Study of Antibacterial Activities of the extracts of Neem, Pomegranate, and Tulsi and Essential Oils against *Salmonella Typhi*

Submitted By
Tazreen Binte Ehsan
15126001

A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of Bachelor of Science in Microbiology

Department of Mathematics and Natural Sciences
Brac University
January 2022

Declaration

It is hereby declared that,

1. The thesis submitted is my original work while completing my degree at Brac University.

2. The thesis does not contain material previously published or written by a third party, exceptwhere this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material that has been accepted, or submitted, for any otherdegree or diploma at a university or other institution.

4. I have acknowledged all main sources of help

Tazreen Binte Ehsan
15126001

Approval

The thesis titled "Comprehensive study of antibacterial activities of medicinal plants and essential oils against *Salmonella typhi"* submitted by Tazreen Binte Ehsan (15126001) has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology on * January 2022.

Examining Committee:

Supervisor**:**

Mahboob Hossain, PhD
Professor
Department of Mathematics and Natural Sciences

Program Coordinator:

Mahbubul Hasan Siddiqee
Assistant Professor
Department of Mathematics and Natural Sciences

Department Head:

Prof. Dr. A F M Yusuf Haider
Professor and Chairperson

Ethics Statement

The data presented in this thesis are acquired from conducted experiments. Pieces of information that are taken from other sources are appropriately cited through accurate referencing.

Abstract

The microorganism occupied for this research was*Salmonella*,itisa gram-negative, rod-shaped, facultative anaerobic bacteriumthat belongs to the family Enterobacteriaceae.

Recently it has been observed that an increasing number of bacteria are building resistance to synthetic antibiotics which hampers controlling infective health conditions. It's familiar to everyone that most antibiotics no longer function; infections are getting worst or impossible to manage. It is time to discover a substitute for antibiotics from organic sources

Medicinal plants are considered as the abundant bioresource of drugs for conventional medicines, food supplements, and chemical essence for artificial drugs. Nowadays, researchers are progressively focusing their consideration in investigating herbal products due to the higher resistance of microorganisms against the presently used antibiotics and pharmaceutical companies are seeking options for the costly production of synthetic drugs. Medicinal plants can be the method to alternate this condition as most of them are secure with minor side effects. In the present work, ethanol and aqueous extracts of Neem (Azadirachta indica), Tulsi (Ocimum sanctum), and Pomegranate (Punica granatum) were impinged to microbial sensitive test using disk diffusion method

The greatest and notable antibacterial activity (zone of inhibition) was noticed with ethanolic extract of Neem (Azadirachta indica) extract against Salmonella (12mm) which is the most effective. No antimicrobial action was found with aqueous extracts of all the three medicinal plants against the chosen bacteria. However, three conventional antibiotics were employed as a positive control against the chosen bacteria which had demonstrated antimicrobial action.

Four oils (thyme oil,rosemary oil,orange oil and clove oil) were chosen for this study to consider the impact of different concentrations on hindrance of *Salmonella*.Thyme,Orange,Clove and Rosemary oil have been utilized to alleviate stomach torment for centuries. It makes a difference to calm the nerves and help in assimilation in case you eat something that doesn't concur with you. Put one or two drops of the oils in a refreshment to utilize it for stomach torment purposes Past reports of in vitro study appears that thyme, orange, rosemary, and clove oils were a bit successful against *Salmonella*.. Nowadays, researchers are progressively turning their focus in investigating herbal products to combat the increasing occurrences of microbial drug resistance.

This study of antibacterial activity against chosen pathogen was done by the Disk diffusion method and Agar well diffusion method. In most cases, the inhibition of bacterial growth after one day of incubation demonstrated better results. Thyme oil showed the best inhibition against the bacteria chosen for this research whereas Rosemary oil also showed maximum and orange oil showed minimum inhibition against *S.typhi*

Dedicated
To my loved and dear ones

Acknowledgement

I would like to begin with saying thanks to the Almighty Allah for keeping me in great wellbeing and giving me the quality to thrust through.

My heartfelt gratitude also go to **Professor A F M Yusuf Haider**, Ph.D, Chairperson, Department of Mathematics and Natural Sciences, BRAC University, **Prof. A. A. Ziauddin Ahmad**, Former Chairperson, Department of Mathematics and Natural Sciences for granting me consent to continue my research work in my preferred topic at BRAC University Microbiology Laboratory.Moreover,I would like to express my gratitude to my supervisor, **Dr. Mahboob Hossain**, Professor, Microbiology Program, Department of Mathematics and Natural Sciences for his valuable guidance andproper supervision .I feel greatly thankful to get a supervisor like him who helped me gather proper knowledge about my topic which led the successful finish of my thesis.

Furthermore, Iam also very much thankful to my departmental teachers especially **Fahareen-Binta Mosharraf** who were extremely helpful and provided invaluable assistance, support and guidance. I thank them all for their generous and affectionate care. Iam also very much overwhelmed by the immense help and support provided by**Nowreen Hossain**, Teaching Assistant, BRAC University who always guided me properly with utmost patience in every step from the very first day, till the end of this project. Mycordial thanks **Asma Binte Afzal**, laboratory officer of Microbiology and Biotechnology Laboratories, BRAC University and associate **Salman Khan** for their cooperation and assistance.Besides, I am also indebted to all my lab mates specifically **Tasfia Tawhid, Honse Ara, Saima Istiaque Priyam**and **Faria**

Ferdousi for offering me a helping hand whenever I asked for their assistance on any matter related to the thesis work.

Last, but not the least, I would like to thank my parents who had been a great source of encouragement and inspiration to me throughout my life.

Sincerely,
Tazreen Binte Ehsan
Department of Mathematics and Natural Sciences

Table of Contents

List of Figures

List of Tables

List of Acronyms

CFU*-Colony Forming Unit*

IMViC*- Indole, Methyl Red, Voges-Proskauer, Citrate*

WHO*-World Health Organization*

MHA- *Muller Hinton Agar*

NA- *Nutrient Agar*

CA-MRSA*- Community Acquired-Methicillin resistant Staphylococcus aureus*

TSI- *Triple Sugar Iron*

MR- *Methyl Red*

MRSA- *Methicillin-resistant Staphylococcus aureus*

SSTIs- *skin and soft-tissue infections*

MSSA- *Methicillin sensitive Staphylococcus aureus*

VP- *Voges-Proskauer*

TSB- *Trypticase Soy Broth*

MDR-*Multi Drug Resistant*

MBC-*Minimum Bactericidal Concentration*

MIC-*Minimum Inhibitory Concentration*

TNTC *-Too numerous to count*

AI *-Activity Index*

HA-MRSA *- Hospital-associated- Methicillin-resistant Staphylococcus aureus*

PBP *- penicillin-binding protein*

CHAPTER 1

INTRODUCTION

1. Introduction and Literature Review:

1.1 Overview

Salmonella typhi is gram-negative rod-shaped bacteria. *Salmonella* typhi is a strain of bacteria that lives only in humans. It causes a bacterial infection of the intestinal tract and occasionally the bloodstream which is called typhoid fever. Antibiotics used on typhoid patients include ampicillin, trimethoprim- sulfamethoxazole, or chloramphenicol. Due to the overuse of such antibiotics, the species have started to develop drug resistance over the past few years (Pollack, 2003). The world seems to be running out of antibiotics. While any antimicrobial resistance is concerning, the increasing incidence of antibiotic-resistant Gram-negative bacteria has become a particular problem as strains resistant to multiple antibiotics are becoming common and no new drugs to treat these infections will be available in the near future (Schneider *et al*., 2017). There's a larger problem—the problem of resistance is also due to an abuse of antibiotics. Many people will go to a doctor and demand an antibiotic when they have a cold or the flu, for which these antibacterial compounds are useless. Moreover, the huge significance of medicinal plants in human wellbeing cannot be neglected. These plants have healing/therapeutic properties in one or any of their organs. The use of these plants is increasing worldwide. They are used in several conditions to augment and maintain human health**.** In sustainable human health management, medicinal plants have played a vital role which has led to the growing interest in alternative therapies and therapeutic use of plants. This is because; it is very cheap in comparison to the synthetic industrial forms of medication. Herbal oilsor essential oils have recently emerged as an effective topical antimicrobial agent active against a wide range of organisms.Essential oils have great medicinal benefits as they contain the essence of different herbs and flowers in concentrated form. The aroma molecules are very potent organic plant chemicals that make the surroundings free from disease, bacteria, viruses, and fungus. Their versatile character of antibacterial, antiviral, anti-inflammatory nature along with immune booster body with a hormonal, glandular, emotional, circulatory, calming effect, memory, and alertness enhancer, is well documented by many scientists(Ali *et al*., 2015). Essential oils are a rich source of

biologically active compounds. There has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants, particularly essential oils. Therefore, it is reasonable to expect a variety of plant compounds in these oils with specific as well as general antimicrobial activity and antibiotic potential

1.2 The Gram-negative bacteria selected for this study

1.2.1 *Salmonella*

The species *Salmonella*was envisioned by Karl Eberth in the Peyer's patches and spleens of typhoid patients in 1880. Four years later, another scientist named Georg Theodor Gaffky successfully grew this pathogen in pure culture. One year after that another scientist named Theobald Smith discovered which later known as *Salmonella enterica.* During that time, Smith used to work in the veterinary division of US department of agriculture as a research laboratory assistant .This division was under Daniel Elmer Salmon, who was a veterinary pathologist. Basically, *Salmonella* Choleraesuis was guessed to be the causative agent of hog cholera, so both Salmon and Smith titled it "Hog-cholerabacillus". Until 1900 the term *Salmonella* was not in use, then Joseph Leon Lignières suggested that the pathogen discovered by Salmon's group should be called *Salmonella* in his honor.

Salmonella is the driving cause of bacterial food-borne contamination causes approximately 1.2 million cases of human Salmonellosis each year. The foremost commonly involved source of foodborne Salmonellosis is consumption and dealing of undercooked poultry items.

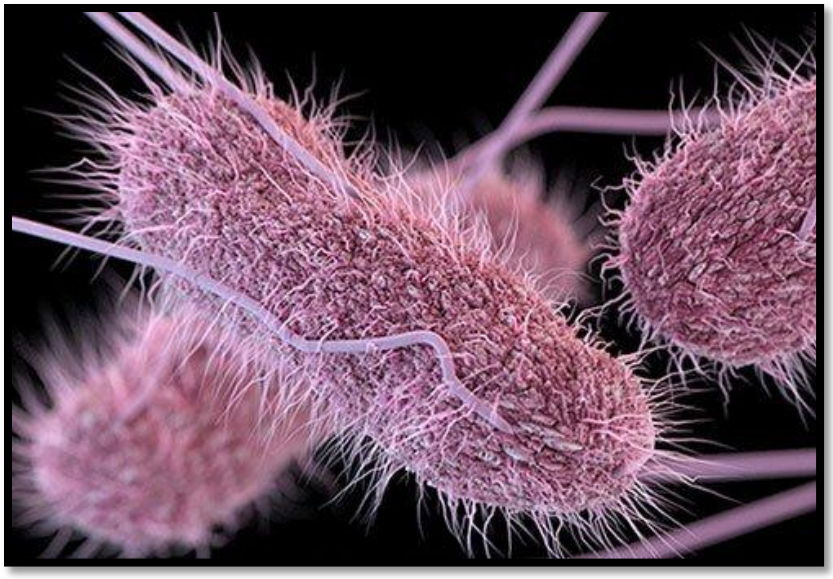


Figure A: *Salmonella*

Salmonella is a genus of rod-shaped (bacillus) Gram-negative microbes of the family Enterobacteriaceae.*Salmonellaenterica* and *Salmonellabongori* are the two species of *Salmonella*.*S.enterica* is the sort species and is further classified into six subspecies that incorporate over 2,600 serotypes. *Salmonella* was named after Daniel Elmer Salmon (1850–1914), an American veterinary surgeon.

Salmonellosis

Thespecies*Salmonella* are intracellular pathogens certain serotypes causing sickness. Non-typhoidal serotypes can be exchanged from animal-to-human and from human-to-human.By invading only, the gastrointestinal tract they cause salmonelloisis. However, in some areas like Sub-Saharan Africa, there is non-typhoidal *Salmonella*which can be invasive and can also cause paratyphoid fever, which can be recovered with immediate treatment of antibiotics.The transmission of typhoidal serotypes can only be possible from human to human causing food-borne infection, typhoid fever, and paratyphoid fever.*Salmonella* causes typhoid by invading the bloodstream and in addition spreads throughout the body invades different organs and releases endotoxins.This may lead to fatal hypovolemic shock and septic shock which will require intensive care including antibiotics.

Taxonomy

*Salmonella*belongs to the family of Enterobacteriaceae. The genus is comprised of two species, S. *bongori* and S. *enterica*, the last-mentioned of which is isolated into six subspecies: *S. e. enterica, S. e. salamae, S. e. arizonae, S. e. diarizonae, S. e. houtenae, and S. e. indica.* The taxonomic groupcomprises of more than 2500 serotypes (too serovars) characterized on the premise of the somatic O (lipopolysaccharide) and flagellar H antigens (the Kauffman–White classification). The total title of a serotype is given as, for the case, *Salmonella* enterica subsp. enterica serotype Typhimurium, but can be truncated to *SalmonellaTyphimurium*. The additionaldistinction of strains to help clinical and epidemiological examination may be accomplished by anti-microbial sensitivity testing and by other molecular biology procedures such as Pulse-field gel electrophoresis, multilocus sequence analysis, and, progressively, entire genome sequencing.Historically, *Salmonella*e have been clinically classified as invasive (typhoidal) or noninvasive (nontyphoidal *Salmonella*e) on the basis of host preference and illness appearances in humans.

Detection, culture, and growth conditions

Most subspecies of *Salmonella* deliver hydrogen sulfide,which can promptly be identified by developing them on media containing ferrous sulfate, such as is utilized within the triple sugar iron test. Most of the isolates exist in two stages, a motile stage, and a non-motile stage.

By using a Craigie tube or ditch plate cultures that are non-motile upon primary culture can be switched to the motile phase.For the enrichment of *Salmonella* species, RVS broth can be used for better detection in a clinical sample.*Salmonella* can moreover be identified and subtyped utilizing multiplexor real-time polymerase chain reaction (qPCR) from extracted *Salmonella* DNA.

For chicken, pork, tomatoes, and melons scientific models of *Salmonella* development energy have been advanced.*Salmonella*replicates asexually with a cell division interim of 40 minutes. *Salmonella* species mostly lead host-associated ways of life, but the microbes were found to be able to continue in a washroom setting for weeks resulting in contamination, and are often kept isolated from water sources, which act as bacterial stores and may offer assistance to encourage

transmission between hosts. *Salmonella* is infamous for its capacity to outlive drying up and can endure for a long time in dry situations and nourishments

By freezing,*Salmonella* cannot be destroyed,but their demolition can be accelerated byUV light and heat.They die after being warmed to 55 °C (131 °F) for 90 min, or to 60 °C (140 °F) for 12 min.Within the gastrointestinal tracts of people and creatures, especially reptiles *Salmonella* species can be found *Salmonella* can be transferred to people from different creatures because the species resides on the skin of different animals who live in water or land if the person meets the species.Food and water can to be sullied with the microscopic organisms if they come in contact with the feces of tainted individuals or animals.

Pathogenicity

The microscopic species *Salmonella* are facultative intracellular pathogens.*Salmonella* can attack diverse kinds of cells, consisting of epithelial cells, M cells, macrophages, and dendritic cells.As a facultative anaerobic life form, *Salmonella*utilizes oxygen to form ATP in an aerobic environment (i.e., when oxygen is accessible). Nevertheless, in an anaerobic environment (i.e., when oxygen isn't accessible) *Salmonella* produces ATP by fermentation; by substituting one or more of four less productive electron acceptors than oxygen at the conclusion of the electron transport chain: sulfate, nitrate, sulfur, or fumarate. Most diseases are due to the consumption of edibles sullied by creature feces, or by human feces, such as by a food-service laborer at a commercial diner. *Salmonella* serotypes can be isolated into two fundamental groups—typhoidal and non-typhoidal. Nontyphoidal serotypes are more common, and as a rule cause self-limiting gastrointestinal diseases**.** *Salmonella*can cause infection in a wide range of creatures and are zoonotic, that is they can be passed on between people and other creatures.Typhoidal serotypes comprise of *SalmonellaTyphi* and *SalmonellaParatyphi A,* which are adjusted to humans and don’t occur in other creatures.

Epidemiology

Due to being considered intermittent, between 60% to 80% of *Salmonella* contaminations cases go undiagnosed.In March 2010, an information investigation was completed to gauge a rate of 1140 per 100,000 person-years. Within the same investigation, 93.8 million cases of gastroenteritis were due to *Salmonella* diseases. At the 5th percentile, the evaluated sum was

61.8 million cases and at the 95th percentile, the assessed sum was 131.6 million cases. The evaluated number of passing due to *Salmonella*was roughly 155,000 deaths. In 2014, in nations such as Bulgaria and Portugal, children beneath 4 were 32 and 82 times more likely, respectively, to have a *Salmonella* infection. Those who are most sensitive to contamination are children, pregnant ladies, elderly individuals, and those with lacking immune systems.

Avariety of foods and nourishments can be included as risk factors for *Salmonella* infections including meats such as chicken and pork have the chance to be infected. An assortment of vegetables and grows mightalso have *Salmonella*. In conclusion, an assortment of handled nourishments such as chicken chunks and pot pies might also contain these bacteria.

Molecular mechanisms of infection

Components of disease contrast between typhoidal and nontyphoidal serotypes, owing to their diverse targets within the body and the distinctive indications that they cause. Both categories must enter by crossing the obstruction made by the intestinal cell wall, but once they have passed this obstruction, they utilize diverse techniques to cause contamination.

1.3 Description of Medicinal Plants

The medicinal herb is another name for medicinal plants,it has beeninvented and utilized in conventional pharmaceutical practices since ancient times. Plants incorporate hundreds of chemical compounds for works consisting of defense against crawlies, organisms, infections, and herbivorous mammals. Various phytochemicals with potential or already formed biological activities have been found. Although, since a single plant contains broadly different phytochemicals, the impacts of employing an entire plant as a pharmaceutical are not certain.Furthermore, the phytochemical substance and pharmacological activities, if any, of numerous plants having therapeutic potential stay unassessed thorough logical inquire about to characterize adequacy and safety.

the Atharva Veda, the Rig Veda, and the Sushruta Samhita has utilized hundreds of pharmacologically dynamic herbs and flavors such as turmeric, which contains curcumin.

Therefore, it can be said that medicinal plant plays a vital role in human civilization from a very old time.

1.3.1 *Azadirachtaindica*(Neem)

Azadirachta indica, commonly known as Neem is a tall evergreen tree whose bark is hard, rough, and scaly. Its leaves are alternate, flowers are small and white in color. Azadirachta indica tree belongs to the family Meliaceae. Originally from Southeast Asia, Neem is also found in tropical and semitropical regions like India, Bangladesh, Pakistan, and Nepal Neem trees,moreover, develop in islands found within the southern portion of Iran.Neem fruit and seeds are the sources of neem oil.

The most important active constituent of Neem is azadirachtin and the most characteristic metabolites of this family are called limonoids, which are tetranortriterpenoides having broad biological activity. The Chemical constituents contain many biologically active compounds that can be extracted from Neem, including alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids, and ketones. Azadirachtin is a mixture of seven isomeric compounds labeled as azadirachtin A-G and azadirachtin E is more effective

Other compounds that have a biological activity are salannin, volatile oils, meliantriol and nimbin. Flavonoids, flavono-glycosides, dihydrochalocones, tannins and others are also important constituents of bark, leaves, fruits, and flowers of Neem

Azadirachtin, a major compound of the neem has potent anti- fedent, growth and reproductive regulating properties. Likewise, nimbin, a limonoid from neem, is also involved in improving pesticide properties

Binomial name

Azadirachta indica

Figure B: The external morphology of Neem leaves

Figure B(i) : Dried powder of Neem leaves

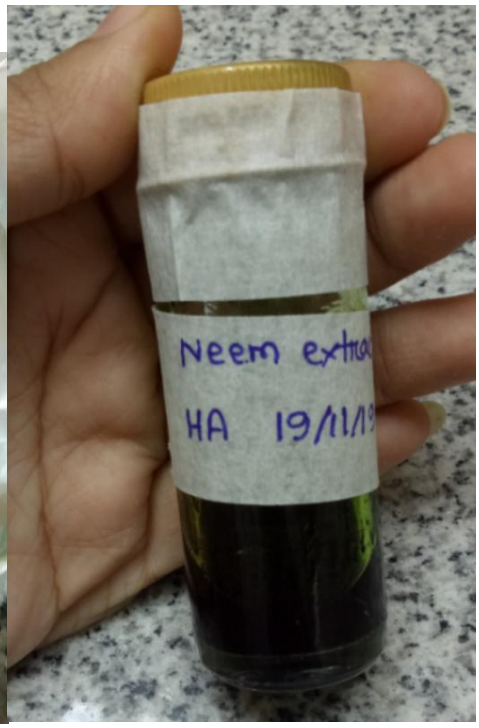


Figure B(ii) : Extract of Neem leaves

1.3.2 ***Ocimumtenuiflorum*(Tulsi)**

Ocimum tenuiflorum (also known as *Ocimum sanctum*), commonly used as holy basil or tulsi, is an aromatizedperenial plant within the family Lamiaceae. It isa localplant in the Indian subcontinent and commonly nurtured plant all through the Southeast Asian tropics.Tulsi is nurtured for devout and traditional medication purposes, and also for its oil. It has great benefits when consumed as a herbal tea, commonly utilized in Ayurveda.The type of *Ocimum tenuiflorum* used in Thai food is known as Thai holy basil (Thai: กะเพรา kaphrao); it isn't the same as Thai basil, which may be a type of *Ocimum basilicum***.**

The plant is an erect, many-branched subshrub, 30–60 cm (12–24 in) tall with shaggy stems. Consisting of green or purple leaves; they are plain-simple, petioled, with an ovate, up to 5 cm (2.0 in)-length edge, which more often has a marginally toothed edge; they are emphatically scented and have a decussate phyllotaxy. The purplish blossoms are put in near whorls on stretched racemes**.**

Tulsi (Sanskrit: -Surasa) has been utilized in Ayurveda and Siddha for its assumed treatment of infections. The plant is really popular for its enhancing specialties, we can use it also to treat skin problems like acne, and boost the immunesystem, it can also help to diminish blood sugar levels, it is exceptionally useful for diabetics patients.It alsoact as an organic warrior against all classes of contaminations. Tulsi extractsderived from the plant are an exceptionally vital and compelling antioxidant that can cure different sicknesses, such as wounds and boils, migraine, common cold, intestinal sickness, inflammation,cough, heart diseases, kidney stones, stomach infections, pimples, and skin breakouts etc .

Figure C: The external morphology of Tulsi leaves

Figure C(i): Dried powder of Tulsi leaves

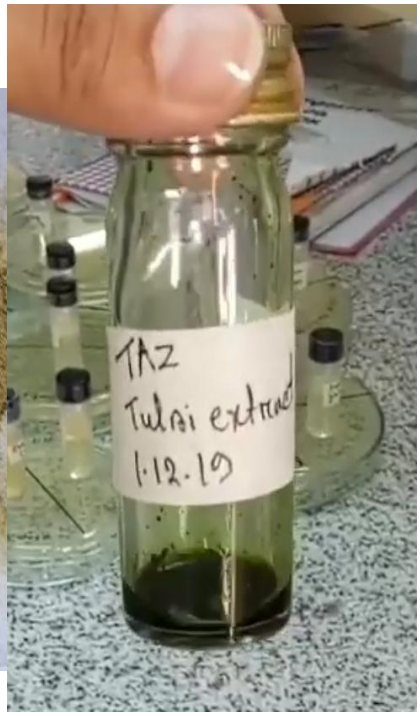


Figure C(ii): Extract of Tulsi leaves

1.3.3***Punicagranatum*** **(pomegranate)**

The pomegranate (*Punica granatum*) is a fruit in the family Lythraceae, subfamily Punicoideae, that develops between 5 and 10 m (16 and 33 ft) tall.

The pomegranate developed in the locale region expanding from Iran to northern India and has been cultivated since antiquated times all through the Mediterranean locale. It was presented into Spanish America in the late 16th century and into California by Spanish settlers in 1769.

Pomegranate is ordinarily in season in the Northern Side of the equator from October to February, and in the Southern Half of the globe from March to May. The fruit,juice, pomegranates are utilized in baking, cooking, juice mixes, dinner garnishes, smoothies, and alcoholic refreshments, such as cocktails and wine.

The bark of the stem and root contains a few alkaloids counting isopelletierine which is dynamic against tapeworms. Either a decoction of the bark, which is exceptionally severe, or the more secure, insoluble Pelletierine Tannate may be utilized. Overdoses are emetic and laxative, creating widening of pupils, the obscurity of locating, strong shortcoming, and paralysis. Because of their tannin substance, extricates of the bark clear out, juvenile natural product and natural product skin have been given as astringents to stop the runs, diarrhea, and hemorrhages. Dried, pulverized bloom buds are utilized as a cure for bronchitis. In Mexico, a decoction of the blossoms is swished to diminish verbal and throat irritation. Clears out, seeds, roots, and bark have shown hypotensive, antispasmodic, and anthelmintic action in bioassay.

Pomegranate Nutrition Value per 100 g

(Source from USDA)

	Nutrient Value
Energy	83 Kcal
Carbohydrates	18.70 g
Protein	1.67 g
Total Fat	1.17 g
Cholesterol	0 mg
Dietary Fiber	4 g

Electrolytes	Nutrient Value
Sodium	3 mg
Potassium	236 mg

Minerals	Nutrient Value
Calcium	10 mg
Copper	18%
Iron	0.30 mg
Magnesium	12 mg
Manganese	0.119 mg
Phosphorus	36 mg
Selenium	0.5 µg
Zinc	0.35 mg

Vitamins	Nutrient Value
Folates	38 µg
Niacin	0.293 mg
Pantothenic acid	0.135 mg
Pyridoxine	0.075 mg
Riboflavin	0.053 mg
Thiamin	0.067 mg
Vitamin A	0 IU
Vitamin C	10.2 mg
Vitamin E	0.60 mg
Vitamin K	16.4 µg

Binomial name

Punica granatum

Figure D: The external morphology of Pomegranate

Figure D(i) : Dried powder of Pomegranate

Figure D(ii): Extract of Pomegranate

1.4 Therapeutic use of these plants

Neem leaf is effective in treating eczema, ringworm, acne, anti-inflammatory, antihyperglycemic properties, and it is used to heal chronic wounds, diabetic foot, and gangrene developing conditions. It is believed to remove toxins from the body, neutralize free radicals and purify the blood. It is used as an anticancer agent and it has hepato-renal protective activity and hypolipidemic effects. Neem extract has been reported to have anti-fungal, antibacterial, antiprotozoal, and antiviral activity. It is also considered a natural insecticide/pesticide plant and the quality of pesticide and pharmacological products depend upon the contents of azadirachtin and Nimbin in the plant. Accordingly, all parts of this plant are useful and have been used in the treatment of diseases ranging from teeth decay, ulcers, swollen liver, malaria, dysentery, diarrhea etc. They possess astringent, purgative anti-inflammatory, moderate anti-tumor, and bactericidal effects Almost every part of the tree has been in use since ancient times to treat several human ailments and also as a household pesticide. The extract from bark, leaves, fruits, and root have been used to control leprosy, intestinal helminthiasis, and respiratory disorders in children

Nowadays Tulsi is used in many countries for various ailments. (Abeysinghe, P.D., Wijesinghe, K.G.G., Tachida, H., & Yoshda, T. 2009) Tulsi is considered an appetizer in Ayurveda and also useful in gastric troubles. The Native Americans used it in several traditional ways. In Europe, it's traditionally used to treat flatulence, digestion problems, gall bladder problems and coughs. The oil was extracted and rubbed into the skin for aches and pains

Pomegranate, *Punica granatumL.*, is an antiquated, enchanted, one-of-a-kind natural product borne on a little, long-living tree developed all through the Mediterranean locale, as distant north as the Himalayas, in Southeast Asia, and in California and Arizona within the Joined together States (Arunkumar, A., Dhyani, A., & Joshi, G. 2019).. In expansion to its antiquated authentic employments, pomegranate is utilized in a few frameworks of pharmaceutical fora an assortment of afflictions.Synergistic activity of the pomegranate constituents shows up to be predominant to that of single constituents.

1.5 Antimicrobial properties of these plants

The antimicrobial activity of Neem showed that the ethyl acetate extract and butanol fraction presented greater activity against *Streptococcusmutans* and *Streptococcus mitis* presenting a MIC = 50 µg/ml for these strains, and the strain *Enteroccocus faecalis*, the hydroethanolic extract, and aqueous fraction were most promising samples with a MIC = 50 µg/ml and MIC = 25 µg/ml, respectively (Galeane *et al.*, 2017). Minimum Bactericidal Concentration (MBC) value of 5 mg/l was obtained with Azadirachta indica against *S. typhi* and *K. pneumoniae* (Joshi et al., 2011). Azadirachta indica leaf extract has antibacterial activity against dental pathogens (Tesso*et al.*, 2015).

The ethanol extract of Tulsi showed the highest antimicrobial activity against *Shigella sonnei,* while the lowest antimicrobial activity was observed by ethanol extract of Tulsi against *Citrobacter* spp and by Acetone extract of Tulsi against *Shigella dysentarie*.The minimal inhibitory concentration (MIC) of Tulsi extract was determined by broth microdilution assay. The highest (MIC) value (8, 16, 32, 32 and 32 µg/ml) was observed against *Bacillus fastidiosus, Staphylococcus aureus , Proteus mirabilis , Proteus vulgaris and Salmonella choleraesuis respectively, while Escherichia coli, Pseudomonas aeruginosa , Klebsiella pneumoniae* and*Serratia odorifera* ranked next (MIC 64, 128, 256 and 512 µg /ml) respectively

1.6 Effect of plant extracts on the human body

Herbal treatment is still used for many health problems. Herbs are safe, less toxic, economical, and a reliable key natural resource of drugs all over the world . Antibiotics, which are considered as a remedy against pathogens with ignoring their influences on the microflora, could lead to creating a new disease by disturbing the microbial ecosystem of the human body and developing new generations of antibiotics resistant pathogens. The biological interactions of the microflora in the human body are important in keeping the somatic ecophysiological balance (Thilakarathne, R., Madushanka, G., & Navaratne, S. 2018). Many studies reported that antibiotics therapy directly influences the normal flora's niches in the human body. In contrast, phytotherapy using plant products could get rid of the pathogens and maintain the normal flora of the human body

1.7MIC and MBC measurement of plant extract

The potency of plant extracts can be determined by measuring the minimum inhibitory concentrations (MIC) value which is the lowest concentration of antimicrobials required to inhibit the growth of a test organism within a definite period, usually within 18-24 hours . MIC measurement by serial broth dilution method is the most preferred one which requires various dilutions of the antimicrobial compound in a suitable solvent. The selection of appropriate solvent is very important as it plays the most important role and has major influences on MIC measurements. The most common solvents used in MIC measurements are ethanol, methanol, and DMSO. The minimum bactericidal concentration (MBC) value was determined by spread plating a small amount of broth from MIC-containing tubes onto freshly prepared nutrient agar media. The plates were incubated further for 18 hours at 37°C. The highest dilution that produced no single bacterial colony on the nutrient agar plates was taken as MBC

1.8 Essential Oils as treatment agent

Essential oils have great medicinal benefits as they contain the essence of herbs and flowers in concentrated form. The aroma molecules are very potent organic plant chemicals that make the surroundings free from disease, bacteria, viruses, and fungus. Their versatile character of antibacterial, antiviral, anti-inflammatory nature along with immune booster body with a hormonal, glandular, emotional, circulatory, calming effect, memory, and alertness enhancer, is well documented by many scientists Essential oils are a rich source of biologically active compounds. There has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils. Therefore, it is reasonable to expect a variety of plant compounds in these oils with specific as well as general antimicrobial activity and antibiotic potential

1.9 Oils used for this study

1.9.1 Clove Oil

Clove bud oil is a pale-yellow fluid with a warm, hot, sweet, and solid smell. The thickness of the clove oil is medium. In comparison, clover leaf oil is dim brown with a crude, burnt-woody odor and clove stem oil may be a pale-yellow fluid with a solid spicy-woody smell.

Chemical composition of Clove Oil

Clove oil is supposedly a blend of distinctive compounds, with the three primary dynamic fixings being eugenol, eugenol acetic acid derivation, and caryophyllene. Jirovetz et al. (2006) found 23 constituents, with eugenol (76.8 %), taken after by β-caryophyllene (17.4 %), α-humulene (2.1 %), and eugenol acetic acid derivation (1.2 %) as the most components. Santin et al. (2010) detailed eugenol (89.6 %), β-caryophyllene (8.6 %) and eugenol acetic acid derivation (1.7 %) whereas Alma et al. (2007) detailed eugenol (87 %), eugenol acetic acid derivation (8 %) and caryophyllene (3.56 %). Variety in components and composition depends on assortment, agro-ecological condition, pre-treatments, handling, and strategies of extraction

Table 1 Characteristics of Clove Oil

Compound	Bud (%)	Range	Stem (%)	Range	Leaf (%)	Range
Essential oil	18	15–20	6	5–10	3–4	1–4
Specific gravity	10 425	1004–1057	10 495	1048–1056	1030	1030–1060
Refractive index	15 296	1528–1538	15 320	1534–1538	15 295	1520–1540
Optical rotation	– 1.1	0-(– 1.58)	– 1.05	0-(– 1.50)	– 1.58	– 1.58
Total eugenol content	80	85–93	85	89–85	82	78–93
Solubility in ethanol	1:2	1:2	1:2	1:2	1:2	1:2
True eugenol	61.62		80		77.1	
Eugenol acetate	18.72		2.1		trace	
β-Caryophyllene	15.27		12.70		17.02	

Medicinal Uses of Clove Oil

- **Moisturizes Dry Skin and Helps Other Skin Problems**

  Clove oil for dry skin could be an incredibly common cure.

- **Reduces Pain**

  Numerous individuals know about the recuperating qualities of clove oil for toothaches and other tooth torments.

- **Soothes Irritation**

  Clove bud oil is frequently utilized as the dynamic fixing to alleviate the tingling and burning regularly related toencountering harm oak or harm ivy.

- **Improves Blood Circulation**

Clove oil is utilized in different types of balms and ointment to assist in progress in blood circulation. Since balm is used to relieve muscle hurts and pains, cloves are the main ingredient that is very helpful in making the blood flow through
the zone and help diminish the pain.

- **Reduces Stomach Pain**

Cloves have been utilized to alleviate stomach torment for centuries. It makes a difference to calm the nerves and help in assimilation in case you eat something that doesn't concur with you. Put one or two drops of the oil in a refreshment to utilize it for stomach torment purposes.

1.9.2 Thyme Oil

Thyme oil has antifungal, anti-inflammatory, and antibacterial properties. It's commonly utilized as an additive in nourishments, makeup, and toiletries. It can too be found as a fixing in mouthwash.

Chemical composition of Thyme Oil

Thyme Oil was developed in Romania. The basic oil was isolated in a abdicate of 1.25% by steam refining from the airborne portion of the plant and along these lines analyzed by GC-MS. There are three major components which are p-cymene, γ-terpinene, and thymol.(Yoon, J.-H., & Baek, S. J. 2005) Its antimicrobial movement was assessed on seven common food-related microorganisms and bacterias by utilizing the disk diffusion method. The result illustrates that the Thymus vulgaris fundamental oil tried has solid antimicrobial properties and may within the future speak to a modern source of natural antiseptics with applications within the pharmaceutical and nourishment industry**.**

Table 2 Characteristics of Thyme Oil

Component	%	Component	%	Component	%
Thymol	39.44	Geranyl acetate	0.44	Nerolidol	0.077
P-Cymene	23.6	Bisabolene	0.27	(-) – Globulol	0.02
γ – Terpinene	12.51	3-Eicosene	0.26	Hexadecanoic acid	0.019
Ledol	2.24	Farnesyl	0.52	7-Tetradecene	0.032
Aromadenrrene	2.12	Phytol	0.21	Heptacosane	0.056
Caryophyllene	0.94	β- Pinene	0.2	P-menth 2-en-1ol	0.10
Farnesyl acetate	0.63	Thujanol	0.17	5-α-Pregn-16-en-20 one	0.36
Linalyl acetate	0.55	Camphor	0.17	Octadecanoic methyl ester	0.14
α – Pinene	0.68	Ethyl butyrate	0.63	Ethyl chrysanthemumate	0.005
α – Thujone	0.52	Caryophyllene oxide	0.13	2-pentadecanone6,10,14trimethyl	0.07

Medicinal Uses of Thyme Oil

- **Alopecia areata**

  Thyme oil blended with other basic oils and a carrier oil, coupled with knead, may be utilized as a treatment to avoid hair loss.

- **Breast cancer**

  One exceptionally preparatory ponder found that wild thyme extricate may, in the long run, appear guarantee at battling breast cancer

- **Coughs and respiratory tract infections**

  The thymol substance in thyme oil is thought to have antispasmodic properties. When blended with primrose, thyme basic oil has been appeared to be compelling at lessening hacks and lessening the duration of respiratory tract diseases, such as the common cold.

- **Heart disease**

  The carvacrol in thyme oil was a useful anti-inflammatory agent with cardioprotective capabilities, making it possibly useful for individuals with heart disease.

- **Oral health**

  The thymol in thyme oil is successful in reducing inflammation and infection. According to recent studies, thymol's anti-inflammatory and antibacterial properties make it useful for oral health. The compound is the main ingredient in a few dental items, including Listerine Cool Mint mouthwash.

1.9.3 Rosemary Oil

Rosemary Essential Oil's chemical composition consists of the following main constituents: α - Pinene, Camphor, 1,8-Cineol, Camphene, Limonene, and Linalool.

Chemical composition of Rosemary Oil

The composition of the basic oil of Rosemary was analyzed by gas chromatography-mass spectrometry (GC–MS). 22 components, which constitute 97.41% of the oil, were recognized. The major constituents were 1,8-Cineole (26.54%) and α-Pinene (20.14%). Least inhibitory concentrations (MICs), negligible bactericidal concentration (MBC), and time-kill energetic forms against three Gram-positive microscopic organisms (*Staphylococcus epidermidis, Staphylococcus aureus* and *Bacillus subtilis),* three Gram-negative microbes (*Proteus vulgaris, Pseudomonas aeruginosa* and *Escherichia coli*) and two organisms (*Candida albican*s and *Aspergillus niger*) were decided for the oil, 1,8-Cineole and α-Pinene. The oil appeared articulated antibacterial and antifungal action than 1,8-Cineole and α-Pinene against all of the tried organisms(Xiao, S.,& Zhang, Y. 2020). Besides, the survival rates and morphological changes of S. aureus after treatment with diverse concentrations of the fundamental oil were evaluated by stream cytometry (FCM) and (AFM).

Table 3 Characteristics of Rosemary Oil

Number of compounds	Compound	%	RI
1	Tricyclene	0.2	919
2	α-Thujene	0.1	923
3	**α-Pinene**	**11.0**	**932**
4	**Camphene**	**5.2**	**944**
5	Sabinene	0.1	966
6	**β-Pinene**	**9.2**	**971**
7	**Myrcene**	**1.2**	**983**
8	α-Phellandrene	0.2	997
9	Car-3-ene	0.1	1005
10	α-Terpinolene	0.1	1010
11	*p*-Cymene	1.3	1017
12	**1,8-Cineole**	**46.4**	**1027**
13	**Limonene**	**1.0**	**1027**
14	γ-Terpinene	1.0	1050
15	*trans*-Sabinene	tr	1054
16	Terpinolene	0.2	1079
17	Linalool	0.5	1087
18	α-Campholenol	tr	1096
19	*endo*-Fenchol	tr	1102
20	**Camphor**	**11.4**	**1124**

Medicinal Uses of Rosemary Oil

1.9.4 Orange Oil

Orange basic oil is extricated from the skin of the sweet orange, Citrus sinensis. Usually done by a strategy is known as cold pressing, which employments weight to crush the oils from the skin. Some of the time, the clears out and blossoms from the orange plant can be utilized as well.

Chemical composition of Orange Oil

The main components of orange oil are monoterpenes (limonene 94.00%, α-pinene 0.54%, sabinene 0.74%, ß-myrcene 1.18%), taken after by oxygenated compounds such as alcohols (linalool 0.89% and α-terpineol 0.06%) and aldehydes (citral-Z 0.09%, citral-E 0.14%, citronellal 0.07%)

Table 4 Characteristics of Orange Oil

Variable	Orange seed oil	Orange peel oil
pH	4.2	5.2
Colour	Golden-yellowish	Brownish -yellow
Percentage yield	38%	30%
Specific density	0.997 g/cm^3	0.778 g/cm^3
Refractive Index	1.46	1.47
Smoke point	140℃	149℃
Flash point	150℃	160℃
Cloud point	13℃	16℃
Pour point	7℃	10 ℃
Viscosity 100℃	3.8185cst	0.9622cst
Viscosity 40℃	11.968cst	1.9766cst
Acid value	23.6 mgKOH/g	25.1 mgKOH/g
Peroxide value	18.00 mgKOH/g	5.40 mgKOH/g
Free fatty acid	11.86% as oleic acid	12.61% as oleic acid
Saponification value	222.58 mgKOH/g	41.25 mgKOH/g
Ester value	178.24 mgKOH/g	28.96 mgKOH/g
Iodine value	78.83 I_2g/100 g	120.10 I_2g/100 g

Medicinal Uses of Orange Oil

1.10 Antibiotics selected for the study

A list of antibiotic disc used for this study is given below. [The brief overview has been taken from (PubMed Health**)] :**

- **Amoxicilin(AMC)** -Amoxicillin/clavulanic corrosive, too known as co-amoxiclav or amox-clav, is an anti-microbial pharmaceutical utilized for the treatment of several bacterial infections.It may be a combination comprising of amoxicillin, a β-lactam anti-microbial, and potassium clavulanate, a β-lactamase inhibitor.It is particularly utilized for otitis media, streptococcal pharyngitis, pneumonia, cellulitis, urinary tract diseases, and creature bites. It is taken by mouth or by infusion into a vein.
- **Ampicillin (AMP) -** Ampicillin was found in 1958 and came into commercial utilize in 1961. Ampicillin is an anti-microbial utilized to avoid and treat a few bacterial contaminations, such as respiratory tract diseases, urinary tract contaminations, meningitis, salmonellosis, and endocarditis.
- **Co-trimoxazole (COT)** - Co-trimoxazole is an antibacterial specialist. Co-trimoxazole is compelling in vitro against a wide extend of gram-positive and gram-negative living beings. It isn't dynamic against *Mycobacterium tuberculosis, mycoplasma or Treponema pallidum, Pseudomonas aeruginosa* is as a rule harsh.

- **Ceftriaxone (CTR)** - Ceftriaxone (ceftriaxone sodium and dextrose) Infusion is an antibacterial sedate utilized to treat conditions such as lower respiratory tract contaminations, skin, and skin structure diseases, urinary tract contaminations, pelvic fiery infection, bacterial septicemia, bone and joint diseases, and meningitis. Ceftriaxone is accessible in nonexclusive frame.
- **Chloramphenicol(C)** - Chloramphenicol is an anti-microbial valuable for the treatment of several bacterial diseases. This incorporates utilize as an eye treatment to treat conjunctivitis.It is utilized to treat meningitis, torment, cholera, and typhoid fever.

1.11Effects of antibiotics on human body

Allopathic antibiotics are synthetic substances that destroy microorganisms or inhibit their growth and are used extensively to treat diseases in animals and humans. Some people may show allergic reactions to some antibiotics. These reactions may be mild like rashes appearing on the skin or may be very serious and can even be fatal. Allopathic system of medicine mostly makes use of chemicals as medicines. It takes more than a few years of testing and trials on animals and humans before allopathic medicine is made available in the market. Its effects, side-effects, efficiency, fixing recommended dose, etc. are extensively studied on scientific lines before it is made available in a market. Prolonged antibiotic treatment can lead to damaging side effects in patients, including ototoxicity, nephrotoxicity, and tendinopathy, yet the mechanisms underlying the effects of antibiotics in mammalian systems remain blurred(Yoon, J.-H., & Baek, S. J. 2005). Some ofthe antibiotics can create reactive oxygen species (ROS) throughout the body. The ROS is toxic to healthy cells and can possibly harm bacterial cells. The ROS start to kill energy sources as well as the mitochondria in human cells. The ROS are thought to bind to bacterial DNA and dissociate it, therefore killing the bacteria, as the bacteria no longer have genetic information

1.12Objectives of the study

There are huge benefits in the study of plants with medicinal value. The present study aims to compare the antimicrobial activity by agar well diffusion method of ethanolic, methanolic and aqueous extracts of medicinal plant extracts and essential oils on the bacterial strain Salmonella

Typhi and among them, the most efficient extracts are identified, provided that the concentration of all the extracts were same (0.1g/ml) except Mint, whose concentration was 0.15g/ml.. In this investigation, the antimicrobial activity test of some common commercial antibiotics against the selected bacteria was also done to serve as a positive control and to compare the antimicrobial potency of these plant extracts with that of the antibiotics. Considering these, the specific objectives of this study included the following:

- Preparation of different types of extracts for the medicinal plants using three different solvents: ethanol, methanol, and distilled water.
- Observation of antimicrobial activity of the extracts of the medicinal plants and the oils against the organisms.
- Comparison of antimicrobial activity of the plant extracts and the oils with commercial antibiotics.
- Determination of the most efficient plant extract among the medicinal plants by the highest activity index value and then finding out the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of that extract.

CHAPTER2

Materials and Methods

2.1 Sample collection

In this research, 8 environmental samples of *Salmonella typhi*were used. All these species were collected from several environmental locations of Dhaka city.

Table 5: Sample collection

Sample no.	Source	Location	Presence of *Salmonella* species
1	Raw fish water(Rui)	Banani kacha bazar	**5/6**
2	Raw fish water(Shing)	Uttara 11no sector kacha bazar	**5/6**
3	Raw Crab water	Uttara 11no sector kacha bazar	**2/5**
4	Raw Prawn water	Banani kacha bazar	**3/6**
5	Lake water	Dhanmondi lake	**0/3**
6	Jheel water	Hatir jheel	**0/2**
7	Raw fish water (Khachki)	Kochukhet bazar	**4/5**
8	Raw fish water (Mola)	Kochukhet bazar	**3/5**
9	Raw Beef	Banani kacha bazar	**1/2**
10	Raw Milk	Milkvita milk	**0/1**
11	Raw Shrimp water	Niketon local bazar	**4/5**

Isolation and identification of the reference strains

To isolate and detect *S.typhi*from the collected samples, one selective media was used which isxylose lysine deoxycholate (XLD). The confirmation of *S. typhi* is guaranteed by the colonymorphology and the cultural characteristics of the inoculated samples on this media.

Xylose Lysine Deoxycholate (XLD)

Xylose Lysine Deoxycholate (XLD) Agar may be a particular medium for the segregation of *Salmonella* spp from clinical examples and nourishment tests. XLD Agar was initially defined by Taylor for the segregation and recognizable proof of Shigella from stool examples. The pathogens are separated not as it were from the non-pathogenic lactose fermenters but too from numerous non-pathogens which don't ferment lactose or sucrose.

Additionally, the medium was defined to extend the recurrence of the development of the more fussy pathogens, which in other details have frequently fizzled to develop due to the incorporation of unreasonably poisonous inhibitors.

XLD Agar is included in the USP microbial limit test for testing for the presence or absence of Salmonella and is recommended for the testing of foods, dairy products and water

Preparation of XLD

- An appropriate amount of dried XLD powder was suspended in distilled water (as mentioned in the container) and was boiled in water-bath for 30 mins.
- Heat with frequent agitation until the medium boils. Before pouring in the petri-dish the temperature was brought to 50º C

Culture preparation and identification

- Sample to be tested was inoculated into on a medium-sized Petri plate and incubated at 37°C for 24 hours.
- After the 24 hours incubation, the morphology, and cultural characteristics of the colonies on the media were observed to identify and ensure the presence of *Salmonellatyphi*.
- The appearance of red colony with black centers indicated a positive result for the presence of *S.typhi*.

2.2 Biochemical Confirmation of the *S.typhi* isolates

Biochemical tests were carried out for the further confirmation of the selected *S.typhi* isolates according to the procedures described in the Microbiology Laboratory.

2.2.1 Methyl Red (MR) test

Bacterial isolates were inoculated into 5ml of dextrose phosphate broth (MR-VP broth) and incubated at 37ºC for 48 hours. After 48 hours of incubation, the pH of the medium was checked by the addition of five drops of MR reagent.

2.2.2 Voges-Proskauer (VP) Test

Bacterial isolates were inoculated into 5ml of dextrose phosphate broth and incubated at 37°C for 48 hours. Following the incubation, 10 drops of Barritt's reagent A were added to each broth culture and were shaken. Instantly, 10 drops of Barritt's reagent B were added and the cultures were shaken again. The cultures were then kept aside for 15 minutes for the reaction to happen. After 15 minutes, the colors of the cultures were reviewed, and the results were recorded.

2.2.3 Triple Sugar Iron (TSI) test

A single bacterial colony of each of the isolates was selected from each nutrient agar plate by a needle and stabbed into 6 ml of TSI agar. Caps of the tubes were loosened and incubated at 35°C overnight and were noted after 18-24 hours for carbohydrate fermentation, CO_2, and H_2S production.

2.2.4 Citrate Utilization Test

A single colony collected from the bacterial isolates was stabbed into the slant of 3ml of Simmon's citrate agar and incubated at 37°C for 24 hours. After 24 hours of incubation, the color change of the media was observed, and the results were noted.

2.2.5Indole Test

The selected isolates were inoculated in 6ml of peptone water broth and incubated overnight at 37°C. After overnight incubation, five drops of Kovac's reagent were added. Then the colors of the cultures were examined, and the results were noted. The formation of a rose red ring at the top of the liquid surface gives a positive result. A negative result can have a yellow or brown layer.Usually,*S.typhi*indicates a negative result for the indole production test.

2.2.6Oxidase test

Two drops of oxidase reagent (p- Amino dimethylaniline oxalate) were added to the filter papers.The filter papers were labeled according to the sample being tested. A loopful of each bacterialisolate to be tested was streaked onto the filter paper. A positive reaction might turn the paper from violet to purple within a few seconds.

2.2.7Catalase test

A drop of the catalase reagent (hydrogen peroxide) was taken on autoclaved glass slides. The glass slides were labeled according to the sample to be tested. A colony for each of the bacterial isolates was taken from a nutrient agar plate and placed on the reagent drops on the glass slides. An instant bubble formation indicates a positive result.

2.3 Antibiotic disk diffusion

The antibiotic disk diffusion test is done to identify antimicrobial susceptibility in the bacterial isolates and to ensure susceptibility to drugs of choice for infections caused by these bacteria. In this research, the effectiveness of five different commercially available antibiotics was determined.The list of antibiotics used is as follows:

Table 6: Sensitivity measurement of six different antibiotics against *S. typhi*

Provided antibiotic disks	An identification number and amount	Resistant	Intermediate	Sensitive
Amoxicilin	AMC (30 µg)	≤13	15-16	≥17
Ampicillin	AMP (25µg)	≤13	14-16	≥17
Co-trimoxazole	COT (25µg)	≤10	11-15	≥16
Ceftriaxone	CTR (30µg)	<19	20-22	>23

Chloramphenicol	C (30µg)	≤12	13-17	≥18

Inoculation on the MHA plates

- For eight test samples, eight different MHA (Muller-Hinton Agar) plates with proper labeling of the samples were prepared
- Autoclaved cotton swab was dipped into the bacterial suspensions and rotated so that it is completely wet with the suspension.
- The test tubes having the bacterial suspension were vortexed before dipping the cotton swab.
- The swab was then streaked several times on the dried surface of the MHA plate to make a pure lawn assuring the contact of the cotton of the swab with all the edges of the plate.
- The agar plate was being rotated 90 degrees each time it was being streaked, to assure the even distribution of the inoculums
- The plates were next allowed to dry out.

Placement of the antibiotic disks

- A burnt sterile forcep was used on an MHA plate to insert the antibiotic discs.
- On the surface of the inoculated MHA plate, five different sterile antibiotic discs were placed.
- Each of the discs was slightly pressed with the forceps on the MHA plate so that it sticks properly to the agar surface
- The disks were not placed close to the edge of the plates was as the zones will not be fully round and lead to an error of the test as the measurement cannot be taken properly
- The MHA plates were next inverted and incubated at 37^0 C for one day

Measurement of the zone of inhibition

- Following the incubation, the zone of inhibition for each of the antibiotics was observed on the MHA plate
- The size of zones for each antibiotic were measured cautiously in millimeters (mm) using a ruler
- All the measurements were taken viewing the back of the Petri dish
- The zone size was noted on the recording sheet in a chart

Preparation of stock sample

When sufficient strains of microorganisms are created, proper preservation is a must for use in thefuture. If the cultures are not properly preserved, their characteristic features may decrease after a specific time.

In this research, two procedures have been employed for maintaining the organisms in viable conditions over a long period.

Long term preservation

The media Trypticase Soy broth (TSB) was prepared in a sterile cryovial. For long-term preservation, 500µl of bacterial culture was grown in Trypticase Soy Broth at 37^0 C for 6 hours. After the incubation period, the cryovial was stored at -20^0 C, and 500µl of sterile glycerol was added to the broth culture.

Short term preservation

- Stock: 3 ml of T1N1 media was prepared in sterile vials. Colonies from the bacterial samples to be preserved were touched by a needle from nutrient agar plates and stabbed onto thebutt of the vials. Then the vials were incubated at 37^0 C for 6 hours. Following theincubation period, 200µl of paraffin oil was added on the surface of the medium contained in each of the vials. All the vials were labeled carefully and preserved at room temperature.
- **Subculture**: The chosen colonies from nutrient agar were re-cultured onto fresh NA platesfor further stocking and biochemical tests.

2.4 Gram Staining

This is a common, vital, and most utilized differential method in microbiology, which was presented by Danish Bacteriologist Hans Christian Gram in 1884. This test separates the microscopic organisms into Gram-Positive and Gram-Negative organisms, which makes a difference in the classification and separations of microorganisms.

- Prepare the smear of suspension on the clean slide with a loopful of sample.
- Then air dry and heat fix
- Crystal violet was poured and kept for about 1 minutes and rinse with water.
- Flood the gram's iodine for 1 minute and wash with water.
- Then, wash with 95% alcohol or acetone for about 5-10 seconds and rinse with water.
- Add safranin for about 45 seconds and wash with water.
- Air dry, blot dry and observe under microscope.

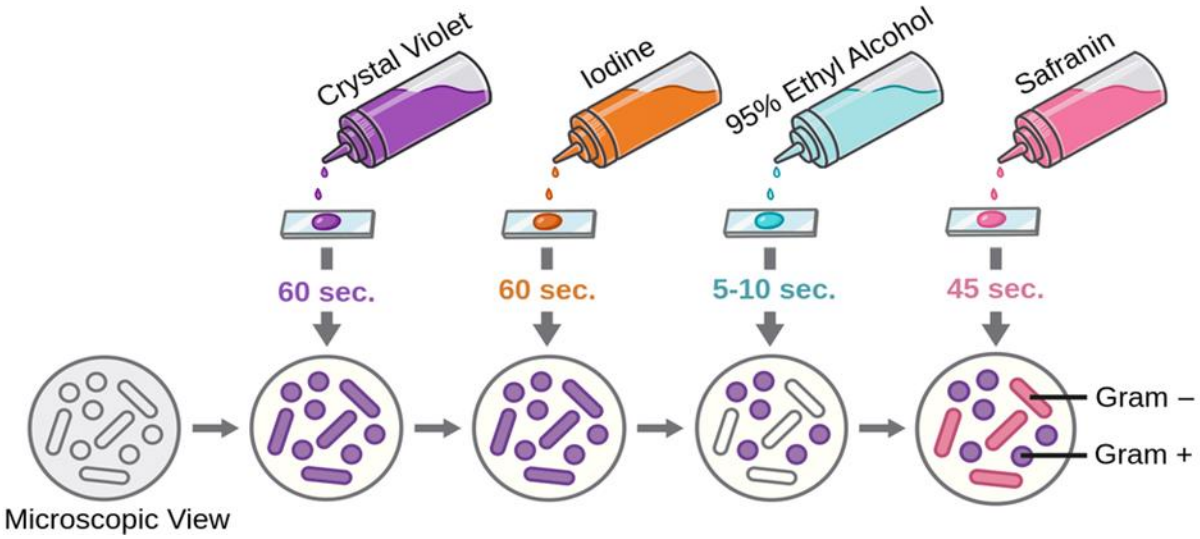


Fig E: Workflow of gram staining method

CHAPTER3

Results

3.1 Confirmation of *Salmonella typhi*

Eleven different environmental samples were collected from several locations of Dhaka city. The samples were enriched in LB broth and after enrichment, the culture broth was subjected to a two folds dilution.The samples from the appropriate dilutions were next spread plated on the Nutrient agar to determine the heterotrophic count. For the separation of *Salmonella* species, one selective Media- XLD was used. From 11 samples, 40 isolates have been selected and culture on the selective media ensured the presence of 20 *Salmonella* isolates. Colonies showing typical morphological characteristics of *Salmonella* were next taken for biochemical tests. A series of biochemical tests- IMVIC, TSI test, Gram staining, etc. confirmed the presence of *Salmonella*. *typhi*. Through the biochemical tests, 8 *Salmonella.typhi* isolates were confirmed from the 11 *Salmonella*isolates

Table 7: Total Aerobic (TA) count of the 8 samples collected on nutrient agar

Sample No.	Sample number and name	Dilution*		CFU/ml
		10^{-1}	10^{-2}	10^{-3}
1.	Raw fish water (Rui)	TNTC	58	$5.8x10^4$
2.	Raw fish water (Shing)	TNTC	62	$6.2X10^4$
3.	Raw Crab water	TNTC	75	$7.5X10^4$
4.	Raw Prawn water	TNTC	57	$5.7X10^4$
5.	Raw fish water (Khachki)	TNTC	65	$6.5X10^4$
6.	Raw fish water (Mola)	TNTC	69	$6.9X10^4$
7.	Raw Beef	TNTC	51	$5.1X10^4$
8.	Raw Shrimp water	TNTC	75	$7.5X10^4$

TNTC= Too Numerous to Count * 100 µl sample was added on each plate

Table 8:Colony characteristics of eight*Salmonella* isolates on selective media

Isolate no.	Shape	Elevation	Margin	Consistency	Colony (MSA)
1.	Circular	Convex	Entire	Smooth	Red colonies with black centers.
2.	Circular	Convex	Entire	Smooth	Large black colonies.
3.	Circular	Convex	Entire	Smooth	Black colonies.
4.	Circular	Convex	Entire	Smooth	Red colonies with black centers.
5.	Circular	Convex	Entire	Smooth	Large black colonies.
6.	Circular	Convex	Entire	Smooth	Black colonies.
7.	Circular	Convex	Entire	Smooth	Black colonies.
8.	Circular	Convex	Entire	Smooth	Large black colonies.

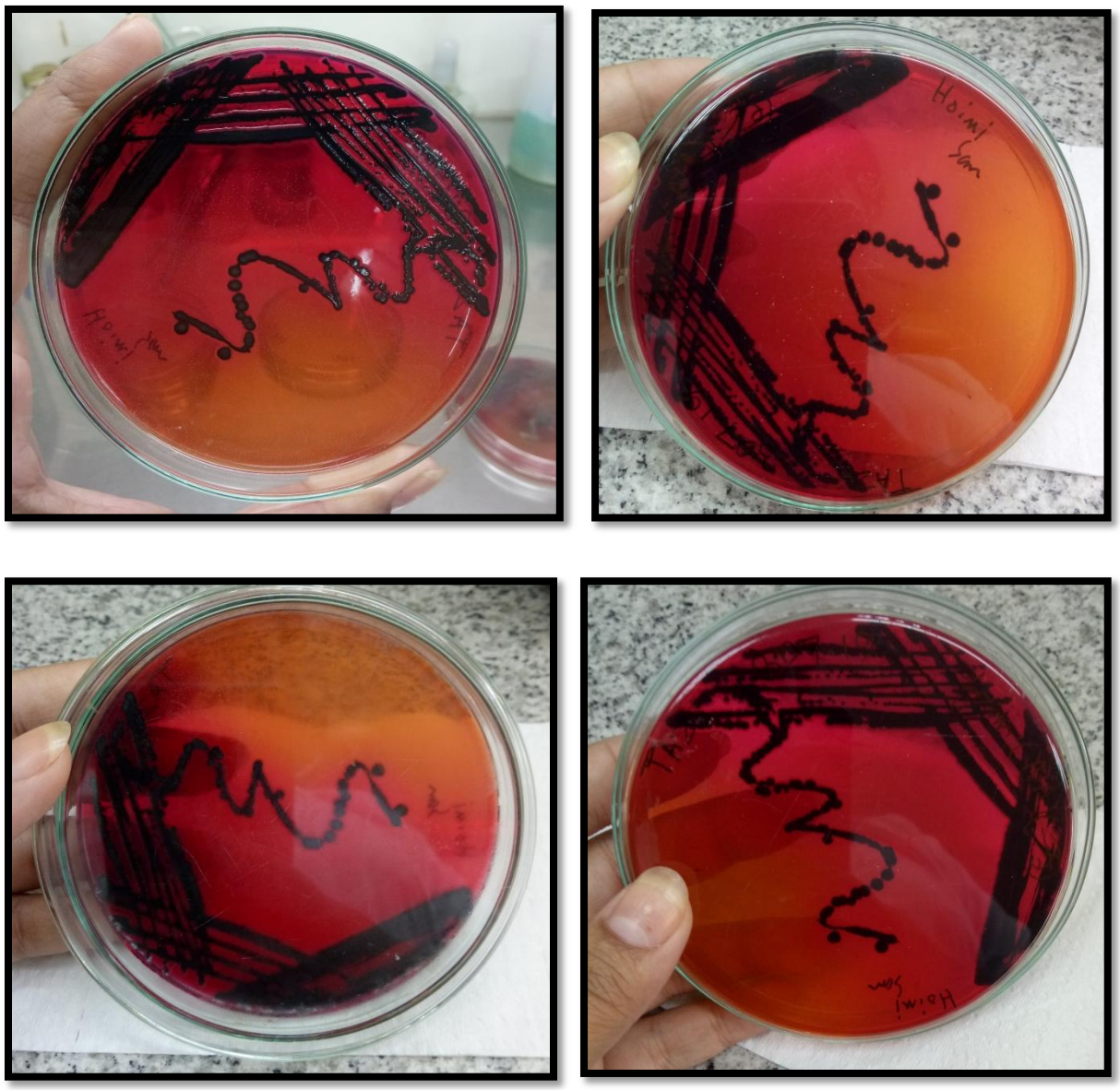


Fig F: Growth of *Salmonella.typhi* on XLD Agar

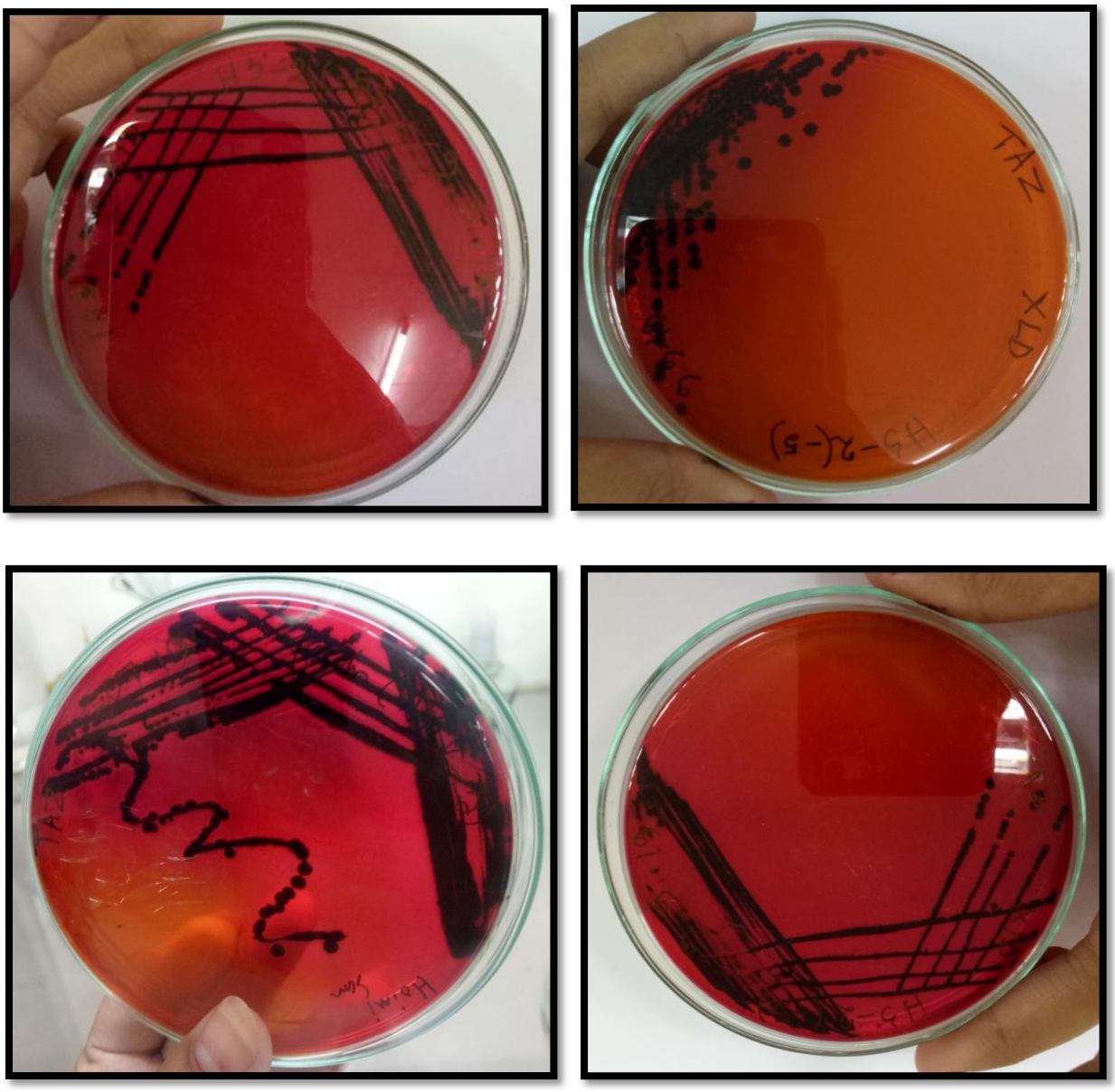


Fig F: Growth of *Salmonella.typhi* on XLD Agar media.

3.2: <u>Biochemical Identification</u>: Biochemical tests were performed for the 8 selected *Salmonella* isolates. These, 8 isolates gave standard results for the *S. typhi*.Standard results of the biochemical tests of the isolates are as follows:

Table 9: Biochemical Identification Results

Isolate no.	Methyl Red test	Voges - Proskauer test	Citrate utilization test	MIU		Indole test	Oxidase test	Catalase test	Triple Sugar Iron (TSI) test			
				Motility	Urease				Slant	Butt	H_2S	Gas
1.	+	–	–	+	–	–	–	+	K	A	+	–
2.	+	–	–	+	–	–	–	+	K	A	+	–
3.	+	–	–	+	–	–	–	+	K	A	+	–
4.	+	–	–	+	–	–	–	+	K	A	+	–
5.	+	–	–	+	+	–	–	+	K	A	+	–
6.	+	–	–	+	–	–	–	+	A	A	+	–
7.	+	–	–	+	+	–	–	+	K	A	+	–
8.	+	–	–	+	–	–	–	+	K	A	+	–

KEY: (A) = acidic condition (Yellow), (K) = alkaline condition(Red), (+) = positive, (-) = negative.

The Methyl Red test for 8 isolates showed positive results. Voges-Proskauer test for 8 isolates showed a negative result. Citrate utilization test for8 isolates showed negative results. Motility test for 8 isolates showed positive result and urease test of 6 isolates among the 8 isolates showed negative result and 2 showed a positive result. Indole test for 8 isolates showed negative results. Oxidase test for 8 isolates showed negative results. Catalase test for 8 isolates showed positive results. TSI test for 8 isolates showed alkali /acid formation in (red) slant and (yellow) butt, blackening of the medium indicated the production of H_2S and no Gas production took place.

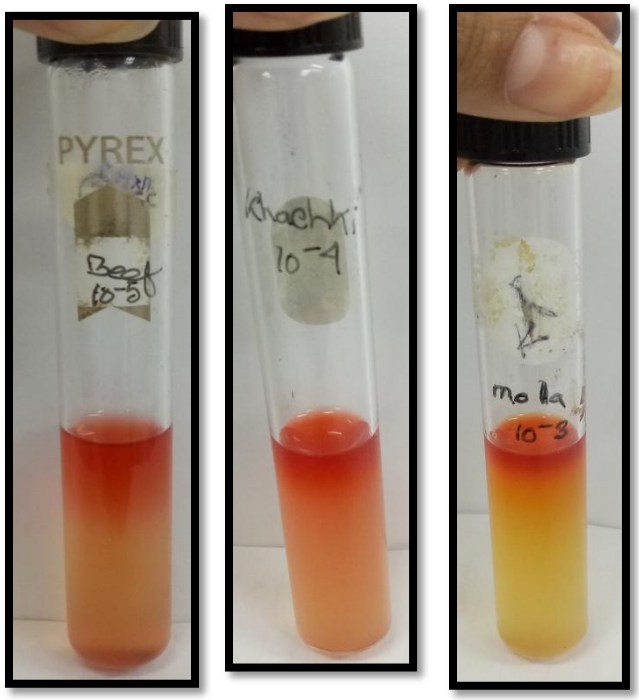


Fig G: Methyl Red (MR) test of isolated *S.typhi* sources showed red color formation which indicates a positive result.

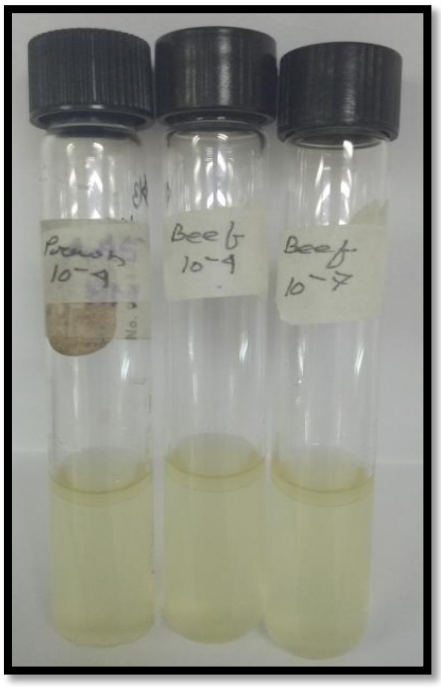


Fig H: Voges Proskauer (VP) test here represents negative result showing the lack of pink-red color on the top.

Fig I: Citrate Utilization test of isolated *S.typhi*. did not change the original green colour of the media which indicates negative result of the test.

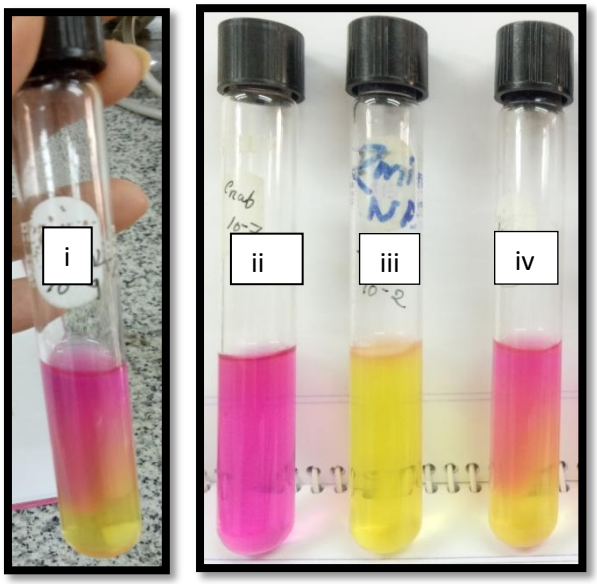


Fig J: Motility test for *S.typhi* is positive for all the sources and urease test for (i),(iii),(vi) is negative and for (ii) is positive.

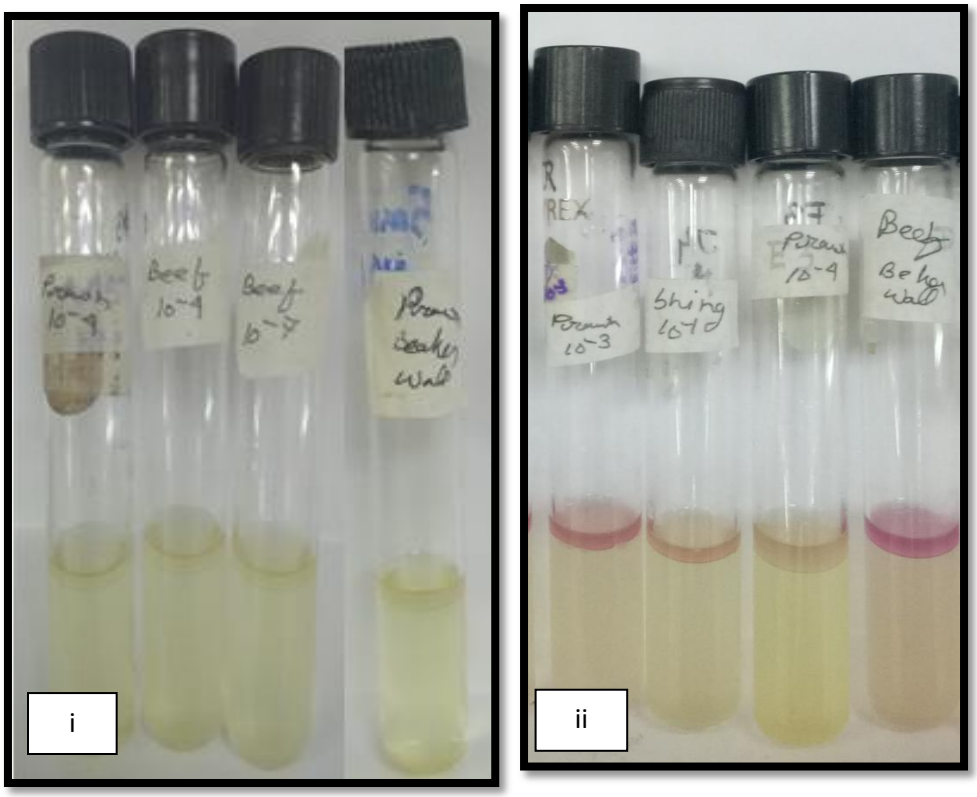


Fig K: Indole test showed negative result in (i) and Indole test showed positive result by forming a red rose ring in (ii)

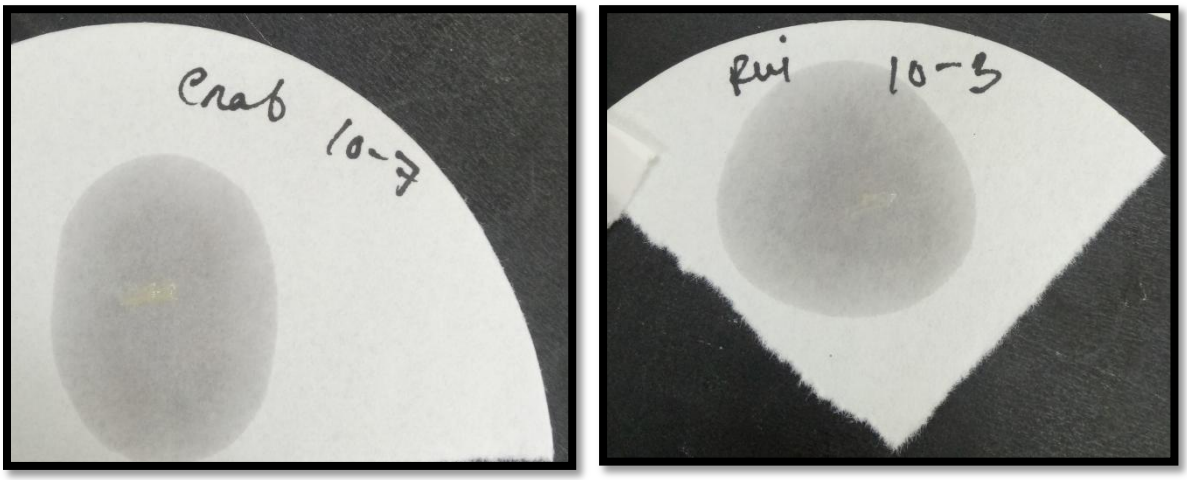


Fig L: Oxidase test results of isolated *S.typhi*showed negative results.

Fig M:Catalase test result of isolated *S.typhi* showed positive result by forming bubbles.

Fig N: Triple sugar iron (TSI) test shows an alkaline/acid reaction forming red slant/yellow butt on the *S.typhi* isolates and blackening of the mediumoccurs due to the production of H_2S,and no gas production is seen.

3.3 Morphology confirmation of the isolates through gram staining

The morphology of the confirmed isolates from the biochemical test was monitored through gram staining. *S.typhi* is usually gram-negative organism and would give red color on staining as seen under the microscope.

Table 10: Morphological properties of the isolates from gram staining

Sl no.	Isolates	Shape under microscope	Color after staining	Arrangement	Inference
1.	Raw fish water (Rui)	Rod	Red	Solitary	Gram-negative
2.	Raw fish water (Shing)	Rod	Red	Solitary	Gram-negative
3.	Raw Crab water	Rod	Red	Solitary	Gram-negative
4.	Raw Prawn water	Rod	Red	Solitary	Gram-negative
5.	Raw fish water (Khachki)	Rod	Red	Solitary	Gram-negative
6.	Raw fish water (Mola)	Rod	Red	Solitary	Gram-negative
7.	Raw Beef	Rod	Red	Solitary	Gram-negative
8.	Raw Shrimp water	Rod	Red	Solitary	Gram-negative

After performing all the biochemical and morphological tests,all the 8 isolates were chosen because they gave similar results when compared with the standard results. The research work from here was next performed with these isolates and their antibacterial resistance pattern were checked, observed, and compared.

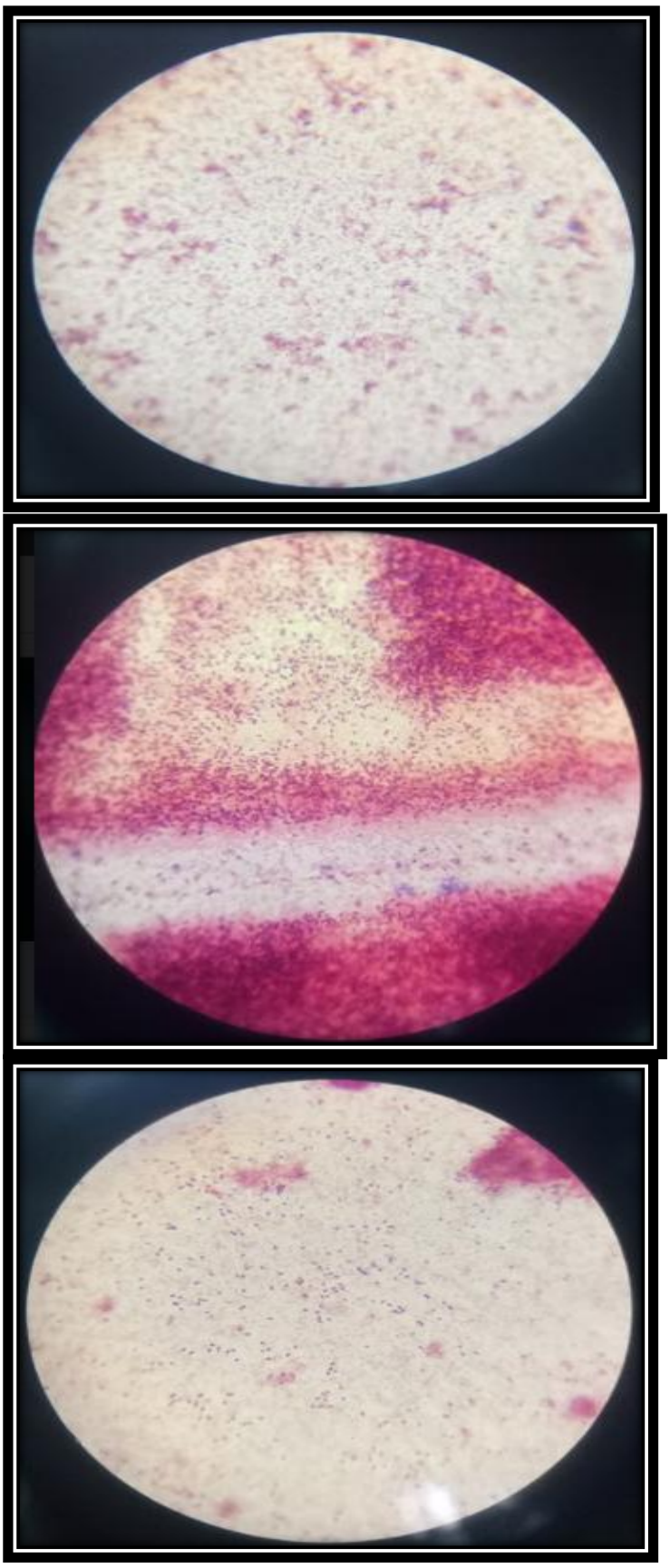


Fig O: Gram staining result of *S.typhi* showing negative result.

3.4: Selective antimicrobial activity test using antibiogram method

The confirmed isolates were chosen to perform the standard disc diffusion test. The test was conducted with six commercial antibiotics- Ampicillin(AMP 25), Amoxicilin(AMC 30),Cotrimoxazole (COT 25),Ceftriaxone (CTR 30),Chloramphenicol(C 30).The size of the zone of inhibition determines the resistance and sensitivity of the organisms to the respective antibiotics. The antibiotic disk diffusion test carried out for each of the isolates showed the following results.

Table 11: Antibiotic susceptibility test of isolated *Salmonella typhi* (Raw fish water from Banani kacha bazar)

Antibiotic disks	Zone of inhibition in diameter(mm)	Inference
AMC 30	16	Resistant
AMP 25	23	Sensitive
COT 25	14	Intermediate
CTR 30	15	Resistant
C 30	13	Intermediate

Table 12: Antibiotic susceptibility test of isolated *Salmonella typhi* (Raw fish water from Uttara 11no sector kacha bazar)

Antibiotic disks	Zone of inhibition in diameter(mm)	Inference
AMC 30	12	Resistant
AMP 25	18	Sensitive
COT 25	17	Sensitive
CTR 30	22	Intermediate
C 30	13	Intermediate

Table 13: Antibiotic susceptibility test of isolated *Salmonella typhi* (Raw Khachki fish water from Kochukhet bazar)

Antibiotic disks	Zone of inhibition in diameter(mm)	Inference
AMC 30	16	Intermediate
AMP 25	25	Sensitive
COT 25	24	Sensitive
CTR 30	17	Resistant
C 30	13	Intermediate

Table 14: Antibiotic susceptibility test of isolated *Salmonella typhi* (Raw Shrimp water from Niketon local bazar)

Antibiotic disks	Zone of inhibition in diameter(mm)	Inference
AMC 30	12	Resistant
AMP 25	26	Sensitive
COT 25	28	Sensitive
CTR 30	21	Intermediate
C 30	16	Intermediate

Table 15: Antibiotic susceptibility test of isolated *Salmonella typhi* (Raw beef from Banani kacha bazar)

Antibiotic disks	Zone of inhibition in diameter(mm)	Inference
AMC 30	15	Intermediate
AMP 25	21	Sensitive
COT 25	19	Sensitive
CTR 30	21	Intermediate
C 30	22	Sensitive

Table 16: Antibiotic susceptibility test of isolated *Salmonella typhi* (Raw Crab water from Uttara 11no sector kacha bazar)

Antibiotic disks	Zone of inhibition in diameter(mm)	Inference
AMC 30	9	Resistant
AMP 25	16	Intermediate
COT 25	14	Intermediate
CTR 30	20	Intermediate
C 30	18	Sensitive

Table 17: Antibiotic susceptibility test of isolated *Salmonella typhi* (Raw Prawn water Banani kacha bazar)

Antibiotic disks	Zone of inhibition in diameter(mm)	Inference
AMC 30	19	Sensitive
AMP 25	17	Sensitive
COT 25	17	Sensitive
CTR 30	24	Sensitive
C 30	20	Sensitive

Table 18: Antibiotic susceptibility test of isolated *Salmonella typhi* (Raw Mola fish water from Kochukhet bazar)

Antibiotic disks	Zone of inhibition in diameter(mm)	Inference
AMC 30	15	Intermediate
AMP 25	29	Sensitive
COT 25	20	Sensitive
CTR 30	15	Resistant
C 30	14	Intermediate

3.5 Plant extracts obtained from different solvents

The table below outlined the number of crude extracts obtained from three different medicinal plant samples using two different solvents: ethanol and distilled water.

Table 19: Number of plant extracts obtained using ethanol

Plant sample	Type of solvent	The volume of solvent(ml)	Amount of powder(gm)	Amount of crude extract(gm)
Neem	Ethanol	100	10	0.47
Tulsi	Ethanol	100	10	0.66
Pomegranate	Ethanol	100	10	0.59

Table 20: Number of plant extracts obtained using distilled water

Plant sample	Type of solvent	The volume of solvent(ml)	Amount of powder(gm)	Amount of crude extract(gm)
Neem	Distilled water	100	10	0.54
Tulsi	Distilled water	100	10	0.63
Pomegranate	Distilled water	100	10	1.10

3.6 Observation of antibacterial activity of ethanolic, and aqueous extracts of selected plants with allopathic antibiotics

In this research, *Salmonella typhi*was used for comparing the antimicrobial properties of ethanolic and aqueous extracts of the three medicinal plants: Neem, Tulsi, and Pomegranate. During the antimicrobial assay, a positive control in the form of the antibiotic disc was used on the petri plate as well as different extracts of each of the three medicinal plants to assess and compare the activity of the extracts with that of the antibiotic disc against the bacteria.

Table 21: Zone of inhibition produced by antibiotic, ethanol, and aqueous extract of neem leaf against *Salmonella typhi*

Name of bacteria	Name of antibiotic	Zone of inhibition(mm)		
		Antibiotic disk	Ethanolic extract	Aqueous extract
Salmonella typhi	Co-trimoxazole	16	*12	0
	Ampicillin	27		
	Chloramphenicol	25		

*The Highest zone of inhibition by the extract

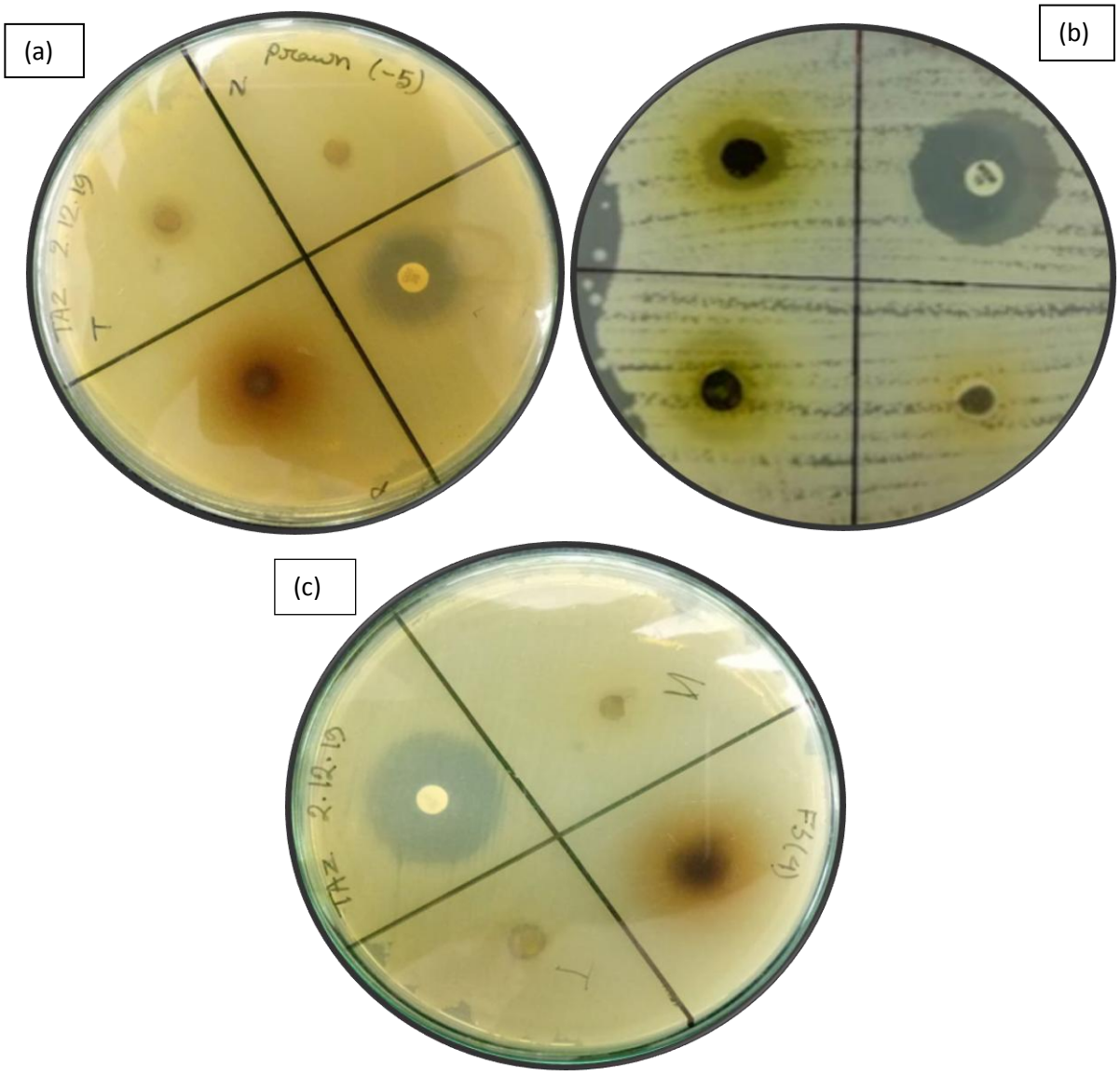
Table 22: Zone of inhibition produced by antibiotic, ethanol, and aqueous extract of Tulsi leaf against *Salmonella typhi*

Name of bacteria	Name of antibiotic	Zone of inhibition(mm)		
		Antibiotic disk	Ethanolic extract	Aqueous extract
Salmonella typhi	Co-trimoxazole	16	8	0
	Ampicillin	27		
	Chloramphenicol	25		

Table 23: Zone of inhibition produced by antibiotic, ethanol, and aqueous extract of pomegranate peel against *Salmonella typhi*

Name of bacteria	Name of antibiotic	Zone of inhibition(mm)		
		Antibiotic disk	Ethanolic extract	Aqueous extract
	Co-trimoxazole	16	5	0

Salmonella typhi	Ampicillin	27		
	Chloramphenicol	25		



FigP: Antibiotic susceptibility test result of the confirmed *S. typhi* isolates compared with the medicinal plant extracts (a) Antibiotic susceptibility of Neem, Tulsi, and Pomegranate compared tothe conventional antibiotic Co-trimoxazole (b) Antibiotic susceptibility of Neem compared to the conventional antibiotic Ampicillin. (c) Antibiotic susceptibility of Tulsi, Neem, and Pomegranate compared to the conventional antibiotic Chloramphenicol.

3.7 Comparative study of antibacterial activity by showing activity index

Activity index (AI) values are the estimated measure of the potency of antimicrobial activity of plant extracts by quantitatively comparing them to the respective standard antibiotics. In this study, the AI values are calculated for the ethanolic extract of three different medicinal plants against three different antibiotics, named: Co-trimoxazole, Ampicillin, and Chloramphenicol

Using the formula,

$$\text{Activity Index (AI)} = \frac{\textit{Zone of inhibition of extract}}{\textit{Zone of inhibition of antibiotic}}$$

AI values as shown in the following graphs have been calculated for the ethanolic extracts of all the three medicinal plants against the selected bacteria.

Activity index of plant extract to antibiotics against *Salmonella typhi*

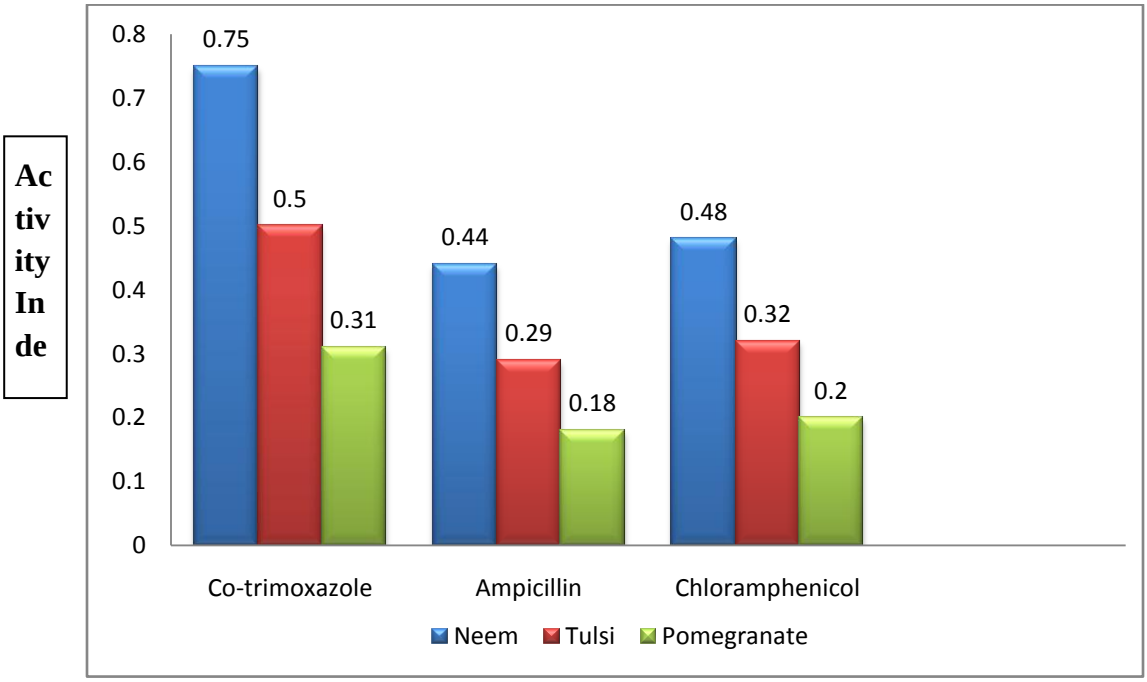


Fig Q: Activity Index of Neem,Tulsi and Pomegranate to antibiotics against *Salmonella typhi* .

3.8 Observation of antibacterial activity of Clove oil, Thyme oil, Rosemary oil and Orange oil with allopathic antibiotics

A similar quantity of four types of oil was taken and serially diluted in saline. Then the same amount of diluted suspension was taken and spread on MHAcontaining a similar amount of bacterial lawn. After incubation, fewer colonies emerged on the agar plate containing oils than

saline which determines the antimicrobial activity of these oils against the selected pathogen. Pathogens were incubated with oils for 24 hours. The number of colonies from each plate was essential to determine the inhibition rate.

3.9 Comparison among four types of oil and conventional Antibiotics

Comparing antimicrobial activity of four types of oil and conventional antibiotics was identified and the zone of inhibition was observed, and the diameter (mm) was calculated as follows:

Table 24: Zone of inhibition in response to four types of oil and conventional antibiotic disks

		Zone of inhibition(mm)				
Name of organism	Name of Antibiotics	Antibiotic disk	Thyme Oil 50µl (a)	Rosemary Oil 50µl (b)	Orange Oil 50µl (c)	Clove Oil 50µl (d)
Salmonella. typhi	Co-trimoxazole	16	8	6	5	7
	Ampicillin	21				
	Chloramphenicol	24				

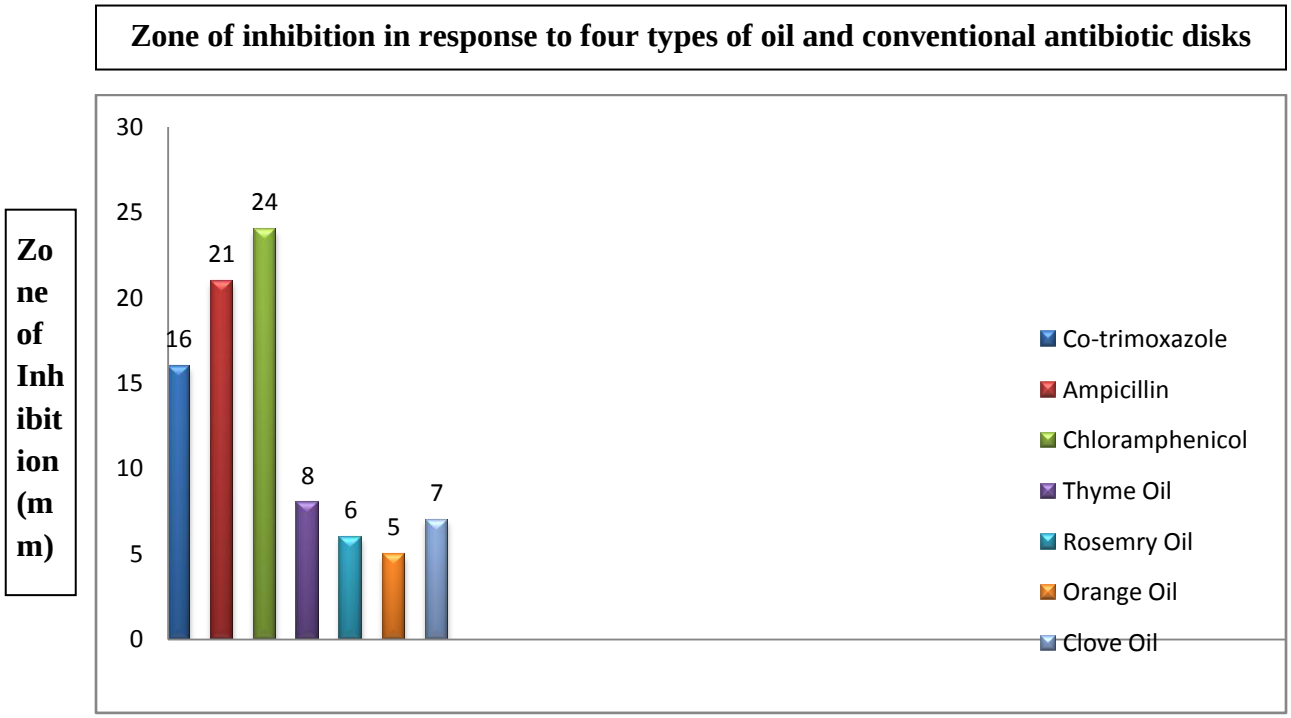
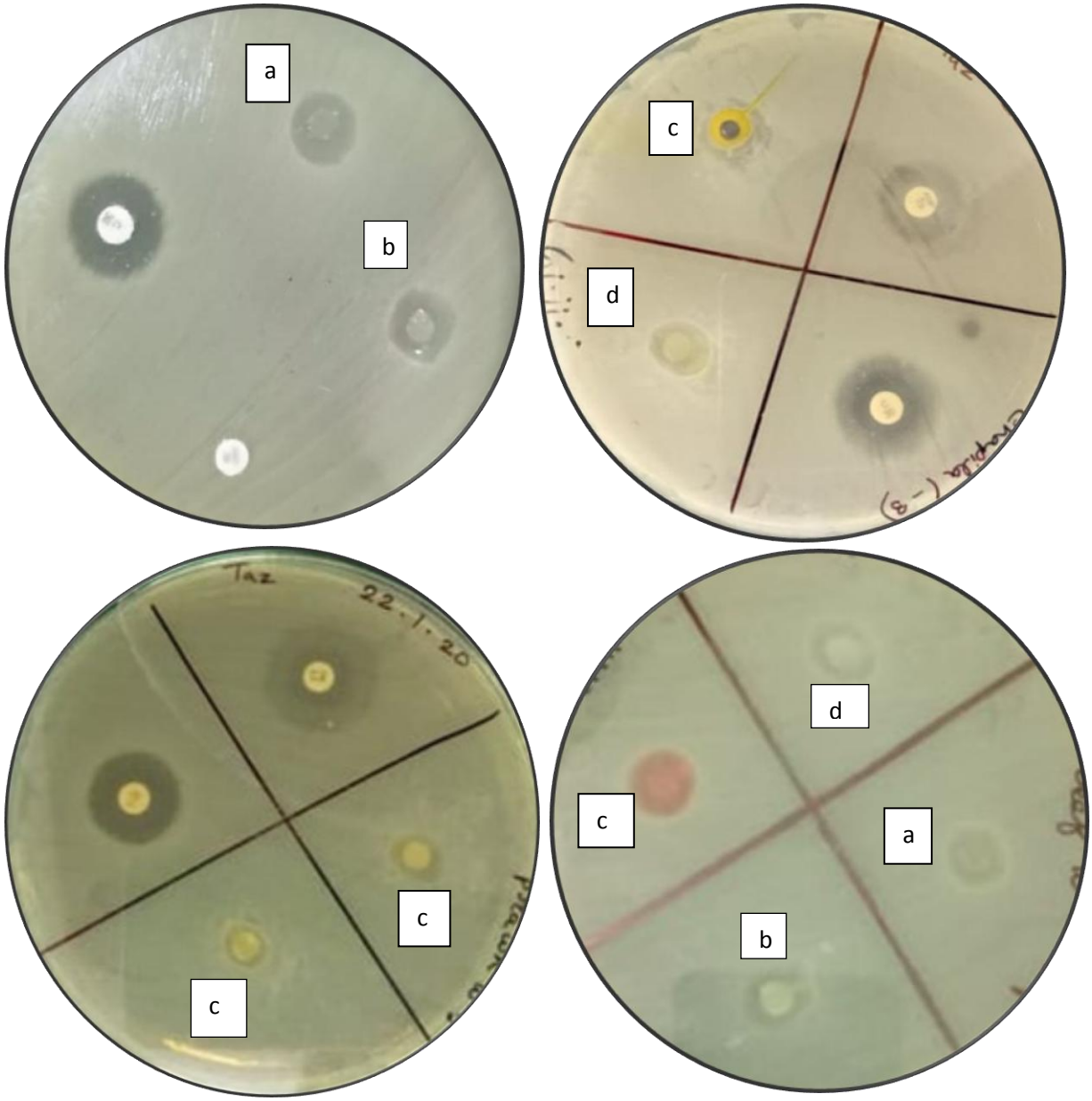


Fig R: Zone of inhibition in response to Four types of oil and conventional antibiotic disks. In which Thyme oil showed the best antimicrobial activity (8mm) against *S. typhi* compared to the other oils. Rosemary oil also showed antimicrobial activity of (6 mm) against *S. typhi*. Orange oil also showed antimicrobial activity of (5 mm) and Clove oil showed antimicrobial activity of (7 mm).


a
b
c
d
Taz
22-1-20
c
c
d
c
a
b

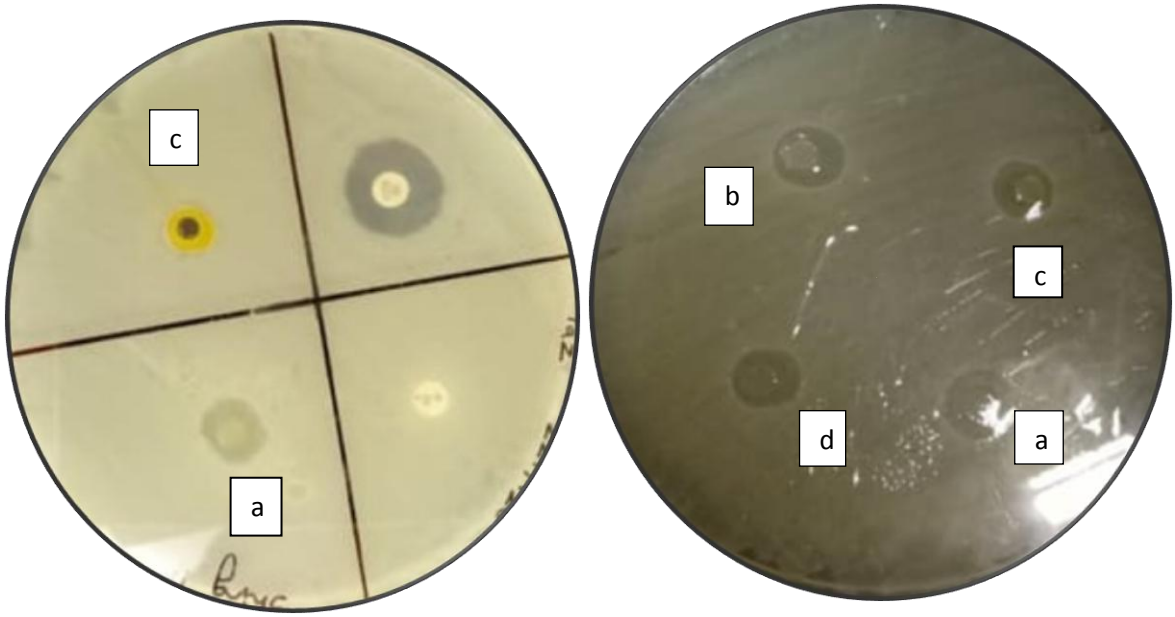


Fig S: Antibiotic susceptibility of (a)Thyme Oil, (b)Rosemary Oil, (c)Orange Oil, (d)Clove Oil compared to the conventional antibiotic Co-trimoxazole, Ampicillin and Chloramphenicol.

4. Discussion

The objective of the research was to prepare three different types of extracts from Neem, Tulsi,and pomegranate using two different solvents: ethanol and distilled water to determine the antimicrobial activity of the various extracts of the three medicinal plants against Salmonella typhi. Nowadays, researchers are progressively turning their focus in investigating herbal products to combat the increasing occurrences of microbial drug resistance.Many researchers have revealed the antioxidant activity of the Azadirachta indica. Antioxidant activity of the flavonoids (orientin and vicenin) in vivo was declared in a major depletion in the radiation-induced lipid peroxidation in mouse liver.The inhibition zone and their comparison by the ethanolic extracts of all the three medicinal plants showed that the highest zone of inhibition (12mm) (Table 21) was determined by Neem against *Salmonellatyphi*. On the other hand, there is no zone of inhibition shown by pomegranate(Livermore, D. M. 2000)..

Different bio-actives for example asteroids, sugars, triterpenoids, alkaloids, reducing sugars, tannins, flavonoids, sesquiterpene lactones are accountable for the in-built antimicrobial propertiesAlthough the ethanolic extracts of some medicinal plants have shown antimicrobial

activity,but the aqueous extract of these medicinal plants did not show any antimicrobial activity. This might be due to the appearance of some of the chemical elements in these plant extracts whose solubility may vary in different solvents. In this research, ethanolic extract of Neem sample had shown significantly better antibacterial activities compared to aqueous extract, where they considered that, it may be due to organic properties of ethanol and for its high ability to dissolve more organic and active antibacterial compositions. However, in the common households of Bangladeshi people, Neem and Tulsi are not generally eaten in raw form, rather they are usually eaten in a mixture with water. Normally it is not eaten in a mixture with ethanol or methanol. Neem leaves are typically not eaten but used externally on the body as a homemade herbal remedy for skin allergies by either boiling the leaves in water to make an aqueous extract or made into a semi-solid paste using water by mortar and pestle. To reduce toxins from the body, enhance body immunity and improve gut health the Neem leaf powder is used. Also, it can produce the bioconversion of nanoparticles from their equivalent at comparatively smaller amounts because of the native availability of the Neem plant. So, the in vitro antimicrobial effect shown in this study may consider that these plant extracts may not be as effective against any of these organisms in vivo. The concentrations of the extracts can be changed, or different extraction methods can be performed to produce different substantial results. So, by considering the overall outcomes of the antimicrobial effects of the three medicinal plant extracts of this study, it can be noted that further thorough experimental in-vitro study mustbe done which may lead to clinical trials, giving rise to production of herbal medicines using these medicinal plant extracts.

A comparison of the antibacterial activity of the medicinal plant extracts and allopathic antibiotics was done in this study by calculating the activity index values. The AI values are the approximate potency of antimicrobial activity of plant extracts by quantitatively comparing them to the respective standard antibiotics. (Sarmiento *et al*.2020) High AI values indicate that the extracts have excellent activity against the bacteria in comparison with the standard antibiotics. From this study, the activity index obtained from the ethanolic extract of Neem was the remarkable one. However, the AI values were very low which were acquired for the antibiotics that were highly effective against the test bacteria and this indicated that the antibiotics can be more effective than the natural plant extracts unless the microorganisms develop resistance to them. This difference in the efficacy of bacterial growth inhibition by the natural plant

extractsand the allopathic antibiotics can be there for the different mechanisms of interactions of these antimicrobial agents on the bacteria. The emergence of antibiotic-resistant organisms is rising firmly and undoubtedly becoming overall consideration for researchers. The requirement of replacement to antibiotics is increased to restrain many antibiotic-resistant strains. The latest antibiotics should discover, or current antibiotics need to be altered so that they may acquire a wide spectrum of activities. Application of antibacterial activity of organic products like different extracts of plants, essential oils, herbs might be a great replacement to restrain these microorganisms.

The objective of the research was to assess the antibacterial properties of some essential oils and to figure out which elements helps in this issue. Essential oils are volatile, complex blends delivered by fragrant plants as secondary metabolites. Most oils are made up of 20 to 60 compounds from an assortment of chemical classes, overwhelmingly terpenes, and their subordinates. Regularly the antimicrobial action of the oil is due to the complex intuition among these compounds, although the bioactivity of the oil would be closely related to the most component. As a rule, combinations of fundamental oils or their filtered major components targets multiple biochemical forms within the microscopic organisms driving to a synergistic, added substance, or in some cases indeed opposing impacts. The conceivable component of activity of this oil mix was due to intuition between the phenolic compounds from thyme oil and the alkyl bunches from the orange oil.Phenolic compounds are hydrophobic and antimicrobial. The major phenolic compounds of thyme oil are carvacrol and thymol. The hydrophobicity of the phenolics empowers them to join the lipid bilayer of the cytoplasmic film, driving to the spillage of particles and other basic particles, coming about in passing of the cell. The major component of orange oil is limonene , a sort of alkyl gather that's more dynamic than other shapes, such as p-cymene. Alkyl substitution of phenolic compounds renders an expanded antimicrobial action The expansive assortment of dynamic compounds too anticipates organisms from creating resistance. Clove and Rosemary oil have been utilized to alleviate stomach torment for centuries. It makes a difference to calm the nerves and help in assimilation in case you eat something that doesn’t concur with you. Put one or two drops of the oils in a refreshment to utilize it for stomach torment purposes Past reports of in vitro study appears that thyme, orange, rosemary, and clove oils were a bit successful against Salmonella. Hence, these 4 oils were

chosen for this study to consider the impact of different concentrations on hindrance of Salmonella.(Thilakarathne, R., Madushanka, G., & Navaratne, S. 2018).

In this study, the significance of antimicrobial and the safeness and contagiousness of the oils are reviewed. Essential oils are condensed fickle liquids removed from plants. These are extensively applied in detoxification, preparing foods, and in drug therapies specifically. Both Gram-negative and Gram-positive bacteria are to some extent can be killed by essential oils which have antibacterial activity

Conclusion

Overall, the organic solvent extracts of Neem, Tulsi, weresomewhat effective antibacterial agent but compared to allopathic antibiotics these are not that effective against *Salmonellatyphi*The potential value of these medicinal plant extracts is not on the rise when compared to that of allopathic antibiotics but as some bacteria have established resistance against allopathic antibiotics,are vulnerable to the natural plant extracts. It is expected that the outcomes of this research may inspire other researchers to design clinical trials and to come up with a less expensive antibacterial agent that may be effective for people from developing countries like Bangladesh. In addition, the potency of Thyme oil, Rosemary oil, orange oil and Clove oil to be applied as organic antimicrobic agent is recommended as antimicrobial activity against *Salmonella typhi*. The antibiotics would never be up for employment in the first place. But the medicinal plants and the essential oils used in this research are quite efficient against the selected bacteria.

Recommendation

This research can be further developed by adopting some measures such as:

- In Silico prediction such as molecular docking of the molecules of these potential medicinalplants found in this research work with *S. typhi* along with molecular dynamics and molecularmechanics can be applied for further in-depth analysis.
- Besides medicinal plants and essential oils, other crops (such as. jute) can be considered as apotential resource of antibiotics against *S. typhi*.

- Different antibiotics, which are ribosomal synthesized antimicrobial peptides can beinvestigated from different sources of the environment to determine potential antibiotics of *S.typhi*

7 References

A, O. P., Oluwadunsin, O., & Benjamin, O. (2015). Antibacterial Activity of Neem Against Isolated Bacteria 5(19), 131–138.

Abdallah, E. M. (2016). An Overview of the Effects of Antibiotics and Medicinal Plant Extractson the. Nova Journal of Medical and Biological Sciences, 1-6.

Abeysinghe, P.D., Wijesinghe, K.G.G., Tachida, H., & Yoshda, T. (2009). Molecular Characterization of Pomegranate Accessions and Evaluation of Genetic Relatedness of Cinnamon Species in Sri Lanka Based on TrnL Intron Region,Intergenic Spacers Between trnT-trnL, trnL-trnF, trnH -psbA and nuclear ITS 12. Research Journal of Agriculture and Biological Sciences, 5(6), 1079–1088.

Ali, A., Shahid, M. A., Hossain, M. D., & Islam, M. N. (2019). Antibacterial bi-layered polyvinyl alcohol (PVA)-chitosan blend nanofibrous mat loaded with Azadirachta indica (neem) extract. International journal of biological macromolecules, 138, 13-20.

Antuna-Puente, B., Feve, B., Fellahi, S., & Bastard, J.-P. (2007). Obésité, inflammation et insulinorésistance: quel rôle pour les adipokines? Therapie, 62(4), 285-292.

Arunkumar, A., Dhyani, A., & Joshi, G. (2019).Santalum album.The IUCN Red List of Threatened Species 2019: e. T31852A2807668.

Asgeirsson, H., Thalme, A., & Weiland, O. (2018). Staphylococcus aureus bacteraemia and endocarditis– epidemiology and outcome: a review. Infectious Diseases, 50(3), 175-192.

Ayliffe, G. (1997). The progressive intercontinental spread of methicillin-resistant Staphylococcus aureus.Clinical infectious diseases, 24(Supplement_1), S74-S79.

Adebowale, B. O., Gbenga, B. L., & Yewande, F. (2014).Morphology , Functional and Pasting Properties of Tulsi Starches Prepared by Four Different Drying Methods, 4(12), 1439–1450.

Al-daihan, S., Al-faham, M., Al-shawi, N., Almayman, R., Brnawi, A., & Bhat, R. (2013).Antibacterial activity and phytochemical screening of some medicinal plants

commonlyused in Saudi Arabia against selected pathogenic microorganisms. Journal of King Saud University - Science, 25(2), 115–120.

Al-sum, B. A., & Al-arfaj, A. A. (2013).Antimicrobial activity of the aqueous extract of mintplant, 2(3), 110–113.

Arutselvi, R., Balasaravanan, T., Ponmurugan, P., Saranji, N. M., & Suresh, P. (2012).Phytochemical screening and comparative study of anti microbial activity of leaves andrhizomes of turmeric varieties, 2(2), 212–219.

Bhargava, S., Dhabhai, K., Batra, A., Sharma, A., & Malhotra, B. (2012). Journal of Chemical and Pharmaceutical Research , 2012 , 4 (1): 360-364 Research Article Zingiber Officinale :Chemical and phytochemical screening and evaluation of its antimicrobial activities, 4(1),360–364.

Bouhdid, S., Abrini, J., Zhiri, A., Espuny, M., & Manresa, A. (2009). Investigation of functional and morphological changes in Pseudomonas aeruginosa and Staphylococcus aureus cells induced by Origanum compactum essential oil. Journal of applied microbiology, 106(5), 1558-1568.

Braga, L., Shupp, J., Cummings, C., Jett, M., Takahashi, J., Carmo, L., . . . Nascimento, A. (2005). Pomegranate extract inhibits Staphylococcus aureus growth and subsequent enterotoxin production. Journal of ethnopharmacology, 96(1-2), 335-339.

Burits, M., & Bucar, F. (2000).Antioxidant activity of Orange essential oil.Phytotherapy research, 14(5), 323-328.

Calderon, R. L., Mood, E. W., & Dufour, A. P. (1991). Health effects of swimmers and nonpoint sources of contaminated water. International journal of environmental health research, 1(1), 21-31.

Ds, B. (2014). Studies on Antimicrobial Potential and Phytochemical Analysis of Mint Leaves Extracts, 4(July).Salmonella typhi(2015). Ministry for Primary Industries, (January).

E. Scallan, P.M. Angulo, F.J. Tauxe, R.V. Hoekstra, M. Robert Foodborne illness acquired in the United States–Unspecified agents Emerg. Infect. Dis., 17 (2011), pp. 16-2221192849

Galeane, M. C., Martins, C. H. G., Massuco, J., Bauab, T. M., & Sacramento, L. V. S. (2017). Phytochemical screening ofSalmonella typhi A .Juss for antimicrobial activity, 11(4), 117–122.

H.J. Dorman, S. Deans Antimicrobial agents from plants: Antibacterial activity of plant volatile oilsJ. Appl. Microbiol., 88 (2000), pp. 308-31610736000

Hasan, N. A., Nawahwi, M. Z., & Malek, H. A. (2013). Antimicrobial activity of Tulsi,Mint extract. Sains Malaysiana, 42(2), 143–147.

Hassan, S. A., Barthwal, R., Nair, M. S., & Haque, S. S. (2012). Aqueous Extract of Pomegranate : A Potential Therapeutic Agent for Streptozotocin- Induced Type 1 Diabetes Mellitus (T1DM) Rats, 11(June), 429–435.

Holetz, F. B., Pessini, G. L., Sanches, N. R., Cortez, D. A. G., Nakamura, C. V., & Dias Filho, B. P. (2002). Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Memórias do Instituto Oswaldo Cruz, 97(7), 1027-1031.

Hosseini, B., Saedisomeolia, A., Wood, L. G., Yaseri, M., & Tavasoli, S. (2016). Effects of pomegranate extract supplementation on inflammation in overweight and obese individuals: A randomized controlled clinical trial. Complementary therapies in clinical practice, 22, 44-50.

Hu, W., Li, C., Dai, J., Cui, H., & Lin, L. (2019). Antibacterial activity and mechanism of Litsea cubeba essential oil against methicillin-resistant Staphylococcus aureus (MRSA). Industrial Crops and Products, 130, 34-41.

Hussain, A. I., Anwar, F., Sherazi, S. T. H., & Przybylski, R. (2008). Chemical composition, antioxidant and antimicrobial activities of basil (Ocimum basilicum) essential oils depends on seasonal variations. Food chemistry, 108(3), 986-995.

Ishtiaq, S., Ashraf, M., Hayat, M. Q., & Asrar, M. (2013). Phytochemical Analysis of Mint and its Antibacterial Activity against Clinical Isolates Identified by Ribotyping, 7,1151–1156.

Islam, K., Rowsni, A. A., Khan, M., & Kabir, S. (2014).ANTIMICROBIAL ACTIVITY OF Pomegranate EXTRACTS AGAINST FOOD-BORNE PATHOGENIC BACTERIA, 3(3), 867–871.

Jain, S., Angadi, V., & Shankaranarayana, K. (2003). Edaphic, environmental and genetic factors associated with growth and adaptability of sandal (Santalum album L.) in provenances. Sandalwood research newsletter, 17, 6-7.

Jain, S., Mohan, R., & Rai, R. (2015). Ocimum sanctum as an herbal medicine: A Review. International Journal of Maxillofacial Research, 1(1), 1-12.

Jenks, P., Laurent, M., McQuarry, S., & Watkins, R. (2014). Clinical and economic burden of surgical site infection (SSI) and predicted financial consequences of elimination of SSI from an English hospital. Journal of Hospital Infection, 86(1), 24-33.

Joshi, R. K., Setzer, W. N., & Da Silva, J. K. (2017). Phytoconstituents, traditional medicinal uses and bioactivities of Tulsi (Ocimum sanctum Linn.): a review. American Journal of Essential Oils and Natural Products, 5(1), 18-21

Kaasch, A. J., Barlow, G., Edgeworth, J. D., Fowler Jr, V. G., Hellmich, M., Hopkins, S., . . . RodriguezBaño, J. (2014). Staphylococcus aureus bloodstream infection: a pooled analysis of five prospective, observational studies. Journal of Infection, 68(3), 242-251.

Kadariya, J., Smith, T. C., & Thapaliya, D. (2014). Staphylococcus aureus and staphylococcal food-borne disease: an ongoing challenge in public health. BioMed research international, 2014.

Kahlmeter, G., Brown, D., Goldstein, F., MacGowan, A., Mouton, J., Odenholt, I., . . . Soriano, F. (2006). European Committee on Antimicrobial Susceptibility Testing (EUCAST) technical notes on antimicrobial susceptibility testing

Kulkarni, K. V., & Adavirao, B. V. (2018). A review on: Indian traditional shrub Tulsi (Ocimum sanctum): the unique medicinal plant. Journal of Medicinal Plants Studies, 6(2), 106-110.

K.A. Hammer, C. Carson, T. Riley Antimicrobial activity of essential oils and other plant extractsJ. Appl. Microbiol., 86 (1999), pp. 985-99010438227

Kunwar, D.; Bhatta, S.; Chaudhary, R.; Rijal, K.R. Antibiotic susceptibility pattern of nalidixic acid resistant Salmonella isolates in shree Birendra hospital chhauni. TUJM 2017, 4, 11–14. [CrossRef]

Karkey, A.; Arjyal, A.; Anders, K.L.; Boni, M.F.; Dongol, S.; Koirala, S.; My, P.V.; Nga, T.V.; Clements, A.C.; Holt, K.E.; et al. The burden and characteristics of enteric fever at a healthcare facility in a densely populated area of Kathmandu.PLoS ONE 2010, 5, e13988. [CrossRef] Environment and Public Health Organization Typhoid: The Neglected Urgent in Nepal. 2019. Available online: http://enpho. org/featured/typhoid-the-neglected-urgent-in-nepal/ (accessed on 27 August 2020).

Laupland, K. B., Church, D. L., Mucenski, M., Sutherland, L. R., & Davies, H. D. (2003). Populationbased study of the epidemiology of and the risk factors for invasive Staphylococcus aureus infections. The Journal of infectious diseases, 187(9), 1452-1459.

Laupland, K. B., Lyytikäinen, O., Sgaard, M., Kennedy, K., Knudsen, J. D., Ostergaard, C., . . . Collignon, P. (2013). The changing epidemiology of Staphylococcus aureus bloodstream infection: a multinational population-based surveillance study. Clinical microbiology and infection, 19(5), 465-471.

Lee, B. Y., Singh, A., David, M. Z., Bartsch, S. M., Slayton, R. B., Huang, S. S., . . . Lauderdale, D. S. (2013). The economic burden of community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA). Clinical microbiology and infection, 19(6), 528-536.

Lee, K. A., Moon, S.-H., Lee, J.-Y., Kim, K.-T., Park, Y.-S., & Paik, H.-D. (2013). Antibacterial activity of a novel flavonoid, 7-O-butyl naringenin, against methicillin-resistant Staphylococcus aureus (MRSA). Food Science and Biotechnology, 22(6), 1725-1728. 38

Liu, Y., Xu, Z., Yang, Z., Sun, J., & Ma, L. (2016). Characterization of community-associated Staphylococcus aureus from skin and soft-tissue infections: a multicenter study in China. Emerging microbes & infections, 5(1), 1-11.

Livermore, D. M. (2000). Antibiotic resistance in staphylococci. International journal of antimicrobial agents, 16, 3-10.

Lowy, F. D. (2003). Antimicrobial resistance: the example of Salmonella typhi The Journal of clinical investigation, 111(9), 1265-1273.

M. Friedman, P.R. Henika, R.E. Mandrell Bactericidal activities of plant essential oils and some of their isolated constituents against Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, and Salmonella typhi J. Food Prot., 65 (2002), pp. 1545-1560 12380738

M. Oussalah, S. Caillet, L. Saucier, M. Lacroix Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: E. coli O157: H7, Salmonella Typhimurium, Staphylococcus aureus, and Listeria monocytogenes Food Contr., 18 (2007), pp. 414-420

Machado, T., Pinto, A., Pinto, M., Leal, I., Silva, M., Amaral, A., . . . Netto-dosSantos, K. (2003). In vitro activity of Brazilian medicinal plants, naturally occurring naphthoquinones and their analogues, against methicillin-resistant Staphylococcus aureus. International journal of antimicrobial agents, 21(3), 279-284.

Masotti, V., Juteau, F., Bessière, J. M., & Viano, J. (2003). Seasonal and phenological variations of the essential oil from the narrow endemic species Artemisia molinieri and its biological activities. Journal of agricultural and food chemistry, 51(24), 7115-7121.

Miro, J. M., Anguera, I., Cabell, C. H., Chen, A. Y., Stafford, J. A., Corey, G. R., . . . Abrutyn, E. (2005). Staphylococcus aureus native valve infective endocarditis: report of 566 episodes from the International Collaboration on Endocarditis Merged Database. Clinical infectious diseases, 41(4), 507-514.

Misra, B. B., & Dey, S. (2012). Comparative phytochemical analysis and antibacterial efficacy of in vitro and in vivo extracts from E ast I ndian sandalwood tree (S antalum album L.). Letters in applied microbiology, 55(6), 476-486.

Mondal, S., Mirdha, B. R., & Mahapatra, S. C. (2009). The science behind sacredness of Tulsi (Ocimum sanctum Linn.). Indian J Physiol Pharmacol, 53(4), 291-306.

Murdoch, D. R., Corey, G. R., Hoen, B., Miró, J. M., Fowler, V. G., Bayer, A. S., . . . Moreillon, P. (2009). Clinical presentation, etiology, and outcome of infective endocarditis in the

21st century: the International Collaboration on Endocarditis–Prospective Cohort Study. Archives of internal medicine, 169(5), 463-473.

Nordmann, P.; Poirel, L. Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. J. Antimicrob. Chemother. 2005, 56, 463–469. [CrossRef] [PubMed]

Ogston, A. (1882). Micrococcus poisoning.Journal of anatomy and physiology, 16(Pt 4), 526.

Ogston, A. (1984). On abscesses. Reviews of Infectious Diseases, 6(1), 122-128. Organization, W. H. (2003). Guidelines for safe recreational water environments: Coastal and fresh waters (Vol. 1): World Health Organization.

Pandey, G., & Madhuri, S. (2010). Pharmacological activities of Ocimum sanctum (tulsi): a review. Int J Pharm Sci Rev Res, 5(1), 61-66.

Papadakis, J., Mavridou, A., Richardson, S., Lampiri, M., & Marcelou, U. (1997). Bather-related microbial and yeast populations in sand and seawater. Water Research, 31(4), 799-804.

Papadopoulos, P., Papadopoulos, T., Angelidis, A. S., Boukouvala, E., Zdragas, A., Papa, A., . . . Sergelidis, D. (2018). Prevalence of Staphylococcus aureus and of methicillin-resistant S. aureus (MRSA) along the production chain of dairy products in north-western Greece.Food microbiology, 69, 43-50.

Parthipan, P., Narenkumar, J., Elumalai, P., Preethi, P. S., Nanthini, A. U. R., Agrawal, A., & Rajasekar, A. (2017). Neem extract as a green inhibitor for microbiologically influenced corrosion of carbon steel API 5LX in a hypersaline environments. Journal of Molecular Liquids, 240, 121-127. Prajapati, N. D.,

Purohit, S., Sharma, A. K., & Kumar, T. (2003).Handbook of medicinal plants.A complete source book. Agrobios: India), Shyam Printing Press, Jodhpur.

Prashanth, D., Asha, M., & Amit, A. (2001).Antibacterial activity of Punica granatum. Fitoterapia, 72(2), 171-173. Purohit, P. (2018). Reviving the royal tree Santalum album Linn.: Santalaceae. J Med Plants Stu, 6(2), 273-276.

Raut, J. S., & Karuppayil, S. M. (2014).A status review on the medicinal properties of essential oils. Industrial Crops and Products, 62, 250-264. 39

Rybak, M., Lomaestro, B., Rotschafer, J. C., Moellering, R., Craig, W., Billeter, M., . . . Levine, D. P. (2009). Therapeutic monitoring of vancomycin in adult patients: a consensus review of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists. American Journal of Health-System Pharmacy, 66(1), 82-98.

Rybak, M. J., & LaPlante, K. L. (2005). Community- associated methicillin- resistant Staphylococcus aureus: a review. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 25(1), 74-85.

Salman, M. T., Khan, R. A., & Shukla, I. (2016).Antibacterial activity of Nigella Sativa Linn.seeds against multiple antibiotics resistant clinical strains of Staphylococcus aureus. Int. Arch. BioMed Clin. Res, 2, 96-99.

Sarmiento, W. C., Maramba, C. C., & Gonzales, M. L. M. (2011).An in-vitro study on the antibacterial effect of neem (Azadirachta indica) leaf extract on methicillin-sensitive and Methicillin-resistant Staphylococcus aureus. PIDSP J, 12(1), 40-45.

Šavikin, K., Živković, J., Alimpić, A., Zdunić, G., Janković, T., Duletić-Laušević, S., & Menković, N. (2018). Activity guided fractionation of pomegranate extract and its antioxidant, antidiabetic and antineurodegenerative properties. Industrial Crops and Products, 113, 142-149.

Selton-Suty, C., Célard, M., Le Moing, V., Doco-Lecompte, T., Chirouze, C., Iung, B., . . . Bouvet, A. (2012). Preeminence of Staphylococcus aureus in infective endocarditis: a 1-year populationbased survey. Clinical infectious diseases, 54(9), 1230-1239.

Seyfried, P. L., Tobin, R. S., Brown, N. E., & Ness, P. F. (1985).A prospective study of swimmingrelated illness. II. Morbidity and the microbiological quality of water. American journal of public health, 75(9), 1071-1075.

Shokeen, P., Bala, M., Singh, M., & Tandon, V. (2008).In vitro activity of eugenol, an active component from Ocimum sanctum, against multiresistant and susceptible strains of Neisseria gonorrhoeae.International journal of antimicrobial agents, 32(2), 174-179.

Sizemore, R. K., Caldwell, J. J., & Kendrick, A. S. (1990).Alternate gram staining technique using a fluorescent lectin.Applied and environmental microbiology, 56(7), 2245-2247. Sohail, M. F., Rehman, M., Hussain, S. Z., Huma, Z.-e., Shahnaz, G., Qureshi, O. S., . . . Webster, T. J. (2020). Green synthesis of zinc oxide nanoparticles by Neem extract as multi-facet therapeutic agents. Journal of Drug Delivery Science and Technology, 59, 101911.

Subasinghe, S. (2013). Sandalwood research: a global perspective. Journal of Tropical Forestry and Environment, 3(1), 1-8. Teixeira, B., Marques, A., Ramos, C., Neng, N. R., Nogueira, J. M.,

Saraiva, J. A., & Nunes, M. L. (2013).Chemical composition and antibacterial and antioxidant properties of commercial essential oils. Industrial Crops and Products, 43, 587-595.

Tesso, H., Nisha, A., & Kumsa, K. (2015).Antibacterial activity and phytochemical screening of some important medicinal plants against human diarrheal pathogens in Adama city, Ethiopia. Int J Microbiol Immunol Res, 3(3), 029-035.

Thilakarathne, R., Madushanka, G., & Navaratne, S. (2018). Determination of composition of fatty acid profile of Ethiopian and Indian black cumin oil (Nigella sativa).

Tleyjeh, I. M., Abdel-Latif, A., Rahbi, H., Scott, C. G., Bailey, K. R., Steckelberg, J. M., . . . Baddour, L. M. (2007). A systematic review of population-based studies of infective endocarditis. Chest, 132(3), 1025-1035.

Tripathi, D. K., Singh, V. P., Chauhan, D. K., Prasad, S. M., & Dubey, N. K. (2014). Role of macronutrients in plant growth and acclimation: recent advances and future prospective. Improvement of crops in the era of climatic changes, 197-216.

Tsuchiya, H., Sato, M., Miyazaki, T., Fujiwara, S., Tanigaki, S., Ohyama, M., . . . Iinuma, M. (1996). Comparative study on the antibacterial activity of phytochemical flavanones

against methicillinresistant Staphylococcus aureus.Journal of ethnopharmacology, 50(1), 27-34. 40

Vijayaram, S., Kannan, S., Saravanan, K. M., Vasantharaj, S., Sathiyavimal, S., & Palanisamy, S. P. (2016). Preliminary phytochemical screening, Antibacterial potential and GCMS analysis of two medicinal plant extracts. Pakistan journal of pharmaceutical sciences, 29(3).

Vogel, M., Schmitz, R. P., Hagel, S., Pletz, M. W., Gagelmann, N., Scherag, A., . . . Brunkhorst, F. M. (2016). Infectious disease consultation for Staphylococcus aureus bacteremia–a systematic review and meta-analysis. Journal of Infection, 72(1), 19-28.

Xiao, S., Cui, P., Shi, W., & Zhang, Y. (2020).Identification of essential oils with activity against stationary phase Staphylococcus aureus.BMC complementary medicine and therapies, 20(1), 1- 10.

Xiao, Y.-H., Giske, C. G., Wei, Z.-Q., Shen, P., Heddini, A., & Li, L.-J.(2011). Epidemiology and characteristics of antimicrobial resistance in China. Drug resistance updates, 14(4-5), 236-250.

Yoon, J.-H., & Baek, S. J. (2005). Molecular targets of dietary polyphenols with anti-inflammatory properties. Yonsei medical journal, 46(5), 585.

Young, N. (2016). The association between marine bathing and infectious diseases-a review.Marine Biological Association of the United Kingdom.Journal of the Marine Biological Association of the United Kingdom, 96(1), 93.

Zarb, P., Coignard, B., Griskeviciene, J., Muller, A., Vankerckhoven, V., Weist, K., . . . Catry, B. (2012). The European Centre for Disease Prevention and Control (ECDC) pilot point prevalence survey of healthcare-associated infections and antimicrobial use. Eurosurveillance, 17(46), 20316.

Zhang, L., Gao, Y., Zhang, Y., Liu, J., & Yu, J. (2010). Changes in bioactive compounds and antioxidant activities in pomegranate leaves. Scientia Horticulturae, 123(4), 543-546.

APPENDIX-I

Media composition

The composition of the media used in the present study has been given below. Unless otherwise mentioned, all the media were autoclaved at 121OC for 15 min.

I. Nutrient Agar (Himedia, India)

Ingredients	Amount (g/L)
Peptic digest of animal tissue	5.0
Beef extract	1.50
Sodium chloride	5.0
Yeast extract	1.50
Agar	1.50

II. Nutrient Broth (Oxoid, England)

Ingredients	Amount (g/L)
Lab-lemcopowder	1.0
Yeast extract	2.0
Peptone	5.0
Sodiumchloride	5.0

III. T1N1 soft agar

Ingredients	Amounts(g/L)

Tryptone	0.6
Sodiumchloride	0.3
Agar	0.42

IV. Simmon's citrate agar (Oxoid, England)

Ingredients	**Amount (g/L)**
Magnesiumsulfate	0.2
Ammonium dihydrogenphosphate	0.2
Ammoniumphosphate	0.8
Sodiumcitrate	2.0
Sodiumchloride	5.0
Agar	15.0
Bactobromthymolblue	0.08

V. MR-VP broth

Ingredients	**Amount(g/L)**
Peptone	7
Dextrose	5
Potassiumphosphate	5

VI. Triple sugar iron agar (Himedia, India)

Ingredients	**Amount(g/L)**

Peptic digest of animal tissue	10.0
Sodium chloride	5.0
Lactose	10.0
Sucrose	10.0
Ferrous sulfate	0.20
Sodium thiosulfate	0.30
Casein enzymatic hydrolysate	10.0

VII. Mannitol Salt agar (Oxoid, England)

Ingredients	**Amount(g/L)**
Peptone	10.0
Mannitol	10.0
Lab-lemco powder	1.0
Sodium chloride	75.0
Phenol red	0.025
Agar	15.0

APPENDIX-II

Instruments

Autoclave	Wisd Laboratory Instruments Made in Korea
Water Bath WiseBat	Wisd Laboratory Instruments DAIHAN Scientific Co., Ltd Made in Korea
Shaking Incubator	Model: JSSI-1000C JS RESEARCH INC. Made in Rep. of Korea
Incubator	Model: DSI 3000 Digisystem Laboratory Instruments Inc. Made in Taiwan
Vortex Mixer	Model: VM-2000 Digisystem Laboratory Instruments Inc. Made in Taiwan
Electronic Balance	RADWAG WagiELEktroniczn e Model: WTB 200
Refrigerator (40ºC)	Model: 0636 Samsung
Laminar flow chamber	SAARC Engineering
Rotary evaporator	Heidolph Made in Germany
Fume hood chamber	-