

**Identifying Phytochemical Constituents of *Coccinia grandis* and
Identifying Microbial Efficacy of *Coccinia grandis* Against
Salmonella spp and *E. coli***



Shanta Chowdhury

Roll No.: 0119/08

Registration No: 658

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**A thesis submitted in the partial fulfillment of the requirements for the degree of
Master of Science in Food Chemistry and Quality Assurance**

**Department of Applied Chemistry and Chemical Technology
Faculty of Food Science and Technology
Chattogram Veterinary and Animal Sciences University Khulshi, Chattogram-
4225**

June 2022

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Shanta Chowdhury

JUNE, 2022

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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.

Mr. Suvanker Saha

Supervisor

Assistant Professor

Department of Applied Chemistry

And Chemical Technology

Mr. Monsur Ahmad

Assistant Professor

Department of Applied

Chemistry and Chemical

Technology

Dr. Shamsul Morshed

Associate Professor

Chairman of the Examination Committee

Department of Applied Chemistry

And Chemical Technology

Faculty of Food Science and Technology

Chattogram Veterinary and Animal Sciences University

Chittagong-4225, Bangladesh

June, 2022

**DEDICATED TO MY
RESPECTED AND BELOVED FAMILY AND TEACHERS**

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Roll number: 0119/08

Reg. number: 658

Department: Applied Chemistry and Chemical Technology

Faculty: Food Science and Technology

Supervisor: Mr. Suvanker Saha

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Mr. Suvanker Saha

Assistant Professor

Department of Applied Chemistry and Chemical Technology

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Abbreviations

ALP	Alkaline Phosphate
EMB	Eosin-methylene blue
<i>et al</i>	<i>Et alii/ et aliae/ et alia</i>
etc	Et cetera
FAO	Food and Agricultural Organization
HCL	Hydrochloric Acid
ALP	Alkaline Phosphate
EMB	Eosin-methylene blue
HNO ₃	Nitric Acid
LDL	Low Density Lipoprotein
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bacterial Concentration
SGPT	Serum glutamic pyruvic transaminase
LDL	Low Density Lipoprotein
TG	Thyroglobulin

Abstract

Medicinal plants are a source of great economic value. *Coccinia grandis* or Telakucha is a famous medicinal plant which is growing all over the world. The Present study was conducted to evaluate the phytochemical screening of ethanolic and methanolic extract of *Coccinia grandis* and the effect of ethanolic extract against *Salmonella spp*, and *E. coli* from Poultry sample (September 2021 to February 2022) at Chittagong Veterinary Animal Science University (CVASU), Bangladesh. Telakucha leaves were collected from different area of Kelishahar under the upazilla patiya which is situated in Chattogram. Then leaves of Telakucha were then immersed in Ethanolic and Methanolic extracts for seven days, Telakucha leaves were conducted phytochemical screening for proving the presence of Alkaloids, Flavonoids, Saponins, Tannins, Phenolic Compound, Glycosides, Carbohydrates, Reducing sugar, Protein and Amino acid, Acidic Compound, Phytosterol and finally Steroids and Terpenes. In the present study, a systematic approach was followed to develop and standardize the process. Phytochemical screening of the extract of telakucha was done by different test and antimicrobial susceptibility test for *E. coli* and *Salmonella spp*. was occurred by Agar Disc Diffusion Method. These bacteria grew in Mueller Hinton agar and observed the sensitivity after 24 hours. In this study the phytochemical component like alkaloids, flavonoid, saponins, phenols, tannins, terpenoids etc are present in Telakucha. This phytochemical constituents contain antimicrobial efficacy that inhibit the growth of two gram negative bacteria such *E. coli* and *Salmonella spp*. All concentration showed positive results after successfully applying the extract of Telakucha in the petri dish. At 40% concentration showed the highest zone of inhibition than other the two concentrations.

Keywords: *Salmonella spp*, *E coli*, Alkaloid, Flavonoids, Saponins, Tannins, Phenolic compound

Chapter-1: INTRODUCTION

1.1 Background of the study

A complete variety of cures for humanity's ills have been furnished by nature. For basic primary healthcare needs, around 80 percent of the total of the world's population relies solely or mostly on traditional medicine (Kunwar and Adhikari, 2005). The therapeutically significant essential oils are abundant in the medicinal plants' secondary metabolites (potential medicines). The most abundant source of medications for modern medical systems, nutraceutical remedies, nutritional supplements, traditional medicines, pharmaceutical intermediates, and chemical entities for synthesized drugs are plants (Hammer, *et al.*, 1999). The traditional medical system is quite beneficial. Owing to the widespread identification of medicinal herbs from ancient pharmacopoeias, plants tend to have a big impact in healthcare despite the advancements established by modern medicine (Adebolu, and Oladimeji, 2005).

Plants have medicinal value due to various their phytochemicals, which have real physiological effects on people. Phytochemicals are substances derived from plants used as food and medicine to treat illnesses and ensured human health (Afolabi, 2007). Numerous ailments are cured by medicinal plants, and this has always encouraged researchers to look for various plant extracts that can be used to create novel antibacterial agents (Anmad, 1998 and Bushra Beegum, 2003). In many regions of the world, spice plants have historically been utilized as food additives, coloring agents, flavoring agents, preservatives, and antiparasitic, anthelmintic, analgesic expectorant, sedative, and anti-diabetic compounds (Lee, *et al.*, 2004). Many sections of the world have long employed spices plants as food additives, coloring, flavoring, preservatives, antiparasitic, anthelmintic, analgesic expectorant, sedative, and anti-diabetic compounds. The main cause of mortality worldwide is highly contagious diseases.

1.2 Description of the problem

Antibiotic resistance has therefore become a global concern, because the advent of multidrug-resistant bacteria jeopardizes the clinical effectiveness of many of the available antibiotics. (Westh, 2004). The recent emergence of strains with reduced sensitivity to antibiotics and the rising incidence of multidrug-resistant bacterium strains enhance the possibility of incurable bacterial illnesses, making the research for

novel infection-control methods more urgent. (Silver, 1993). Even though pharmaceutical companies have developed a number of novel antibiotics over the past several era, microbe resistance to these medications has grown. In affluent nations, traditional medicines made from ingredients found in medicinal plants are used by about 80% of people. Therefore, research into these plants is necessary to understand their characteristics, security, and effectiveness (Ellof, 1998). The medically significant essential oils and secondary metabolites found in medicinal plants are abundant. Many items are recognized by their active components, such as the phenolics found in essential oils (Jansen,1987) and tannin (Saxena, 1994). Strong antibiotic and antifungal medicines are still ineffective against resistant or multi resistant strains, which highlights the ongoing necessity) for the research of new medications that are risk-free, more trustworthy than expensive medications, and that have no unfavorable complications (Silver and Bostian, 1993). The main benefits of using herbal plants therapeutically for different illnesses are their security, economy, effectiveness, and accessibility. Numerous human societies Spices plants have been used for a long time as food additives, coloring, flavoring, preservatives, antiparasitic, and anthelmintic, and more than 35,000 species of plants are utilized for medical purposes globally. To find fresh ideas for effective treatments for microbiological illnesses, researchers are increasingly focusing on traditional medicine (Jantan, 1998).

Because of the worrisome rise in the prevalence of newly emerging and reemerging infectious diseases, new antimicrobial agents with a range of chemical structures and distinct modes of action are always needed and unique modes of the action. The emergence of resistance to the antibiotics currently being used in clinical settings is another major worry (Rojas *et al.*, 2003). Plant-based antimicrobials offer a tremendous amount of medicinal potential.

Alkaloids, steroids, resins, tannins, phenolics, flavonoids, steroids, and combinations of these secondary plant metabolites may have antibacterial properties (Sivaraj *et al*;2011). Saponins, cardenolides, flavonoids, and polyphenols may all have antimicrobial properties. This really offers the foundation for more research on these plants to identify their active components and create new medications. The exudates' phenolic components may thus be what gives the substance its antibacterial effects.

1.3 Significance of the study

Therefore, the research estimated the antimicrobial performance of extracts from the Telakucha plant between two pathogenic bacteria such as *Escherichia coli* and *Salmonella spp* most serious threat to the continued effectiveness of a large portion of contemporary medicine is, perhaps, the development of antibiotic resistance by bacteria that pose a risk to human health. In light of this, this study assessed the antibacterial efficacy of extracts from *Coccinia practice*, including intricate surgical procedures and organ transplants. Antibiotic therapy is one of the cornerstones of contemporary medicine, as most, if not all, members of the medical profession are aware. Many contemporary medical operations would be significantly riskier, if not a complete waste of effort, without adequate measures to limit bacterial infection. Significantly more risk of mortality would result from bacterial infection than it does already. Since many bacteria have become untreatable to different antibiotics, treating bacterial infections has become extremely difficult clinically (Davis, 1994).

1.4 Specific Objectives

- ✓ To verify the presence of saponins, alkaloids, lipids, steroids, tannins, flavonoids, sugars, and amino acids in *Coccinia grandis* extract.
- ✓ To check the antimicrobial susceptibility of *Coccinia grandis*.

Chapter-2: Review of literature

2.1 *Coccinia grandis*

Literature from more than a thousand years ago describes the use of therapeutic herbs in conventional medicine (Chang *et al.*, 2016). Vedic literature explains procedures involves using herbal remedies remedies, that served as the foundation for all other medical sciences that were created on the Indian subcontinent (Pattanayak *et al.*, 2010). Botanicals are the main source of therapies in contemporary supplemental and complementary medicine, as well as the seeds, roots, stems, leaves, and fruit of a plant, all of which may contain bioactive chemicals. (Jiang *et al.*, 2015). Secondary metabolite compounds are the primary bioactive elements in medicinal plants (Singh *et al.*, 2010; Wu *et al.*, 2016). The main positive aspects of medicinal plants are that they are affordable and widely accessible. They also have a number of other advantages and disadvantages. Other definite benefits include its safety and absence of major negative effects when contrasted to other pharmaceuticals (Niu *et al.*, 2011).

Plant metabolism, however, varies greatly. Standardization, strict quality control, and safety evaluation are required prior to the approval of therapeutic plant extracts or products for use in the health system (Mantri *et al.*, 2012; Olarte *et al.*, 2013). The emergence and spread of antibiotic resistance, as well as the creation of new strains of disease-causing organisms, are major concerns for the global health community. The creation of novel drugs or some other potential source of innovative medications is necessary for the fruitful purpose of treating. Popular herbal plants in our neighborhood could be a great source of medications to combat this issue (Shisir *et al.*, 2019). Traditional medicine has employed *Coccinia grandis* as an over-the-counter treatment for a number of ailments. *Coccinia grandis* is an entire plant with pharmacological properties that include antitussive, analgesic, antipyretic, anti-inflammatory, antibacterial, antiulcer, antidiabetic, antioxidant, hypoglycemic, hepatoprotective, antimalarial, antidyslipidemic, anticancer, and mutagenic. The botany, chemical makeup, and pharmacological properties of *Coccinia grandis* are discussed here. The gourd, melon, and pumpkin families are collectively referred to as

Cucurbitaceae. The 960 species that make up the Cucurbitaceous family include *Coccinia grandis*. The tropics are where the family is primarily found. The majority of the Cucurbitaceae family's plants are annual vines (Reddy, 2009).

There are 29 other varieties of *Coccinia*, all of which are restricted to tropical Africa. Mankind mostly use *Coccinia grandis* as a product in a number of nations in Australia, Asia, the Caribbean, the southern United States, and the Pacific Islands. Insects that attack several commercially significant species of the Cucurbitaceae, such as *Coccinia grandis* serves as a host for *Leptoglossus Australis*, *Aphis gossypii* Glover, *Diaphania indica*, *Bactrocera Cucurbitae*, *Liriomyza* spp., and *Aulacophora* spp. (Bamba *et al.*, 2009). Chemical and mechanical control techniques were found to be ineffective, unprofitable, undesirable, and unstable (Muniappan *et al.*, 2009).

Over millennia, the curative advantages of traditional medicinal plants have been understood. For the explanation of their normal methods of action, there is currently a dearth of evidence. *Coccinia grandis* or in the wild, the *Cucurbitaceae* ivy gourd, a native of Bangladesh and other South Asian nations, flourishes as a tendril climber all throughout Bangladesh. Traditional treatments for anorexia, cough, diabetes, wounds, and biliary-hepatic problems include *Coccinia grandis* leaf. It is said to have hepatoprotective, epactorant, anti-mutagenic, anti-microbial, anti-ulcer, antioxidant, and anti-inflammatory activities (Yadav *et al.*, 2010).

2.2 Phytochemical character of *Coccinia grandis*

There are over 12,000 plant secondary metabolites with significant antibacterial potential. These substances belong to one of the main categories of substances, which also includes mixes of phenols, quinines, flavonoids, tannins, terpenoids, and alkaloids (Scultes *et al.*, 1978). This plant can be used to treat a variety of ulcers and itchy skin outbreaks (Behl *et al.*, 1993), including ringworm, psoriasis, small pox, and scabies (Perry *al.*, 1980). Analgesic (Rao *et al.*, 2003), anti-inflammatory (Rao *et al.*, 2003), hepatoprotective (Rao *et al.*, 2003), antioxidant (Venkateshwaran and Pari, 2003), antilithic (Jayaweera, 1980), and antimutagenic (Kusamran *et al.*, 1998) properties are all possessed by *C. indica*.

Numerous plant parts from *Coccinia indica* have long been used in conventional diabetes recovery. Following a review of various publications, it was determined that *Coccinia indica* leaf extracts in methanol and ethanol exhibited an anti-diabetic effect (Manish *et al.*, 2010). As resistance to germs increased as a result of the indiscriminate use of antimicrobial drugs, scientists were compelled to search for novel antibacterial chemicals from a range of sources, including medicinal plants (Karaman *et al.*, 2003).

Medicinal plants, minerals, and organic materials are the sources of many traditional medicines currently in use. The utilization of numerous medicinal plants from the outdated practice of medicine for the curing of different illnesses has drawn more attention in recent years. *Coccinia grandis* has been used as a common domestic cure for a number of illnesses in traditional medicine. *Coccinia grandis* is a plant that has pharmacological properties that include antipyretic, anti-inflammatory, antimicrobial, ulcer-preventive, antidiabetic, antioxidant, hypoglycemic, hepatoprotective, antimalarial, antidyslipidemic, anticancer, antitussive, and mutagenic. A perennial vine with rapid growth that reaches lengths of several meters is called *Coccinia grandis*. On flat surfaces, it can grow into thick mats that easily enclose small trees and bushes. *Coccinia grandis* has medicinal use in a number of its parts. The eye, gonorrhoea, hypolipidemia, skin conditions, and urinary tract infection can all be cooled by *Coccinia grandis* leaf extract. The fruit of *Coccinia grandis* has anti-eczema, anti-tuberculosis, anti-pyretic, hypoglycemic, and analgesic properties. anti-inflammatory. Asthma, bronchitis, GIT problems, urinary tract infections, skin illnesses, expectorant, antispasmodic, and Roots can treat skin conditions, diabetes, high blood sugar, arthritis pain, and urinary tract infections. (Pekamwar 2013; *et al*) In the wild, *Coccinia grandis* grows all throughout Bangladesh and is recognized to have certain medicinal qualities. The secondary metabolite contents of *C. grandis* leaf extract, including its alkaloids, flavonoids, glycosides, saponins, sterol, and tannins, were evaluated qualitatively in the current study. The ethanolic extract of the leaf's antimutagenic, cytotoxic, and anticancer properties were also identified. (Minhajur Rahman *et. al*; 2014) The plant parts of *C. grandis*, including as the roots, leaves, and fruit, are used medicinally for a variety of conditions, including ulcers, ulcerative colitis, jaundice, diabetes, and antipyretic. The leaf is used to treat infective hepatitis and has hypoglycemic, antihyperglycemic, and antioxidant effects.

Since many bacteria have become resistant to antibiotics, treating infectious diseases has become extremely difficult clinically so as treatment for infectious hepatitis (Davis, 1994). Due to the alarming rise in the prevalence of newly emerging infectious diseases, there is a constant and urgent need to discover new antimicrobial agents with unique chemical structures and functionalities. The emergence of resistance to the antibiotics currently being used in clinical settings is another major worry (Rojas *et al.*, 2003). Higher plants generate tens of thousands of distinct chemical compounds with

various biological functions. Plant-based antimicrobials have a huge medicinal promise. Combinations of secondary metabolites from plants, such as fatty acids, alkaloids, steroids, resins, tannins, phenolics, and flavonoids, which may have physiological impact on the health, make up possible plant antimicrobials. *Coccinia indica* fruit chemical constituents were tested for their ability to combat various pathogenic microorganisms. The organic extracts (petroleum ether and methanol) exhibited the maximum activity against the test microorganisms, while the aqueous extracts exhibited little discernible action. *Salmonella paratyphi* A was more resistant and *Staphylococcus aureus* was more vulnerable to the action in gram-positive organisms. According to phytochemical research (Shaheen *et al*; 2009), the extracts contain alkaloids, tannins, saponins, flavonoids, glycosides, and phenols.

2.3 Phytochemicals

Plants require phytochemical ingredients for survival and effective functioning. Phytochemicals are vital metabolites that help plants survive momentary or protracted dangers in their environment while also controlling vital growth and reproduction functions. They protect against herbivores, microbes, and competitors; manage growth (for example, by delaying seed germination until the right moment); and pollinate, fertilize, and control the rhizosphere environment. Phytochemicals are "good" in this regard, at least in terms of the producing plant. Their biological activity, on the other hand, are inherent in them (Russell *et al*; 2007) can have a significant negative impact on other creatures.

Phytochemicals are important bioactive substances that are found in plants. Chemical substances that occur naturally found in plants that, in combination to macronutrients and micronutrients, confer health benefits to humans (Saxena *et al*; 2013). They aid in the protection of plants against disease and injury while also boosting their color, fragrance, and flavor. Phytoconstituents constitute natural chemicals that shield plant cells from environmental hazards like stress and pollution dehydration, UV exposure, and pathogenic attack (Oz and Kafkas, 2017). Medicinal plants include a range of phytonutrients and secondary metabolites that can be utilised alone, in combination, or in synergy to improve health (Briskin, 2000). Phytochemicals originating from plants include alkaloids, flavonoids, tannins, terpenoids, glycosides, saponins, and anthraquinones. (Ajayi *et al*; 2011). Plants and multiple portions of the same plant have

varied phytochemical concentrations. Plants' therapeutic benefits are attributed to compounds such as tannins, alkaloids, flavonoids, saponins, terpenoids, steroids, phlobatannins, glycosides, or other substances. These organic compounds have all been associated to the treatment of a broad range of illnesses. Alkaloids, for instance, are analgesic, antispasmodic, antimalarial, and diuretic. Terpenoids have antiviral, anthelmintic, antibacterial, anticancer, antimalarial, and anti-inflammatory actions, according to research. They are also beneficial for sustainable crop production since they reduce cholesterol synthesis but have antimicrobial property. Saponins have anti-inflammatory, antiviral, and anti-fungal properties, as well as the capability to reduce cholesterol. Astringent actions of phlobatanins Antifungal and antibacterial properties have been observed in glycosides. Antioxidant, anti-allergic, antibacterial, and other characteristics have been identified in phenols and flavonoids (Soni and Sosa, 2013).

2.3.1 Alkaloids

Alkaloids are the most frequent sort of secondary plant metabolite, and they're primarily nitrogen bases made by the peptidic ring's hydrogen atoms with one or more replacements with various radicals, the majority of which include oxygen. These nitrogenous chemicals are commonly utilized as medications, psychostimulants, narcotics, and poisons in plant defense against enteric pathogenic organisms due to their well-known biologic activities (Hao, 2009).

2.3.2 Glycosides

Glycosides are the end products of the condensing of sugars that a mixture of molecules of organic hydroxyl or thiol, the condensation process barely involving the hemiacetal moiety of the carbohydrate. Glycosides are bitter naturally occurring substances only in species belong to the Genitiaceae family. They have nothing chemically in similar, but they both seem to have a bitter taste. Bitters stimulate the discharge of saliva and stomach juices by activating on the gustatory nerves. The lactone group in bitter principles might be diterpene enzymes that catalyze (as in andrographolide) or triterpenoids (as in andrographolide) (as in amarogentin. Due to tannic acid's presence, a lot of bitter substances are employed as astringents, antiprotozoans, or to reduce thyroxine and metabolism. Anthracene glycosides are purgative and used to cure skin conditions. Other examples are chalcone glycoside, which is used to treat cancer,

amarogentin, gentiopicrin, rographolide, ailanthone, and polygalin (Adeosun *et al*; 2016)

2.3.3 Flavonoids

Flavonoids are a kind of polyphenol found in a variety of species of plants. They have more than one benzene ring in their structure, and various studies have shown that they can function as antioxidants or free radical scavengers. (Kim and Lee, 2004). The chemicals are generated from flavans, which are parent compounds. There are an estimated 4,000 flavonoids, some of which are pigments in higher plants. Nearly 70% of plants include flavonoids like quercetin, kaempferol, and quercitrin. Other forms of flavonoids include calchones, catechin, leucoanthocyanidins, anthocyanidins, proanthocyanidins, dihydroflavons, flavones, flavans, flavonols, and others (Omojate Godstime *et al*; 2014).

2.3.4 Polyphenols

Phenolics, phenols, or polyphenolics (or polyphenol extracts) are chemical components found in abundance in plants' fruits as natural color pigments. Phenolics are primarily generated in plants by phenylalanine ammonia lyase from phenylalanine (PAL). These are required by plants and serve a variety of purposes. Plant fight against pathogens and herbivores predators could be the most important function, thus so it is no surprise that they're utilised to treat human pathogenic illnesses (Tovar *et al*; 2002).

2.3.5 Saponins

Saponins are compounds that, when shaken, act like soap create foam in water. An aglycone called sapogenin is created during hydrolysis. Saponins are compounds that, when shaken, act like soap in water and produce foam. During hydrolysis, sapogenin, an aglycone, is produced. Saponins are especially harmful since they produce hemolysis in the blood and have been attributed to animal poisoning. They taste harsh and caustic, and they irritate mucosal membranes (Omojate Godstime *et al*; 2014).

2.3.6 Tannins

Because of the presence of the phenolic group, tannins have antibacterial characteristics. In Ayurveda, tannin-rich plant-based medicines are often used to treat enteric illnesses such as diarrhea (Omojate Godstime *et al*; 2014).

2.4 Pharmacological Activities

2.4.1 Antibacterial

The aqueous extract of *Coccinia grandis* outperformed the ethanol extract in terms of antibacterial activity. The extract's antibacterial properties are primarily due to its polar component. *Bacillus subtilis* and *Sarcina lutea* both are somewhat resistant to *Coccinia cordifolia* chloroform extract. *Staphylococcus aureus* is susceptible to ethyl acetate extracts. Hexane extract efficacious against *Pseudomonas aeruginosa* (Bulbul *et al.*, 2011). *Coccinia grandis* leaf extract was tested for its antibacterial properties against five different bacterial species by (Sivaraj *et al.* in 2011). They included solvents such as acetone, ethanol, aqueous solution, methanol, acetone, and hexane. *S. pigeons*, *B. cereus*, *K. pneumonia*, and *S. aureus* were all successfully eradicated by *Coccinia grandis* ethanol leaf extract (Sivaraj *et al.*, 2011). The antibacterial effect of *Coccinia grandis* extract was tested using six gram negative and gram positive and gram-negative bacteria. Except for *Proteus mirabilis* and *Klebsiella p* all were active against an ethanol extract of the stem. With the exception of *Proteus mirabilis*, all gram positive and gram-negative bacteria are relatively amenable to hexane extract. Only *Staphylococcus aeruginosa* and *Proteus mirabilis* are mildly antimicrobial against ethyl acetate extracts (Farukhh *et al.*, 2008; Tamilselvan *et al.*, 2011).

2.4.2 Anthelmintic

Coccinia grandis has anthelmintic action when extracted in methanol. Anthelmintic action was achieved using posthumous worm pheretime. There are several extract concentrations used. The worm's paralysis is how the methanolic *Coccinia grandis* extract works. The duration till the worm is paralyzed and dies is the activity's yardstick (Tamilselvan *et al.*, 2011).

2.4.3 Antioxidant

Analyzed by Moideen (2011) Flavonoids, which found in the ethanol extract of *Coccinia grandis* root, are what give it its antioxidant properties. The fruit of *Coccinia*

grandis has significant antioxidant activity when extracted with methanol. *Coccinia grandis* has glycoside and flavonoid in its methanol extract. Because of its removal efficiency and hydrogen peroxide filtering capacity, *Coccinia grandis* has antioxidant action (Mongkolsilp *et al.*, 2004 Deshpande *et al.*, 2011). Antioxidant activity is demonstrated in ethanol and methanol extracts (Ashwini *et al.*, 2012) Using petroleum, ethyl acetate and chloroform as solvents, a extract of stem from *Coccinia grandis* indicates antioxidant potential. Petroleum lacks the powerful antioxidant action that ethyl acetate does (Deshpande *et al.*, 2011) The antioxidant principle is present in the methanol extract and leaf powder of *Coccinia grandis* (Mujumder *et al.*, 2008).

2.4.4 Antiulcer

The anti-ulcer efficacy of aqueous extract of *Coccinia grandis* leaves was investigated in experimental rats using pylorus ethanol-induced and pylorus ligation ulcer models. In both models, the ulcer index was calculated. At doses of 500 and 250 mg/kg, aqueous extract of *Coccinia grandis* significantly inhibited the stomach lesions brought on by pylorus ethanol-induced gastric ulcer and pylorus ligation-induced ulcer. The extract significantly reduced stomach acidity, free acidity, and ulcer index (Girish *et al.*, 2011) Manoharan (2010) assessed the antiulcer activity of aqueous, ethanol, and aqueous extracts in pylorus ligation-induced stomach ulcers. The antisecretory mechanism for its antiulcerogenic effect was shown using ethanol extract. 400 mg/kg of plant ethanolic extract antiulcer.

2.4.5 Antimalarial

Excellent antiplasmodial efficacy against *Plasmodium falciparum* is revealed by *Coccinia grandis* extract (Sundaram *et al.*, 2012). The SGPT, SGOT, ALP, total protein, and blood ureanitrogen percentage are all dropped by *Coccinia grandis* aqueous leaf extract. The antimalarial action of *Coccinia grandis* extract is due to its hydrophilic moiety. *Plasmodium berghei*, is a parasite counts in mice are dramatically decreased by it (Samanta *et al.*, 2011). The larvicidal process of *Coccinia grandis*, is proven by its methanolic extract (Rahumann., 2008).

2.4.6 Anti-inflammatory

Deshpande (2011) tested the leaf of *Coccinia grandis* and stem extracts to reduce inflammation in rat paw edema brought on by formaldehyde exposure. Serotonin,

prostaglandins, bradykinin, and histamine are all produced as a result of the cell damage that formaldehyde induces. In comparison to the stem aqueous extracts and standard, which were used to make indomethacin, the aqueous extract of the leaves significantly reduced paw edema. When inflammation caused by formaldehyde is treated with *Coccinia grandis* extract, endogenous mediators like histamine, serotonin, prostaglandins, and bradykinin are produced (Bernard *et al.*, 1998).

2.4.7 Antipyretic

Aggarwal (2011) assessed *Coccinia grandis* extract for antipyretic efficacy in yeast-induced fever at doses of 200 and 100 mg/kg. By affecting prostaglandin production, the extract demonstrated antipyretic efficacy. Prostaglandin is thought to control body temperature. Glycosides, alkaloids, flavonoids, terpenoids, phenols, and tannins are all present in *Coccinia grandis* extract.

2.4.8 Analgesic

The analgesic activity was assessed using the induced acetic acid writhing, immersion of tail and plate models. A methanol extract of *Coccinia grandis* is used to treat acetic acid-induced analgesia. Glycosides, alkaloids, flavonoids, terpenoids, phenols, and tannins were discovered in a methanolic extract of *Coccinia grandis* leaves. The ingredient(s) in the methanol extract of *Coccinia grandis* have analgesic properties. Be mediated by a peripheral, as opposed to a central, process. Acetic acid problems are lessened by *Coccinia grandis* (Aggarwal *et al.*, 2011).

Hypoglycemic In streptozotocin-induced diabetes rats, Mallick (2007) tested the combination extracts of *Musa paradisiaca* and *Coccinia indica* for antidiabetic activity. At 100 or 200 mg/kg, the ethanolic extract of the aerial component lowers lipid parameters and blood glucose levels in diabetic mice where streptozotocin is induced. In diabetic rats where alloxan is induced, chronic treatment of extract of fruits 200 mg/kg for fourteen days lowers blood glucose levels (Gunjan *et al.*, 2010). With continued use, the aqueous extract of *Coccinia indica* decreased levels of protein, urea, cholesterol, and glucose in the blood. In the diabetic rat liver, *Coccinia grandis* increased gluconeogenesis or decreased glycogenolysis. Total protein, SGPT, and SGOT levels are all increased after taking *Coccinia* extract (Doss *et al.*, 2008). In rats with alloxan-induced diabetes, *Coccinia indica* extract of leaf had hypoglycemic effects on cholesterol and blood glucose levels (LDL, TG, VLDL) (Manjula *et al.*,

2007). Using diabetic rats caused by alloxan, the hypoglycemic efficacy of *Coccinia grandis* fruit was studied. The reduced blood glucose level is visible in ethanol extract. Fruit pectin lowers blood sugar levels via reducing the absorption of glucose from the gut, boosting glycogen of liver and reducing the activity of the enzyme glycogen phosphorylase (Ramakrishnan *et al.*, 2011).

Coccinia indica and *salvadora oleoides* leaves combined with methanolic extract demonstrate hypoglycemic action (Saklani *et al.*, 2012). *Coccinia grandis* leaf alcohol extract (Eliza Jose, 2010) And stem can reduce blood glucose levels in healthy rats that haven't eaten in hours (Doss *et al.*, 2008) Triterpenes, alkaloids, flavonoids, and Bcarotene found in *Coccinia* extracts made from ethyl acetate and petroleum ether are what give them their hypoglycemic properties (Ariful Islam *et al.*, 2011).

2.4.9 Antifungal

According to Bhattacharya (2010) *Candida tropicalis* II *Aspergillus niger*, *Saccharomyces cerevisiae*, *Candida tropicalis*, *Aspergillus niger*, *Saccharomyces cerevisiae*, *Cryptococcus neoformans*, and *Candida albicans* ATCC, *Candida albicans*-II, ATCC were all targets of the *Coccinia grandis* leaf extract's antifungal activity. Antifungal production activity by extract of ethanol is somewhat considerable. The extract's nonpolar portions have stronger antifungal effects. Both strains of *Candida albicans* are more susceptible to aqueous extract, while both *Candida albicans* and *Aspergillus Niger* are more sensitive to ethanolic extract (Bhattacharya *et al.*, 2010).

2.4.10 Hepatoprotective

Against CCl₄-induced hepatotoxicity in experimental rats, (Vadivu, 2008) assessed the fruit's extract of *Coccinia grandis* which is alcoholic for hepatoprotective potential. The SGPT, SGOT, and bilirubin levels were dramatically decreased after treatment with a 250 mg/kg ethanolic fruit extract. The extract's hepatoprotective properties could be a result of the flavonoids, which have been found to be prevalent in fruits and have antioxidant properties. *Coccinia grandis* included antioxidants such as flavonoids, triterpenes, and tannin, which may prevent the generation of free radicals and have been linked to hepatoprotective effects. (Vinothkumar *et al*; 2009, Dr. Krishnkumari *et al*; 2011, Sunilson, *et al*; 2009, Anil Kumar *et al*; 2012,).

2.4.11 Antidyslipidemic

By reducing the triglyceride and cholesterol levels in hamsters, (Singha,2007) examined the chloroform preparation of *Coccinia grandis* leaves for antidyslipidemic efficacy. Polyprenol-containing extract of *Coccinia grandis* leaves where chloroform is present, decreases the plasma lipid profile by raising the ratio of high-density lipid cholesterol to total cholesterol. This plant produced the first isolation of C60-polyprenol. At a dose of 50 mg/kg body weight, it effectively diminished blood glycerol by 11%, triglycerides by 42%, and total cholesterol by 25%, in dyslipidemic hamsters fed a high-fat diet. Leaf extracts in aqueous and ethanolic forms can be utilized to treat obesity (Mishra *et al.*2012).

2.4.12 Anticancer

Numerous veggies have been found to lower the incidence of cancer. *Coccinia grandis* is one of them. The antioxidant properties of *Coccinia grandis* are what give it its anticancer properties. The *Coccinia grandis'* antioxidant properties turn the ferrocynaide into ferrous. Water is produced when H₂O salvaged from *Coccinia grandis* reacts (Behera *et al;* 2012). Bhattacharya, 2011 examined the anticancer potential of an aqueous extract of *Coccinia grandis* leaves. A free radical called nitric oxide plays an important role in the pathophysiology of inflammation and pain. The *Coccinia grandis* antioxidant property reduces the amount of nitrite produced during decomposition. The cell produces a relatively weaker graded response. When compared to the reference medicine (vinblastine), *Coccinia grandis* considerably increased the number of non-viable cells and decreased the number of viable cells (Nanasombat *et al;* 2009; Bhattacharya *et al;* 2011).

2.4.13 Antitussive

Analgesic efficacy of the *Coccinia grandis* fruit's methanol extracts was examined by (Pattanayak, 2009). The native Indians have utilized *Coccinia grandis* frequently to treat their asthma and cough. steriod, Alkaloid, triterpenoid, tannin, glycoside, reducing sugar, carbohydrates, are all present in the methanol extracts of *Coccinia grandis* fruit. Methanol extract's antitussive activity has been likened to that of codeine (Antitussive drug). The *Coccinia grandis* fruit extract in methanol demonstrated a dosedependent considerable reduction in cough brought on by a chemical simulation of codeine phosphate. At 90 minutes, the methanol extract inhibits coughing to its greatest extent.

2.4.14 Mutagenic effect

By gradually slowing the formation of mycelia, an aqueous extract of *Coccinia grandis* leaves inhibited growth and mutagenesis on *Neurospora crassa*. This finding suggests that the plant *Coccinia grandis* has mutagenesis effects on *Neurospora crassa*. (Bhuiyan *et al*; 2009).

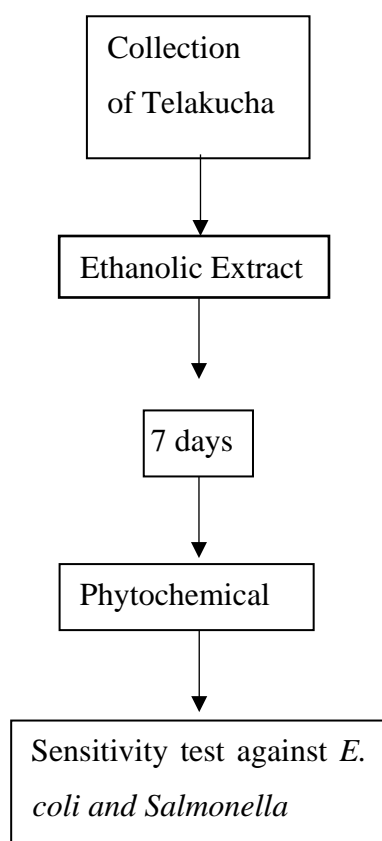
Part used	Medicinal value
Leaves	The treatment of ringworm, psoriasis, and itching when combined with gingely oil; the treatment of sores, skin conditions, and small pox skin eruptions when combined with ghee; the cooling impact on the eyes; and the healing of large ulcers, minor scabies lesions, anuria, and body heat.
Stem	spasm-reducing impact.
Fruit	In addition to being used as a vegetable and treating eczema, green fruit can treat sores on the tongue when swallowed.
Bark	Bark that has been dried possesses cathartic qualities.
Juice	Tuberous roots juice, stems, and leaves is practiced to treat urinary tract infections and other related problems as well as diabetes, intermittent glycosuria, swollen glands, and pityriasis.
Powder	Used to cure gastrointestinal problems, liver complicacies, diarrhea, worm infestation, vomiting, purifies blood, stops the spread of infection throughout the body, works well to treat bronchitis and asthma, and is helpful against chronic cough and colds.
Root	With tubers, you can get rid of phlegm, wheezing, aphthous ulcers, diabetes, skin lesions (Tenia), and joint discomfort.
Decoction	Bronchitis is cured by a stem-and-leaf decoction.

Table 2.1: Numerous medicinal uses of different parts of *Coccinia grandis*

Chapter-3: Materials and methods

3.1 Experimental Design

The study was conducted to determine the efficacy of an ethanolic and methanolic extract of *Coccinia grandis* (Telakucha) against *Salmonella spp* and *E. coli* in broilers and to isolate obtained from the broiler chicken.



3.2 Collection of plant materials

Mature and disease free Telakucha was collected from the medical plants hill areas of my village.

3.3 Drying and grinding

Collected plant leaves were thoroughly cleaned by washing and discarding all the unwanted materials. The aged leaves were discarded. To remove moisture, the leaves were completely dry for (shed drying method) ten days. The leaves which were dried, were processed to a fine powder by a blender. After that, fine dust was obtained, powder was maintained in a staunch plastic bag in the dark until it was needed.

3.4 Preparation of ethanolic extracts

50 g of telakucha were taken into a bottle with 500 ml 80% ethanol. Then the composition was moved about for 10 minutes and left seven days. That mixture was shaken and kept in a cool dark place every day overnight. After 7 days one batches of telakucha was taken for filtration. The whole mixture of telakucha was strained separately through Whatman no.1 filter paper. Then the filtrates were taken into a round bottom flask of rotator vacuum evaporator. After the evaporation, the extracts were preserved in a petri plate and stored in a refrigerator (4°C)

3.5 Collection of *E. coli*, *Salmonella*

E. coli and *Salmonella* were collected from commercial poultry Liver. 1gm of liver was blended with 9 ml of peptone water. Then incubate it for 24 hours at 37°C. After that MacConkey and EMB agar was prepared. By inoculating loop, streaking was done in both agars. Incubate 37°C overnight to observe the final outcome. After 24 hours large pink color in MacConkey and Green Metallic color in EMB was shown then indicates the present of *E. coli*.

Bacterial peptone water should be incubated for *Salmonella* at 42°C and allowed to grow on XLD agar. *Salmonella* is present when the hue is black pink.

3.6. Preparation of Stock

For the preparation of stock, 2-3 colony of bacteria was taken in a 5 ml of Brain heart infusion (BHI) broth which was freshly prepared. Then incubate the broth at 37°C overnight at incubator for bacterial growth. After that, 700 µL was taken from overnight culture + 300 µL of 50% glycerol was mixed in Eppendorf tube. Finally stored the Eppendorf tube at -40-80°C for long time preservation.

3.7 Phytochemical activity test

Phytochemicals present in the leaf extracts were screened as per standard protocols for phytochemical ingredients such as steroids, saponins, alkaloids, tannin, glycosides, phenols, flavonoids, and Terpenes, Phenolic Compound, Glycosides etc.

According to accepted procedures, phytochemical analyses were done on each and every telakucha extract. (2000) Sofowora.

3.7.1 Detection of Alkaloids:

3.7.1.1 Mayer's Test:

Mercuric chloride of 1.36g was dissolved in distilled water of 60ml to create the mayer's reagent. An iodide of 5g solution solved in distilled water of 20ml was added. With distilled water, both of the solutions were combined and diluted to a final volume of 100 ml. A small amount of the test residue was mixed with a few drops of the aforementioned reagent. Alkaloids were present because a precipitate with a cream tint developed.

3.7.1.2 Wagner's reagent:

When it is mixed with the test residue, a brown-reddish precipitate appears, confirming that alkaloids were present.

3.7.2 Detection of Flavonoids

3.7.2.1 Alkaline Reagent Test:

Four to five drops of a NaOH solution were used to treat the extracts. The presence of flavonoids is pointed out by the production of a bright yellow color that fades to colorlessness when diluted acid is added.

3.7.3 Detection of Saponins

3.7.3.1 Foam test:

For a stable, enduring froth, 2g of plant extract was combined with distilled water of 10ml and forcefully shaken. Saponins are present when there is froth on the surface.

3.7.4 Detection of Tannins

3.7.4.1 Lead acetate test

2ml of extract and 2ml of filtered water were combined. This combined solution was shaken thoroughly before adding 0.01g lead acetate. The influence of tannins is indicated by the formation of white cloudiness and precipitate.

3.7.5 Detection of Phenolic Compound

3.7.5.1 Ferric chloride test: Plant extract was added to water and heated at 45–50°C in amounts of around 2 ml. Then 2 ml of FeCl₃ at 0.3 percent were added. Greenish or blueish color production suggests that phenol is present.

3.7.6 Detection of Glycosides

3.7.6.1. Cardiac Glycosides (Keller-Killani test): The test residue was treated with 1 ml of glacial acetic acid 1 ml of concentrated sulfuric acid consist of ferric chloride, and the production of a reddish brown color at the intersection of two layers was monitored. When glycosides is present, the top layer turned bluish green.

3.7.6.2. Anthraquinone Glycosides (Borntrager's test): Since there was no glycoside present, no pink to red color developed when 0.5 ml of diluted ammonia solution and 1 ml of an organic solvent (benzene) were mixed to the test resid.

3.7.7 Detection of Carbohydrates

3.7.7.1. Molisch's test: 10 g of α -naphthol were dissolved in 100 ml of 95 percent alcohol to create the Molisch's reagent. Two drops of Molisch's reagent were added to a few milligrams of the test residue. From the inclined test-side, tube's 1 cc of concentrated sulfuric acid was introduced to this solution. In the presence of sugars, there was no reddish-violet ring at the intersection of the two layers.

3.7.8 Detection of Reducing sugar

3.7.8.1 Benedict's Test: A test tube was filled with 0.5 ml of the plant material's aqueous extract. Benedict's solution (5 ml) was added to the test tube, heated for 5 minutes, and then put to naturally cool down. Without a reducing sugar, no red-colored cuprous oxide precipitate was produced.

3.7.8.2 Fehling's solution Test: Several ml of Fehling's solution was added after a little amount of the test residue had been dissolved in water. Then, this mixture was warmed. Reducing sugars were present if a red cuprous oxide precipitate was formed.

3.7.9 Detection of Protein and Amino Acid

3.7.9.1 Xanthoproteic test: By the side of the test tube, concentrated 0.5ml nitric acid was assembled to the 2 ml of extract. The lack of yellow color indicated that proteins and amino acids were not present.

3.7.10 Detection of Acidic Compound

3.7.9.2 Sodium bicarbonate test: The addition of sodium bicarbonate solution to the alcoholic extract was watched for the development of exuberance.

3.7.11 Detection of Phytosterol

3.7.10.1 Liebermann-Burchard's test: Using the standard medication, as a reference standard, ampicillin was used, and balanced salt discs were used as a control treatment acid. 2 mg of the extract was dissolved in acetic acid of 2ml anhydride, boiled, cooled down and then 1ml of concentrated sulphuric, was added. The test for phytosterols was validated by a brown ring development at the junction.

3.7.12 Detection of Triterpenoids

3.7.12.1 Salkowski test: A few drops of strong sulfuric acid were placed to the side of a test tube, and 2 mg of dry extracts were mixed with 1 ml of chloroform. The test result for triterpenoids was revealed by a reddish-brown tint that appeared at the interface.

3.8 Preparation of stock solutions

In a separate Eppendorf tube, the crude extract of *Coccinia grandis* was diluted in 0.2 percent DMSO (Dimethyl Sulphoide).

3.9 Preparation of Dried Filter paper discs

Each disc was made using a puncher to be roughly 6 mm in diameter from what was once No. 1 filter paper. The manufactured filter paper disc was autoclaved at 121°C for 15 minutes to sterilize it before being impregnated with 60 microliters of the experimental plant's extract. Using sterilized forceps, each disc was excluded from the extract and immediately transferred in an aseptic conditions Petri dish. Before being implemented to the agar plate on a plate, these dishes were thoroughly dried to remove the solvent.

3.10 McFarland Standard preparation

In order to create a solution with precise optical densities, 0.5 McFarland turbidity standards were created by combining 1% sulfuric acid and 1% barium chloride. A suspension was maintained by stirring 0.05 mL of a 1 percent BaCl₂ solution with 9.95 mL of a 1 percent H₂SO₄ solution to create a 0.5 McFarland standard. A 1.5x10⁸ colony-forming unit (CFU/ml) bacterial suspension has an optical density that is comparable to a 0.5 McFarland turbidity standard. Visually contrast the turbidity of the test suspension with that of the McFarland standard in well-lit conditions by contrasting the sharpness of the lines on the Wickersham card. If the test suspension is too thin, add more organisms or incubate the tube until the turbidity is the same as the reference value. Use a sterile pipette for dilution, then add enough broth or saline to the sample to reach the desired turbidity. (1907; McFarland).

3.11 Preparation and components of Agar

Mueller-Hinton Agar (MHA)

To make 1000 mL of Mueller-Hinton agar (MHA), 1000 mL of distilled water was added, along with 15 g of nutritional agar, 10 g of tryptone and 5 g of yeast extract, 5 g of sodium chloride. Boil for 10 minutes, sterilize in an autoclave for 15 minutes, and then subculture the test microorganisms to ensure that all components are thoroughly dissolved.

3.12 Antimicrobial Activity test of plant Extracts

Agar Disc Diffusion Assay

The disc diffusion process was practiced to examine the antibacterial activity of the experimental plant's crude extracts and fractionated components against *Salmonella* and *E. coli*. For each organism, the Sterile Muller Hinton agar was prepared as follows. Sterilized Petri dishes were filled with 20 milliliters of sterile Muller Hinton agar (MHA), which was kept at 45–50°C in a molten condition. 0.1ml of the test organism was put on the Muller Hinton agar (MHA) plate after it had solidified. The filter paper discs were then positioned as instructed by on the agar plate's surface, 15 mm from the plate's edge and evenly spaced apart (Clutter buck *et al*; 2007).). Each container was altered and placed in a 37°C incubator for each disc squeezed required to maintain networks of communication with the agar plate for 24 hours. The diameter was measured in millimeters using just a plastic ruler after 24 hours to determine the susceptibility of the antimicrobials.

Chapter-4: Results

In this study, we can depict that many compounds were absence in methanolic extract except Phenolic compound, Glycosides, Acidic Compound and Steroids and Terpenes. But in ethanolic extract most phytochemical compound was present except tannins, reducing sugar and steroids and terpenes.

Compound Name	Methanol	Ethanol
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Phenolic Compound	+	+
Glycosides	+	+
Carbohydrates	+	+
Reducing sugar	-	-
Protein and Amino Acid	+	-
Acidic Compound	-	+
Phytosterol	-	+
Steroids and Terpenes	+	-

Figure 4. 1: Phytochemical Result of Telakuchi

The findings of a qualitative phytochemical study of the dried powder of *C. indica* leaves are displayed in Table 4.1. The methanolic extract was subjected to phytochemical screening, which found the presence of triterpenoids, alkaloids, steroids, tannins, saponins, ellagic acids, phenols, and glycosides, but not found the reducing sugar, acidic compound and phytosterol.. Again, samples were drawn from *C. indica* were extracted using ethanol, which revealed the presence of alkaloids, terpenoids, flavonoids, phenols, tannins, carbohydrates, glycosides, saponins, and

phytosterols but reducing sugar, protein and amino acids, steroid and terpenes were not present.

Table 4. 1: Zone of Inhibition of Telakucha and Control in 2 different organisms

Sample		<i>E.Coli</i> (ZOI)mm	<i>Salmonella</i> (ZOI)mm
Telakucha	20%	10.34±1.2	9.05±1.54
	30%	11.54±0.85	10±0.03
	40%	14.42±0.76	12±0.65
Control	Amoxicillin	11.54±0.89	N/A
	Ciprofloxacin	N/A	13±0.56

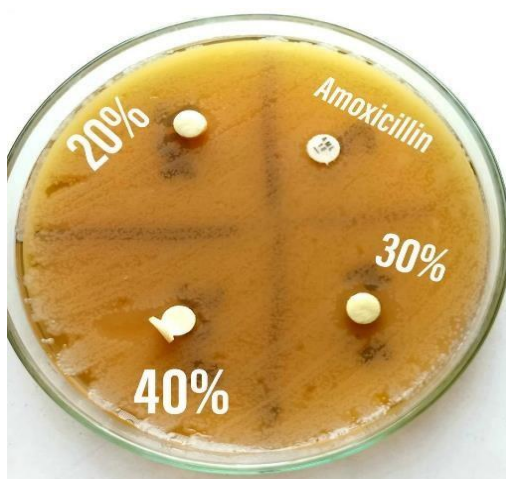


Figure 4.2: Zone of inhibition of Telakucha against *Salmonella*. Spp

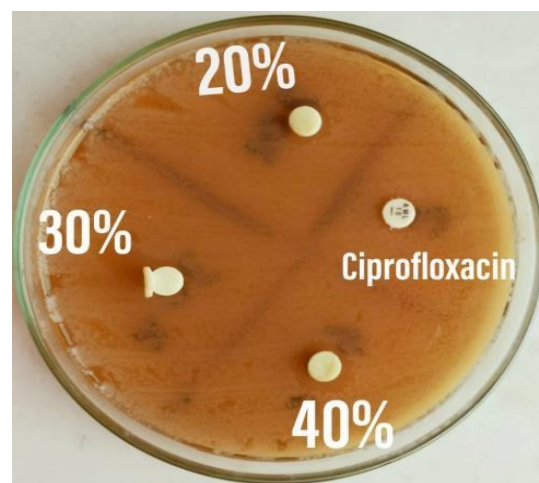


Figure 4.3: Zone of inhibition of Telakucha against *E.coli*

Discussion

This study indicated a considerable difference in antibacterial activity between different dosages obtained with an extract of telakucha.

There are specific components, as well as both primary and secondary metabolites, that are found in medicinal plants, and these provide the antimicrobial effects (Wang, 2016). Depending on the extraction solvents used, the geographic source, harvesting period, storage conditions, and drying all have a significant impact on the bioactivity of plant extracts (Tanver *et al*; 2017)

Previous investigations into the leaves and stem of *C. indica's* antibacterial and antifungal characteristics (Dewanjee *et al*; 2007). also found that methanol and ethyl acetate extracts had a significant amount of activity oppose to various bacteria and fungi, reinforcing the concept that methanol is a better solvent for extraction and detachment of phytochemicals with antimicrobial activity. The present investigation found that water extract had only moderate antibacterial activity, supporting past results that said it is usually preferable to extract and isolate antibacterial components using organic solvents (Varadarajan *et al*; 2007). Thymol and other phenolic substances such as alkaloids, flavonoids, terpenoids, and other that are categorized as antimicrobial substances are a few examples (Rojas *et al*; 1992). According to traditional healers, *C. indica* has been used to cure pneumonia, cough, and diarrhea and colds is supported by these data on antibacterial activity. Even though the current study on these extracts is a contribution to the body of knowledge, further research is required to identify the extracts. Several compounds' alkaloids, flavonoids, saponins, phenols, tannins, terpenoids, and other compounds have been found in plant extracts, including leaf extract. The outcomes Positive results from the phytochemical analysis the plant extracts used in this research indicate that the detected phytochemical substances may represent this plant's bioactive elements demonstrating that plants are important which rich in phytochemicals with significant medical value. Indigenous usage has made extensive use of telakucha. (Tamilselvan *et al*; 2011) founded that phytochemicals such as tannins, flavonoids, protein and amino acid, glycosides, phenols, saponins, alkaloids are present for both methanolic and ethanolic extract. But in one side sterols and acidic compound are absent in the ethanolic extract and another side, lignans were absent in *Coccinia grandis*. In this research, alkaloids, flavoids, saponins, tannins,

phenolic compound, glycosides, carbohydrates, acidic compound were present for methanolic and ethanolic extract. But reducing sugar, acidic compound, phytosterol were negative for ethanolic extract. Protein and amino acid, reducing sugar, steroid & terpenes were absent for ethanolic extract. As a result, this plant's phytochemicals can be employed to treat a variety of human illnesses. The herbs are inexpensive and plentiful all around us. They are very valuable to living things because they don't endanger them or the environment or consumers. Ethanolic extracts of different dosages such as 20%, 30%, 40% show sensitivity against gram-negative bacteria, *E. coli* and *Salmonella spp.* These studies revealed that *Coccinia grandis* ethanolic extract had considerable antibacterial action against *Salmonella spp.* and *E. coli*. The inhibition activity at different concentrations Isolated components from the ethanolic extract of telakucha exhibited various zones of inhibition. The zone of inhibition of antimicrobial screening against *E. coli* and *Salmonella spp.* finds highest in 40% concentration than other two concentration. This indicates having a lower sensitivity zone at high concentrations and a larger inhibition at lower concentrations. According to the (Shivraj *et al*; 2011) founded that antimicrobial efficacy against *E. coli* (40% ethanolic extract) the zone of inhibition is 14.83 ± 0.15 .In this study , it is found that antimicrobial efficacy against *E. coli* the zone of inhibition is 14.42 ± 0.76 which is similar to the value of the research of (Shivraj *et al*;2011) .Then (the ethanolic extract of 40% *Coccinia grandis* showed antimicrobial sensitivity against the another gram negative bacteria which is *Salmonella spp.* and the zone of inhibition is 12 ± 0.65 . According to the study of (Umbreen *et al*; 2008) the zone of inhibition for the ethanolic extract is 6.5.It means that the antimicrobial efficacy of *Coccinia grandis* showed higher susceptibility against *Salmonella spp.*

According to the study, *Coccinia grandis* leaf extract contains a variety of secondary metabolites, including glucose, tannin, flavonoids, saponins, glycosides, terpenoids, fatty acids, and phenol. Saponins have hypotensive, antihyperlipidemic, and cardiodepressant effects (Bairwa *et al.*, 2012). Alkaloids, steroids, flavonoids, tannins, phenols, and other aromatic compounds are among the phytochemical components of plants that act as a defense mechanism against a variety of microbes, insects, and other herbivores (Bonjar *et al*; 2004). In circumstances of heart failure, glycosides can act as cardio stimulants (Sood *et al*; 2005). Tannins have hemostasis and anti-diarrheal effects (Asquith *et al.*, 1986). The anti-inflammatory and immunostimulatory effects

of flavanoids is their result. Alkaloids, glycosides, flavonoids, and saponins are among plants' antibacterial principles, claim (Cragg *et al*; 1999) and Khanna *et al* ; 2003). The defense systems of plants against infections are based on these antimicrobial principles.

Study Limitations

Only two types of bacteria were evaluated in microbial assays and only two types (methanoli and ethanolic) extracts of phytochemicals were tested. Only ethanolic extract was used for the antimicrobial susceptibility. There were no tests done on the fungal growth. No virus and fungal tests were conducted.

Conclusion

In this study the phytochemical component like alkaloids, flavonoids, saponins, phenols, tannins, terpenoids etc are found in Telakucha. This phytochemical constituent contains antimicrobial efficacy that inhibit the growth of two-gram negative bacteria such as *E. coli* and *Salmonella* spp. At 40% concentration the inhibition zone found most than other two concentration. In essence, *Coccinia grandis* is a true miracle of nature since it contains powerful chemical components that have a wide range of therapeutic uses. Additionally, some pharmaceutical companies have developed it as a medication. Wide-ranging written works studies and searches for ethnopharmacology have been conducted all over the world, and they have revealed that *Coccinia grandis* may be a substantial source for many pharmacologically important as well as medicinally important compounds. Again, it is evident from the discussion above that ivy gourd can compete with existing medications to treat several serious conditions. This evaluation offers a perspective on numerous issues that can aid in the advancement of *Coccinia grandis* study in order to get more details about current endeavors. According to the study, the numerous benefits of the adaptable medicinal plant *Coccinia grandis* are a wonderful miracle of nature. The chemical components in the plants that have a distinct psychological effect on the human body are what give them their medical worth. Alkaloids, flavonoids, tannins, and phenolic compounds are the most significant bioactive substances found in plants. If scientists do such screenings of numerous naturally occurring chemical molecules, it will aid in the discovery of new things for humanity.

Recommendation

- ✓ In this study gram positive bacteria should be used.
- ✓ In-vivo analysis should be done in this research.
- ✓ Quantitative analysis should be done for phytochemical analysis.

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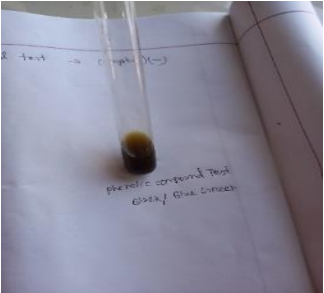
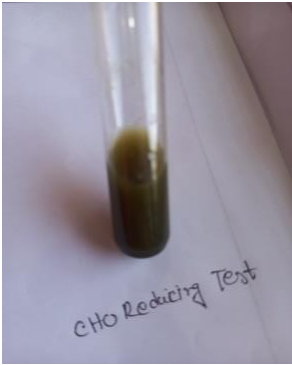

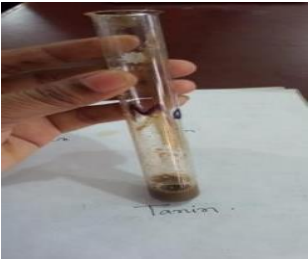


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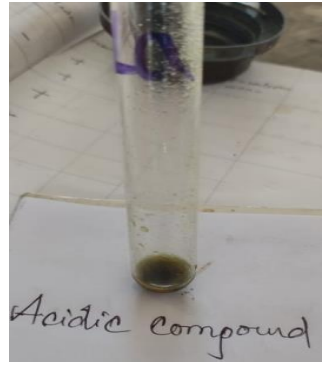
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Appendix

 <p>Dry <i>Coccinia grandis</i> powder</p>	 <p>Absorbed <i>Coccinia grandis</i> in alcohol solution</p>	 <p>Extraction of <i>Coccinia grandis</i></p>
 <p>Heating the extract by Rotary evaporator</p>	 <p>Extract found from <i>Coccinia grandis</i></p>	 <p>Making solution from the extract</p>
 <p>Phenolic test</p>	 <p>CHO test</p>	 <p>Protein test</p>
 <p>Tannin test</p>	 <p>Glycoside test</p>	 <p>Alkaloid test</p>



Protein test



Acidic test



Flavonoid test



Microbial culture growing
process



Growing culture in petri
dish



Identifying the zone of
inhibition

Pictures of some different test

Brief Biography

Shanta Chowdhury has successfully finished the B.Sc. in Food Science and Technology programme at Chattogram Veterinary and Animal Sciences University 's faculty of Food Science and Technology. She passed the Higher Secondary Certificate Exam in 2012 after passing the Secondary School Certificate Exam in 2010 .She is currently a candidate for the Masters of Food Chemistry and Quality Assurance degree through the Faculty of Food Science and Technology at Chattogram Veterinary and Animal Sciences University ,s Department of Applied Chemistry and Chemical Technology. Her research interests include the identifying phytochemical constituents of Telakucha and identifying microbial efficacy of Telakucha which can use in pharmacological purpose which act as an antibiotic agent.