mm	14 mm
Methanol Extract	13 mm	11 mm
Water	07 mm	05 mm

Chapter 5: Discussions

5.1 Nutritional composition of *Centella asiatica*

Proximate data from the study reported that large amount of carbohydrates (42.96%), crude fiber (17.02%) and ash (16.44%) were found in *Centella asiatica*. The result indicates that the key nutrient found in the plant was carbohydrate. The high carbohydrate and ash content reported in this study was consistent with the report by Mertz et al., (2019) on various species of *Centella asiatica* grown in Madagascar with a carbohydrate content of 42.9-52% and an ash content of 13.5-16.4%. For many industries, carbohydrates provide the body with the necessary energy needed to drive cell metabolism as well as a raw material. Compared to the findings of Ranoyona et al., (2019), this research shows similarity in the amount of protein and fat content of the present study. The moisture content of *Centella asiatica* was found in this current study to be an average of 14.17% which is slightly higher than the other values (Mertz et al., 2019). The variation in moisture percentage may be due to different drying processes, regional production or weather condition of the growth area.

5.2 Phytochemical compounds of *Centella asiatica*

In the evaluation of active biological components of plants with medicinal value, phytochemical analysis is very useful. In *Centella asiatica*, steroids, phenols, flavonoids, saponin and tannin were present. Phenols are able to scavenge free radicals and neutralize their effects by avoiding cardiovascular diseases and cancer due to their antioxidant properties (Joshi et al., 2012). Saponin, which cannot be contained in meat and milk, is also rich in plant-based foods. The presence of these phytochemicals in *Centella asiatica* suggests that, with good nutritional and therapeutic value, it could serve significant physiological function.

The average phenol content of CA was 7.68 mg GAE/g, which was marginally lower than the analysis of Zainal et al., (2019). The difference in phenol content may be due to different extraction method, geographical and climatic differences.

The flavonoid content of CA was around 12.90 mg Quercetin equivalent/g which was consistent with the previous study (Zainal et al., 2019).

5.3 Antioxidant activity of *Centella asiatica*

Antioxidant acts in human disease conditions can protect biological systems against harm associated with oxidative stress (Misra et al., 2009). Scavenging radical activity of CA express as IC_{50} value was 33.44 µg/ml which was similar to the previous study (Frederico et al., 2009). The IC_{50} value was 9.22 µg/ml for standard ascorbic acid. The IC_{50} value showed that CA is a rich source of antioxidants, since high antioxidant activity is indicated by a low IC_{50} value. CA may be used both in the therapeutic diet and medical industry as a rich source of antioxidants.

5.4 Antibacterial Activity of *Centella asiatica*

This study deals with two pathogenic bacteria. In this study, the antibiotic potential of two different extract of *Centella asiatica* have been determined against two different microorganisms S. aureus and E. coli. This research showed that ethanol and methanol extracts were found to be very effective in inhibiting the growth of the 11-16 mm inhibition zone of the two tested microorganisms, which was satisfactorily compared to other studies. Ethanol extracts, followed by methanol and water extract, were the most effective in inhibiting these bacteria. The aqueous extract showed the lowest inhibitory activity against the bacteria examined, with an inhibition zone of 5-7 mm. The main objective of this research is to expand the use of herbal biomass in pharmaceutical industries that can be used instead of synthetic antibiotics to prevent diseases.

Chapter 6: Conclusion

Centella asiatica (Thankuni) is a small, annual and meander plant that can be abundantly found mainly in wet areas in Bangladesh i.e., near rivers, ponds and paddy fields, etc. In addition to consuming raw, to combat the bitterness of the leaves, it can be cooked and eaten with coconut milk and sweet potato and can be consumed in various ways, such as tea and juice. The study showed that, in terms of nutritional value, it is a good source of carbohydrates and crude fiber, as well as a great source of minerals. This study showed that *Centella asiatica* had greater antioxidant activity and phenolic content that delayed lipid oxidative degradation. It also showed that the presence of some phytochemicals in the *Centella asiatica* leaves that could be used in the chemical and pharmaceutical industries. *Centella asiatica*'s ethanol extracts had greater antimicrobial activity, followed by methanol and water. *Centella asiatica* extracts were found to be an effective antibacterial agent against S. aureus and *E. coli*. Finally, this study indicated that it is possible to use *Centella asiatica* as an essential source of nutrients and medicine.

Chapter 7: Recommendations and Further Perspectives

These days, the majority of the individuals experience the ill effects of ailing health in our country, in these circumstances *Centella asiatica* could be a decent wellspring of the supplements and energy as these are accessible in rustic territories of Bangladesh. Today, most of the people in our country suffer from malnutrition. Since *Centella asiatica* is high in fiber content, it can be used for constipation and other gastrointestinal health problems. The quality of fiber and minerals suggests that it has potential value for food and could be suggested as a functional food ingredient. In addition, as *Centella asiatica* is a rich source of phenolic content and antioxidants, it can also be used to avoid aging and other health problems associated with free radicals. Natural antioxidants are of great benefit because most synthetic antioxidants currently used have health-hazardous side effects, such as liver damage and carcinogenesis. In order to determine the essential vitamins and other phytochemical compounds of *Centella asiatica*, more study should be performed. We do not know, however, what components are responsible for these antimicrobial and antioxidant activities in plant extracts. Due to time and resource limitations the study was conducted in a small scale and sample had collected from limited area. I analyzed the limited number of samples. Further analysis is required which will provide adequate information on variation in biological active compounds and chemical composition in *Centella asiatica* collected from the various parts of the country. To classify them as biological antioxidants, comprehensive studies on chemical composition, isolation of active constituents and pharmacological evaluations are necessary.

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Appendix A: Photo Gallery

Fresh Thankuni

Sun drying

Grinding

Powdered Sample

Mixing with methanol

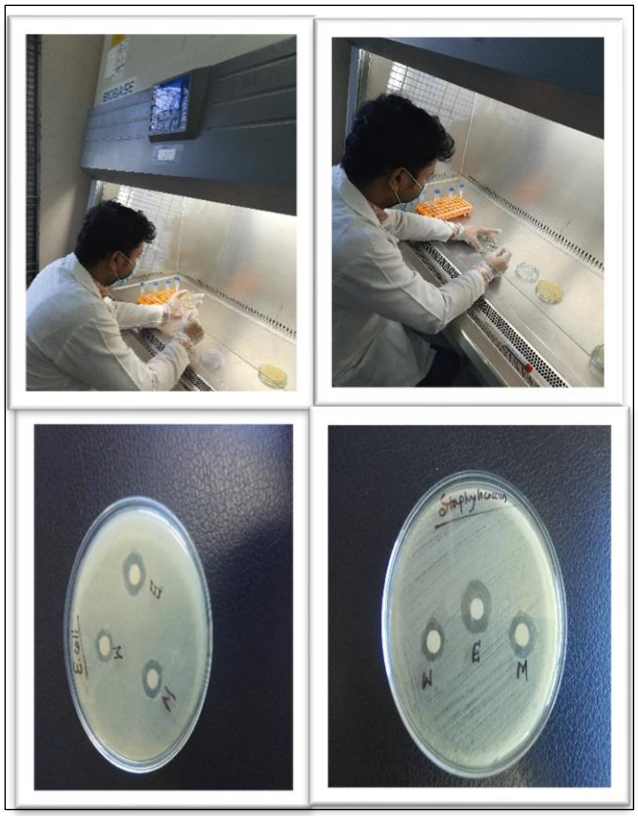
Filtration of extract

Methanolic Extract

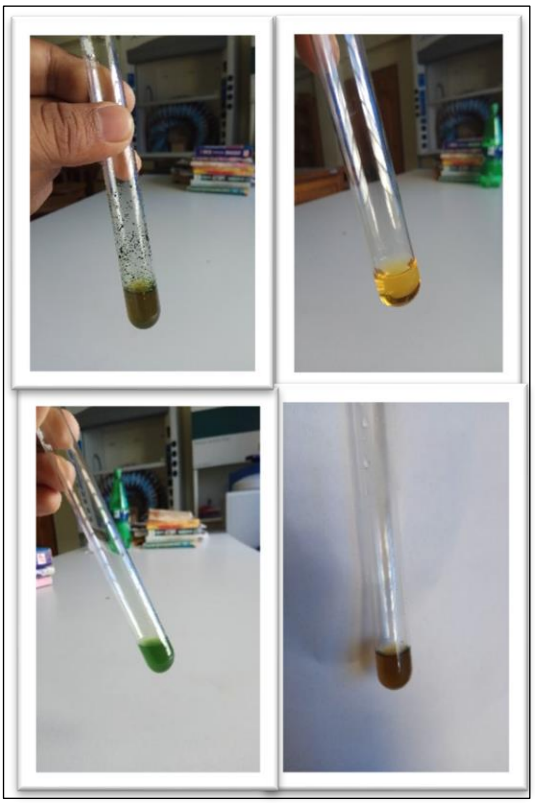
Fig: Preparation of *Centella asiatica* extract

DPHH solution Preparation

Laboratory Works



Antimicrobial Analysis



Phytochemicals Screening

Brief Biography

This is Tapas Dey son of Purnandu Dey and Shilpi Dey who was born in Cox's Bazar Sadar Upazila at Cox's Bazar, Bangladesh. I passed the Secondary School Certificate (SSC) Examination in 2009 from Eidgah Model High School, Cox's Bazar and then Higher Secondary Certificate (HSC) Examination in 2012 from Cox's Bazar Govt. College, Cox's Bazar. I obtained 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, I am 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). I have an enormous interest in improving people's health status through proper guidance and recommendations, and in raising awareness of food safety and nutrition among general people.