ANTIBACTERIAL ACTIVITY AND MEDICINAL PROPERTIES OF GINGER (*Zingiber officinale*) IN BANGLADESH

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SUBMITTED BY

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DECLARATION

I hereby declare that the thesis project titled "**Antibacterial activity and medicinal properties of ginger (*Zingiber officinale*) in Bangladesh"** submitted by me has been carried out under the supervision of **Fahareen Binta Mosharraf**, Senior Lecturer, Microbiology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka. It is further declared that the research work presented here is based on actual and original work carried out by me. Any reference to work done by any other person or institution or any material obtained from other sources have been duly cited and referenced.

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Abstract

The rhizomes of *Zingiber officinale* Roscoe (garden ginger) have been used since primeval times as a traditional remedy for gastrointestinal complaints. The health-promoting perspective of ginger is accredited to its rich phytochemistry. The most active ingredients in ginger are the pungent principles, particularly gingerols and shogaols. The emergence of new pathogenic bacteria resistant to most, if not all, currently existing antimicrobial agents has become a fundamental problem in modern medicine, particularly because of the affiliated increase in immunosuppressed inhabitants worldwide. This observation particularly calls for the *in vitro* examination of crude ginger extract collected from local and imported varieties of ginger available in Bangladesh and provide an update analysis of ginger use against *Salmonella typhi, Bacillus cereus, Vibrio cholerae* and *Staphylococcus aureus* with a focus on the types and presentations of ginger available. We also examine the pharmacokinetic properties of ginger and highlight the type and posology of ginger and its metabolites. However, local variety of *Zingiber officinale* Roscoe has more effective antimicrobial property compared to hybrid varieties.

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Chapter one: Introduction

The natural assets, for example, plants, creatures and microorganisms display puzzling properties wherein their entire parts or subordinates can be utilized for social, financial, ecological, customary medications, logical and chemotherapeutic purposes (Wangchuk *et al*, 2015). The connection amongst plants and people is contemplated in ethnobotany, a field concentrating on the investigation of the indigenous information on how plants are seen, utilized and overseen (Chekole *et al*, 2015). Traditionally, medicinal plants are used to maintain people's health, as well as to prevent, diagnose, improve or treat physical and mental illnesses all over the world. Medicinal plants are accepted to be with healing powers, and individuals have utilized them for a long time. Expected to current medication revelation, customary therapeutic plants have been contemplated and created which is taken after the ethnobotanical lead of indigenous cures utilized by conventional medical system. Traditional medicinal knowledge, particularly utilizing therapeutic plants in the developing countries, has been in existence and use, and has been a part of therapeutic practices. Hence, the investigation of plants and their uses (particularly medical purposes) is a standout amongst the most essential human concerns and has been practiced in the world (Hong *et al* 2015).

Chapter two:
Literature review

2.1 Plant under study: Ginger

General characteristics of ginger plant:

Ginger is a member of the family Zingiberaceae; a small family with more than 45 genera and 800 species. Its scientific name is *Zingiber officinale* (Karuppiah *et al*, 2012). It is a perennial herb, slender perennial plant that reaches the height of two feet and has greenish yellow flowers resembling orchids. The rhizome of ginger plant is horizontal, branched, fleshy, aromatic, white or yellowish to brown. Leaves are narrowly or linear-lanceolate, up to 20 cm long and 1.5-2 cm wide. The dried rhizome of ginger contains approximately 1-4% of volatile oils which are the medicinally active constituents and are also responsible for the characteristic odour and taste. Flowers are produced in a dense spike, yellow green with purple endings. This plant is most likely to be found in South-Eastern Asia (Shareef *et al,* 2016). Fully grown *Zingiber officinale* roots are fibrous and approximately arid. The syrup from ancient ginger rhizomes is exceptionally strong and mostly utilized like spices in Indian recipe, and is as regular ingredient of cooking in many Asian countries, also it makes the taste of many food dishes an extremely delicious. *Zingiber officinale* also plays role as powerful food maintenance (Abdulzahra *et al,* 2014).

2.1.1 Scientific classification of ginger:

Zingiberofficinale was described on the basis of its taxonomic classification:

Kingdom	Plantae – plantes, Planta, Vegetal, plants
Subkingdom	Viridiplantae
Infrakingdom	Streptophyta – land plants
Superdivision	Embryophyta

Division	Tracheophyta – vascular plants
Subdivision	Spermatophytina – spermatophytes
Class	Magnoliopsida
Superorder	Lilianae – monocots, monocotyledons
Order	Zingiberales
Family	Zingiberaceae – Ginger Family
Genus	*Zingibe*r Mill. – ginger
Species	*Zingiber officinale* Roscoe – garden ginger

Table 2.1: Scientific classification of ginger

Figure 2.1: *Zingiber officinale*

2.1.2 Nutrient component:

Fresh ginger contains 80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals, 2.4% fibre and 12.3% carbohydrates. The minerals present in ginger are iron, calcium and phosphorous. It also contains vitamins such as thiamine, riboflavin, niacin and vitamin C. The composition varies with the type, variety, agronomic conditions, curing methods, drying and storage conditions (Gugnani *et al*, 1985).

2.1.3 Phytochemistry:

Ginger is a rich source of volatile oil. Zingiberol, zingiberene, phellandrene and linalool are important constituents of the oil. They account for the aroma of the drug. The pungency of the ginger is due to gingerols and shogaols. Investigations have shown gingerol and shogaol to be mutagenic. Furthermore, ginger contains special group of compounds called diarylheptanoids including gingerenone. The standardization of the medication is based on the presence of pungent principles of the plant (Nagabhushan *et al*, 1987).

2.2 Medicinal importance of ginger:

Cultivated for its edible underground stem (rhizome), ginger has been used since antiquity both as a spice and as a herbal medicine to treat a variety of primarily gastrointestinal ailments, such as nausea, vomiting (emesis), diarrhea and also diverse ailments, including arthritis, muscular aches, and fever (Lete *et al*, 2016). Ginger is truly a world domestic remedy. It is also used in south asia and other places like the ancient China where the fresh and dried roots were considered distinct medicinal products. Fresh ginger has been used for cold induced diseases, asthma, cough, colic, heart palpitation, swelling symptom, loss of appetence and rheumatism. In nineteenth century, ginger served as a well-liked remedy for cough and respiratory illness once the juice of recent ginger was mixed with a bit juice of recent garlic and honey. A paste of fine dried ginger was applied to the temples to alleviate headache and recent ginger was mixed with very little honey, abroach off with a pinch of burnt peacock feathers to alley nausea (Karuppiah *et al*, 2012).

2.3 Some gram positive and gram negative bacteria selected for study:

2.3.1 *Salmonella typhi*:

Salmonella is a genus of rod-shaped Gram- negative non-lactose fermenting Enterobacteriaceae that cause typhoid fever, paratyphoid fever and food borne illness (Ryan and Ray, 2004). *Salmonella typhi* is a strain of bacteria that lives only in humans. It is a motile, facultative anaerobe that is susceptible to various antibiotics. Currently, 107 strains of this organism have been isolated; each of the strains show different types of metabolic characteristics, levels of virulence, and multi-drug resistance genes that complicate treatment in areas that resistance is prevalent. Diagnostic identification can be attained by growth on MacConkey and EMB agars, and the bacteria is strictly non-lactose fermenting. It also produces no gas when grown in TSI media, which is used to differentiate it from other Enterobacteriaceae.

2.3.2 *Bacillus cereus:*

Bacillus cereus is a gram-positive, spore forming, motile, aerobic rod that additionally grows well anaerobically usually found in soil. *B. cereus* causes two different kinds of gastrointestinal disorder: the diarrheal form of food poisoning is caused by complex enterotoxins, created throughout vegetative growth of *B. cereus* within the gut, whereas the emetic toxin is made by growing cells within the food (Granum *et al*, 1997). The spores often contaminate a range of foods, as well as manufacture, meat, eggs, and farm product (Tallent *et al*, 2012). For each forms of gastrointestinal disorder the food concerned has typically been heat treated and living spores are the source of the food poisoning. *B. cereus* isn't a competitive organism however grows well after cooking and cooling (<48°c). The heat treatment can cause spore germination and within the absence of competing flora (Granum *et al*, 1997). Foodborne diseases related to toxins made

by *B. cereus* may result in self-limiting diarrhoea or expulsion. Plate enumeration methods suggested by recognized food authorities to observe the presence of *B. cereus* in potentially contaminated food product do not inhibit alternative gram-positive competitive bacterium (Tallent *et al*, 2012).

2.3.3 *Staphylococcus aureus:*

Staphylococcus aureus is gram-positive microorganism that is cocci-shaped and has a tendency to be organized in clusters that are described as "grape-like." On media, these organisms will grow in up to 100% salt, and colonies are usually golden or yellow (aureus means that golden or yellow). These organisms will grow aerobically or anaerobically (facultative) and at temperatures between 18°C and 40°C. Typical biochemical identification tests embrace catalase positive (all pathogenic *Staphylococcus species*), coagulase positive (to distinguish *Staphylococcus aureus* from alternative *Staphylococcus species*), novobiocin sensitive (to distinguish from *Staphylococcus saprophyticus*), and mannitol fermentation positive (to distinguish from *Staphylococcus epidermidis*) (Taylor and Unacal, 2017). *S. aureus* might colonize the human body as a part of the normal flora. around 30% of healthy folks are inhabited by *S. aureus*, largely within the anterior nares (Akmatov *et al*, 2014). *S. aureus* is additionally a leading reason for hospital-associated (HA) and community-associated (CA) bacterial infections in humans, associating with various delicate skin and soft tissue infections, likewise as dangerous respiratory illness, bacteraemia, osteomyelitis, endocarditis, infection and toxic shock syndrome syndrome (David &Daum, 2010). The increasing prevalence of penicillin resistant *S. aureus* (MRSA) and its ability to resist multiple drugs has posed a significant challenge for infection management (Junie *et al*, 2014).

2.3.4 *Vibrio cholerae:*

Vibrio cholera is a facultative anaerobic, gram negative, non-spore forming curved rod, about 1.04-1.06 µm long. It is a facultative human infectious agent found in coastal waters that causes the acute gastrointestinal illness, cholera, a significant health threat in below developed and developing countries. It is wide acknowledged as one of the most vital water borne pathogen of worldwide economic significance. *Vibrio cholerae* is a member of the family Vibrionaceae; it is oxidase-positive, reduces nitrate, and is motile by means of one, sheathed, polar flagellum. Growth of *V. cholerae* is stirred up by addition of 1% sodium chloride (NaCl). However, a vital distinction from different vibrio spp is that the ability of *V. cholerae* to grow in nutrient broth while not added NaCl.

2.4 Objective of the study:

In this study, six samples of two different types of ginger is used; three from Bangladesh and three from hybrid ginger. The aim of this study is-

- To perform the extraction by using suitable solvent system and perform physiochemical assessment of ginger
- To perform antimicrobial activity of ginger
- To determine the minimum concentration of ginger required for antimicrobial activity
- Find the minimal bactericidal concentration (MBC) of these extracts
- Compare the antimicrobial activity of Bangladeshi and hybrid ginger
- Find the phytochemicals properties of ginger.

Chapter three: Materials and methods

3.1 Working laboratory:

The research project was wet laboratory based and the entire project work was performed in the microbiology laboratories of Department of mathematics and natural sciences, BRAC University, Dhaka, Bangladesh. In this laboratory, biosafety level 2 is followed and all the work was done under laminar flow inside laminar cabinet.

3.2 Collection of samples and processing:

3.2.1 Bangladeshi ginger: Ginger sample was collected from three different cities of Bangladesh respectively Jessore, Dhaka and Chittagong. The samples were washed properly, peeled and °sliced into pieces with aseptic utensils. The sliced pieces were air dried for a few days and then crushed into fine powder using an electric grinder. The fine powder was then stored in a cool and dry place in sterile containers.

3.2.2 Hybrid ginger:

Ginger sample was collected from three different shops of karwan bazar. The samples were processed following methods stated above.

3.3 Preparation of extracts:

For the study, two types of extraction process were followed: ethanolic and methanolic.

3.3.1 Ethanolic extraction:

In a conical flask, 50 grams of each ginger sample was suspended in 500ml of absolute ethanol. The flask was then covered with a clean aluminium foil and kept in a shaker incubator at 37° at 120 rpm for 72 hours. After 72 hours, the suspension was filtered in a conical flask using a sterile whatman filter paper. The filtrate was then evaporated to reduce the amount of solvent till one fourth of the original using a rotary evaporator at a temperature of below 50°. The concentrated solution was poured in a sterile petri dish and kept under fume hood overnight.

On the next day, sticky semi solid extract was scrapped in a autoclaved McCartney bottle. To measure the quantity of the extract, the volume of the empty bottle was subtracted from the total volume of bottle with extract. The bottle was then stored at 4° in a refrigerator.

3.3.2 Methanolic extraction:

In a conical flask, 50 grams of each ginger sample was suspended in 500ml of absolute methanol. The flask was then covered with a clean aluminium foil and kept in a shaker incubator at 37° at 120 rpm for 72 hours. After 72 hours, the suspension was filtered in a conical flask using a sterile whatman filter paper. The filtrate was then evaporated to reduce the amount of solvent till one fourth of the original using a rotary evaporator at a temperature of below 50°. The concentrated solution was poured in a sterile petri dish and kept under fume hood overnight.

On the next day, sticky semi solid extract was scrapped in a autoclaved McCartney bottle. To measure the quantity of the extract, the volume of the empty bottle was subtracted from the total volume of bottle with extract. The bottle was then stored at 4° in a refrigerator.

3.4 Preparation of stock solution of the extracts:

To prepare the stock solution of the extracts, Dimethylsulfoxide (DMSO) was used as solvent. One gram of each extract was dissolved in 1 ml of DMSO. The solution was kept in aluminium foil wrapped McCartney bottles to avoid the molecular modification of DMSO in the presence of light. The stock solution was stored at 4° in a refrigerator.

3.5 Storage and preservation of extracts:

All the extracts were kept in 25 ml McCartney bottles and preserved at 4° in a refrigerator to avoid any external contaminations until use. The caps of McCartney was screwed tightly and labeled properly. The bottles containing stock solution was wrapped with aluminium foil to avoid the contact of light with DMSO.

3.6 Preparation of nutrient agar plates:

Nutrient agar is used as a general purpose medium for the growth of a large variety of non-fastidious microorganisms. It consists of peptone, beef extract and agar. This comparatively simple formulation provides the nutrients necessary for the replication of a large variety of non-fastidious microorganisms. Nutrient Agar/broth is used for the cultivation and maintenance of non-fastidious organisms also as enumeration of organisms in water, sewage, dairy farm product, excretory product and different materials. According to the nutrient agar guide on NA bottle, 28 gm of NA powder is required to prepare 1 litre of NA media. Keeping this a constant, required amount of NA is prepared each time. Required amount of NA powder is measured on an electric balance machine, poured and suspended in distilled water in a conical flask. The flask is then placed in a Bunsen burner with medium flame. Continuous stirring with a glass rod is required to

break down any clump of powder in the flask. The appearance of bubbles indicates that the solution has reached boiling point. The solution must be heated until it is clear. The top of the flask is then covered with aluminium foil and autoclaved at 121°C for 90-120 minutes. After that the agar medium is poured in sterile petri plates inside a laminar flow chamber. The NA medium is let set for solidifying and then labeled properly with name and preparation date. The media plates are then kept in refrigerator until use.

3.7 Subculture of bacteria:

For the research work, ATCC cultures of four microbes were collected from microbiology laboratory of Department of mathematics and natural sciences, BRAC University, Dhaka, Bangladesh. To maintain the purity of the bacterial culture, regular subculture was done. A loopful of bacterial colony was taken by a sterile loop and then streaked on sterile NA medium. The culture plates were then incubated at 37° for 24 hours. After incubation, the plates were wrapped with parafilm and stored in refrigerator at 4° until further use. Fresh 24 hours cultures were prepared and used for each test.

3.8 Preservation and storage of selected bacteria samples

The selected bacterial samples were preserved in order to reuse them for this research purpose a number of times and so the selected bacterial strains were subcultured from the laboratory bacterial stock and then sealed with parafilm to avoid getting contaminated with other samples. Finally, the bacterial samples were stored in the refrigerator at 4ºC until use.

3.9 Maintenance of aseptic condition

All antibacterial assays were conducted inside the laminar hood under complete aseptic conditions. The laminar air flow chamber with HEPA filters was cleaned with 70% ethanol.

3.10 Preparation of 0.85% saline:

To prepare 0.85% saline, 0.85 gram of NaCl was measured and dissolved in 100ml of distilled water. The solution was then transferred in several test tubes using a glass pipette. The caps of the test tubes were closed to 1.5 turns and then autoclaved for sterilization. After autoclave is done, the caps were closed tightly and stored in refrigerator until further use.

3.11 Use of 0.5 McFarland standard:

For the visual determination of the turbidity of bacterial culture, different McFarland standards are used. In microbiology laboratory of Department of mathematics and natural sciences, BRAC University, Dhaka, Bangladesh, McFarland standards 0.5, 1, 2, 3, 4 and 5 were available. For this experiment, McFarland standard 0.5 was used to determine the approximate number of bacteria by visual comparison.

3.12 Preparation of nutrient broth:

Nutrient media are basic culture media used for maintaining microorganisms and to see the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts. Nutrient Broth has the formula originally designed to be used within the standard technique for Examination of Water and sewer water. It is one of the many non-selective media

helpful in routine cultivation of microorganisms (Downes *et al*). It can be used for the cultivation and enumeration of microorganism that are not significantly fastidious. Addition of various biological fluids like horse or sheep blood, serum, egg yolk etc. makes it appropriate for the cultivation of related fastidious organisms. Peptic digest of animal tissue, beef extract and yeast extract give the required nitrogen compounds, carbon, vitamins and additionally some trace ingredients necessary for the growth of microorganism. Sodium chloride maintains the osmotic equilibrium of the medium.

According to the media preparation guide labeled on the bottle, 13 grams of NB powder is needed for the preparation of 1 litre NB. Required amount of NB is measured using an electronic balance machine and dissolved in distilled water. The suspension is heated if needed to dissolve completely. The NB is then transferred to test tubes using a glass pipette. The caps of the test tubes were closed to 1.5 turns and then autoclaved for sterilization. After autoclave is done, the caps were closed tightly and stored in refrigerator until further use.

3.13 Preparation of bacterial culture:

To prepare a bacterial culture of McFarland 0.5 standard, 1 or 2 bacterial colony from a 24 hour culture plate was inoculated in saline in laminar flow cabinet. The mixture was vortexed to make a homogenous suspension and then compared to McFarland 0.5 standard which indicated an approximate cell count of 1.5x10^8.

3.14 Preparation of Mueller Hinton Agar (MHA):

Mueller-Hinton agar (MHA) is the most effective medium to use for routine susceptibility testing using Kirby-Bauer disc diffusion technique for non fastidious bacteria (both aerobe and

facultative anaerobe). Use of media apart from Mueller-Hinton agar couldend in inaccurate results. Mueller-Hinton agar is additionally the quality medium used for many broth dilution testing because the conditions of this medium (i.e. pH, cation concentration and thymidine contents) are well maintained. Mueller Hinton agar (MHA) may be purchased from industrial suppliers or may also be prepared from dehydrated medium.

According to the instruction stated on the bottle, 38 gram of MHA powder is needed to prepare 1 litre of media. Keeping this a constant, required amount of MHA is prepared each time. Required amount of MHA powder is measured on an electric balance machine, poured and suspended in distilled water in a conical flask. The flask is then placed in a bunsen burner with medium flame. Continuous stirring with a glass rod is required to break down any clump of powder in the flask. The appearance of bubbles indicates that the solution has reached boiling point. The solution must be heated until it is clear. The top of the flask is then covered with aluminium foil and autoclaved at 121° for 90-120 minutes. After that the agar medium is poured in sterile petri plates inside a laminar flow chamber. The MHA medium is let set for solidifying and then labeled properly with name and preparation date. The media plates are then kept in refrigerator until further use.

3.15 Determination of MIC and MBC of the extracts:

The MIC is the lowest concentration of a drug that inhibits bacterial growth thus there'll be no turbidness within the culture media. However MBC is the lowest concentration that kills bacteria. Typically the concentration that is taken into account as MBC is above the concentration for MIC. On the other hand, MBC is sometimes conferred as MBC50 or MBC90 which implies the drug concentration that kills 50% and 90%, respectively, of initial bacterial

population. So, to see the MBC one got to cultivate all of the clear tubes/wells on a solid medium like NA or MHA. Then the lowest concentration of a drug that inhibits bacterial growth is going to be considered as MBC.

To determine the MIC and MBC of the plant extracts, undiluted NB was used. In six test tubes each, 3 ml of NB was taken. To this, stock solution of plant extract was given in this following amount: 1000µl, 800µl, 600µl, 400µl, 200µl and 0µl. To adjust the total volume to 4 ml, DMSO was added in this following amount: 0µl, 200µl, 400µl, 600µl, 800µl and 1000µl. After that, 1000µl of bacterial suspension of McFarland standard 0.5 was added to each test tubes to make the total volume 5 ml. The test tubes were incubated for 24 hours at 37°C.

After incubation 100µl from each test tubes were spreaded on MHA plates for the determination of MBC.

3.16 Agar well diffusion method:

The agar diffusion assay is one technique for quantifying the ability of antibiotics to inhibit bacterial growth. Interpretation of results from this assay depends on model-dependent analysis that is predicated on the assumption that antibiotics diffuse freely within the solid nutrient medium. Here it was used to determine the antimicrobial activity of different samples of ginger against four test microorganism. Solvent DMSO was used as a negative control to determine that solvent has no antimicrobial activity to enhance the potency of the plant extracts. For each organism, the activity of six samples was studied. To prepare bacterial suspension, one or two colonies of each test microorganism was inoculated in NB and incubated for 3-4 hours. With autoclaved cotton swabs, lawn culture of each microbe was done on MHA plates inside a laminar flow cabinet. The plates were kept without lids for a little while to soak in the moisture in the

media. With the help of a sterile cork borer, agar was cut to make well for extract and negative control DMSO. Each well was filled with 90µl of each extract of minimum bactericidal concentration and DMSO. The plates were incubated for 24 hours at 37°C. This process was followed for all the four test organisms against two extracts of six samples.

3.16.1 Measurement of agar well diffusion:

The presence of clear zone around well those were filled with extracts signifies the presence of antimicrobial components in extracts. The clear zones were measured from three directions using a millimeter scale and the average of the data was recorded.

3.17 Phytochemical screening:

3.17.1 Detection of phytosterol

Small quantities of various extracts were dissolved in 5ml of chloroform separately. Then this chloroform solution was subjected to the following test to detect the presence of phytosterols.

1. **Salkowski test**: To 1ml of above prepared chloroform solution, few drops of concentrated sulphuric acid was added. Brown colour produced shows the presence of phytosterols.

3.17.2 Detection of alkaloids

Small quantities of various extracts were separately treated with few drops of dilute hydrochloric acid and filtered. The filtrates were used for the following tests.

1. Dragendorff's test: Orange brown precipitate
2. Hager's test: Yellow precipitate

3. Wagner's test: Reddish brown precipitate

3.17.3 Detection of tannin and phenolic compounds

Small quantities of the various extracts were taken separately in water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.

1. 5% ferric chloride: violet colour
2. 1% gelatin + 10% sodium chloride: white precipitate
3. 10% lead acetate: white precipitate

3.18.4 Detection of proteins and free amino acids

Small quantities of various extracts were dissolved in few ml of water and then they were subjected to the following tests.

1. **Biuret test:** To the above prepared extracts equal volume of 5% sodium hydroxide and 1% copper sulphate solution were added. Violet colour produced shows the presence of proteins and free amino acids.
2. **Ninhydrin test:** The extracts were treated with Ninhydrin reagent. Purple colour produced shows the presence of proteins and free amino acids.

3.18.5 Detection of flavonoids

1. **Aqueous sodium hydroxide**: Small quantities of various extracts were dissolved separately in aqueous sodium hydroxide. Appearance of yellow colour indicates the presence of flavonoids.
2. **Conc. sulfuric acid:** To the small portion of each extract, concentrated sulphuric acid was added. Yellow orange colour was obtained shows the presence of flavonoids.

3. **Shinoda's test**: Small quantities of the extracts were dissolved in alcohol. To those pieces of magnesium followed by concentrated hydrochloric acid was added dropwise and heated. Appearance of magenta colour shows the presence of flavonoids.

Chapter four: Results

4.1 Results of MBC

In this study, four types of bacteria- *Salmonella typhi, Bacillus cereus, Staphylococcus aureu* sand *Vibrio cholera* were used for comparing antimicrobial properties of ethanolic and methanolic extracts of three types of local and hybrid ginger.

The purpose of testing minimum inhibitory concentration and minimum bactericidal concentration was to find out the least concentration of ginger extract to inhibit the growth of bacteria and to find out the least concentration of ginger extract to kill all the bacteria present in a suspension.

Ginger extracts, when suspended in NB formed a turbid solution even before adding bacterial culture. So after 24 hours incubation it was not possible to find out MIC neither by visual aid nor with the help of spectrophotometer. So after 24 hours of incubation, 100µl of each test tube was spreaded on sterile MHA plates to find out the MBCs of ginger extracts.

The three samples of local gingers are noted respectively as LT1, LT2 and LT3. The three samples of hybrid gingers are noted as HT1, HT2 and HT3.

4.1.1 Ethanolic extracts

4.1.1.1 LT1 and HT1

Stock solution preparation: 1gm of extract was dissolved in 1 ml DMSO. McFarland standard 0.5 was used for MIC/MBC test.

Test tube number	NB (ml)	Bacterial suspension (ml) in saline	Extract stock solution (µl)		DMSO (µl)		Total volume (ml)	
			Local/ Hybrid	Hybrid (2x)	Local/ Hybrid	Hybrid (2x)	Local/ Hybrid	Hybrid (2x)
1	3	1	1000	2000	none	none	5	6
2	3	1	800	1600	200	400	5	6
3	3	1	600	1200	400	800	5	6
4	3	1	400	800	600	1200	5	6
5	3	1	200	400	800	1600	5	6
6	3	1	none	none	1000	2000	5	6

Table 4.1: This table shows the amount of NB, bacterial suspension, extract, DMSO and total volume present in each test tube

	Salmonella typhi			*Bacillus cereus*			*Staphylococcus aureus*			*Vibrio cholera*		
	Local	Hybrid	Hybrid (2x)	Local	Hybrid	Hybrid (2x)	Local	Hybrid	Hybrid (2x)	Local	Hybrid	Hybrid (2x)
1	-	+	-	-	+	-	+	+	-	-	+	-
2	-	+	-	-	+	-	+	+	+	-	+	-
3	-	+	-	+	+	-	+	+	+	-	+	-
4	-	+	-	+	+	+	+	+	+	-	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+

Table 4.2: This table shows the results of the MBC test of ethanolic extracts for LT1 and HT1 and comparison between them against *Salmonella typhi, Bacillus cereus, Staphylococcus aureus* and *Vibrio cholerae*

The MIC and MBC test of the ethanolic extracts of LT1 and HT1 was done by following table 4.1. After 24 hours incubation of the spreaded MHA plates showed that LT1 was effective on the selected test organisms- *Salmonella typhi*, *Bacillus cereus*, *Staphylococcus aureus* and *Vibrio cholerae*, when the amount of extracts were 400µl or more. However the same result was not obtained from the ethanolic extracts of HT1. To find out the effective concentration for the hybrid ginger, the extract amount was doubled and to make the total volume 6 ml, DMSO was added as per needed. The doubled amount of hybrid ginger showed visible bactericidal activity.

4.1.1.2 LT2 and HT2

Stock solution preparation: 1gm of extract was dissolved in 1 ml DMSO. McFarland standard 0.5 was used for MIC/MBC test.

Test tube number	NB (ml)	Bacterial suspension (ml) in saline	Extract stock solution (µl)		DMSO (µl)		Total volume (ml)	
			Local/ Hybrid	Hybrid (2x)	Local/ Hybrid	Hybrid (2x)	Local/ Hybrid	Hybrid (2x)
1	3	1	1000	2000	none	none	5	6
2	3	1	800	1600	200	400	5	6
3	3	1	600	1200	400	800	5	6
4	3	1	400	800	600	1200	5	6
5	3	1	200	400	800	1600	5	6
6	3	1	None	none	1000	2000	5	6

Table 4.3: This table shows the amount of NB, bacterial suspension, extract, DMSO and total volume present in each test tube

	Salmonella typhi			*Bacillus cereus*			*Staphylococcus aureus*			*Vibrio cholera*		
	Local	Hybrid	Hybrid (2x)	Local	Hybrid	Hybrid (2x)	Local	Hybrid	Hybrid (2x)	Local	Hybrid	Hybrid (2x)
1	-	+	-	-	+	-	-	+	-	-	+	-
2	-	+	-	-	+	-	-	+	-	-	+	-
3	-	+	-	-	+	-	-	+	+	-	+	+
4	+	+	-	-	+	+	+	+	+	-	+	+
5	+	+	+	+	+	+	+	+	+	-	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+

Table 4.4: This table shows the results of the MBC test of ethanolic extracts for LT2 and HT2 and comparison between them against *Salmonella typhi, Bacillus cereus, Staphylococcus aureus* and *Vibrio cholerae*

The MIC and MBC test of the ethanolic extracts of LT2 and HT2 was done by following table 4.3. After 24 hours incubation of the spreaded MHA plates showed that local ginger was effective on the selected test organisms- *Salmonella typhi, Bacillus cereus, Staphylococcus aureus* and *Vibrio cholerae*, when the amount of extracts were 400µl or more. However the same result was not obtained from the ethanolic extracts of hybrid ginger. To find out the effective concentration for the hybrid ginger, the extract amount was doubled and to make the total volume 6 ml, DMSO was added as per needed. The doubled amount of hybrid ginger showed visible bactericidal activity.

4.1.1.3 LT3 and HT3

Stock solution preparation: 1gm of extract was dissolved in 1 ml DMSO. McFarland standard 0.5 was used for MIC/MBC test.

Test tube number	NB (ml)	Bacterial suspension (ml) in saline	Extract stock solution (µl)		DMSO (µl)		Total volume (ml)	
			Local/ Hybrid	Hybrid (2x)	Local/ Hybrid	Hybrid (2x)	Local/ Hybrid	Hybrid (2x)
1	3	1	1000	2000	None	None	5	6
2	3	1	800	1600	200	400	5	6
3	3	1	600	1200	400	800	5	6
4	3	1	400	800	600	1200	5	6
5	3	1	200	400	800	1600	5	6
6	3	1	none	none	1000	2000	5	6

Table 4.5: This table shows the amount of NB, bacterial suspension, extract, DMSO and total volume present in each test tube

	Salmonella typhi			*Bacillus cereus*			*Staphylococcus aureus*			*Vibrio cholera*		
	Local	Hybrid	Hybrid (2x)	Local	Hybrid	Hybrid (2x)	Local	Hybrid	Hybrid (2x)	Local	Hybrid	Hybrid (2x)
1	-	+	-	-	+	-	-	+	-	-	+	-
2	-	+	-	-	+	-	-	+	-	-	+	-
3	-	+	-	-	+	+	-	+	+	-	+	-
4	-	+	+	-	+	+	-	+	+	-	+	+
5	-	+	+	+	+	+	+	+	+	-	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+

Table 4.6: This table shows the results of the MBC test of ethanolic extracts for LT3 and HT3 and comparison between them against *Salmonella typhi*, *Bacillus cereus*, *Staphylococcus aureus* and *Vibrio cholerae*

The MIC and MBC test of the ethanolic extracts of LT3 and HT3 was done by following table 4.5. After 24 hours incubation of the spreaded MHA plates showed that local ginger was effective on the selected test organisms- *Salmonella typhi*, *Bacillus cereus*, *Staphylococcus aureus* and *Vibrio cholerae*, when the amount of extracts were 400µl or more. However the same result was not obtained from the ethanolic extracts of hybrid ginger. To find out the effective concentration for the hybrid ginger, the extract amount was doubled and to make the total volume 6 ml, DMSO was added as per needed. The doubled amount of hybrid ginger showed visible bactericidal activity.

4.1.2 Methanolic extracts

4.1.1.1 LT1 and HT1

Stock solution preparation: 1gm of extract was dissolved in 1 ml DMSO. McFarland standard 0.5 was used for MIC/MBC test.

Test tube number	NB (ml)	Bacterial suspension (ml) in saline	Extract stock solution (µl)		DMSO (µl)		Total volume (ml)	
			Local/ Hybrid	Hybrid (2x)	Local/ Hybrid	Hybrid (2x)	Local/ Hybrid	Hybrid (2x)
1	3	1	1000	2000	none	none	5	6
2	3	1	800	1600	200	400	5	6
3	3	1	600	1200	400	800	5	6
4	3	1	400	800	600	1200	5	6
5	3	1	200	400	800	1600	5	6
6	3	1	none	none	1000	2000	5	6

Table 4.7: This table shows the amount of NB, bacterial suspension, extract, DMSO and total volume present in each test tube

	Salmonella typhi			*Bacillus cereus*			*Staphylococcus aureus*			*Vibrio cholera*		
	Local	Hybrid	Hybrid (2x)	Local	Hybrid	Hybrid (2x)	Local	Hybrid	Hybrid (2x)	Local	Hybrid	Hybrid (2x)
1	-	+	-	-	+	-	-	+	-	-	+	-
2	-	+	-	+	+	-	-	+	-	+	+	-
3	-	+	+	+	+	-	+	+	-	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+

Table 4.8: This table shows the results of the MBC test of methanolic extracts for LT1 and HT1 and comparison between them against *Salmonella typhi, Bacillus cereus, Staphylococcus aureus* and *Vibrio cholerae*

The MIC and MBC test of the methanolic extracts of LT1 and HT1 was done by following table 4.7. After 24 hours incubation of the spreaded MHA plates showed that local ginger was effective on the selected test organisms- *Salmonella typhi, Bacillus cereus, Staphylococcus aureus* and *Vibrio cholerae*, when the amount of extracts were 600µl or more. However the same result was not obtained from the methanolic extracts of hybrid ginger. To find out the effective concentration for the hybrid ginger, the extract amount was doubled and to make the total volume 6 ml, DMSO was added as per needed. The doubled amount of hybrid ginger showed visible bactericidal activity.

4.1.1.2 LT2 and HT2

Stock solution preparation: 1gm of extract was dissolved in 1 ml DMSO. McFarland standard 0.5 was used for MIC/MBC test.

Test tube number	NB (ml)	Bacterial suspension (ml) in saline	Extract stock solution (µl)		DMSO (µl)		Total volume (ml)	
			Local/ Hybrid	Hybrid (2x)	Local/ Hybrid	Hybrid (2x)	Local/ Hybrid	Hybrid (2x)
1	3	1	1000	2000	none	none	5	6
2	3	1	800	1600	200	400	5	6
3	3	1	600	1200	400	800	5	6
4	3	1	400	800	600	1200	5	6
5	3	1	200	400	800	1600	5	6
6	3	1	none	none	1000	2000	5	6

Table 4.9: This table shows the amount of NB, bacterial suspension, extract, DMSO and total volume present in each test tube

	Salmonella typhi			*Bacillus cereus*			*Staphylococcus aureus*			*Vibrio cholera*		
	Local	Hybrid	Hybrid (2x)	Local	Hybrid	Hybrid (2x)	Local	Hybrid	Hybrid (2x)	Local	Hybrid	Hybrid (2x)
1	+	+	-	-	+	-	+	+	-	-	+	-
2	+	+	+	-	+	-	+	+	-	-	+	-
3	+	+	+	-	+	-	+	+	+	+	+	+
4	+	+	+	-	+	+	+	+	+	+	+	+
5	+	+	+	-	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+

Table 4.10: This table shows the results of the MBC test of methanolic extracts for LT2 and HT2 and comparison between them against *Salmonella typhi*, *Bacillus cereus*, *Staphylococcus aureus* and *Vibrio cholerae*

The MIC and MBC test of the methanolic extracts of LT2 and HT2 was done by following table 4.9. After 24 hours incubation of the spreaded MHA plates showed that local ginger was effective on the selected test organisms- *Salmonella typhi, Bacillus cereus, Staphylococcus aureus* and *Vibrio cholerae*, when the amount of extracts were 600µl or more. However the same result was not obtained from the methanolic extracts of hybrid ginger. To find out the effective concentration for the hybrid ginger, the extract amount was doubled and to make the total volume 6 ml, DMSO was added as per needed. The doubled amount of hybrid ginger showed visible bactericidal activity.

4.1.1.3 LT3 and HT3

Stock solution preparation: 1gm of extract was dissolved in 1 ml DMSO. McFarland standard 0.5 was used for MIC/MBC test.

Test tube number	NB (ml)	Bacterial suspension (ml) in saline	Extract stock solution (µl)		DMSO (µl)		Total volume (ml)	
			Local/ Hybrid	Hybrid (2x)	Local/ Hybrid	Hybrid (2x)	Local/ Hybrid	Hybrid (2x)
1	3	1	1000	2000	none	none	5	6
2	3	1	800	1600	200	400	5	6
3	3	1	600	1200	400	800	5	6
4	3	1	400	800	600	1200	5	6
5	3	1	200	400	800	1600	5	6
6	3	1	none	none	1000	2000	5	6

Table 4.11: This table shows the amount of NB, bacterial suspension, extract, DMSO and total volume present in each test tube

	Salmonella typhi			*Bacillus cereus*			*Staphylococcus aureus*			*Vibrio cholera*		
	Local	Hybrid	Hybrid (2x)	Local	Hybrid	Hybrid (2x)	Local	Hybrid	Hybrid (2x)	Local	Hybrid	Hybrid (2x)
1	-	+	-	-	+	-	+	+	-	-	+	-
2	-	+	-	-	+	-	+	+	+	-	+	-
3	-	+	-	+	+	-	+	+	+	+	+	+
4	-	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+

Table 4.12: This table shows the results of the MBC test of the methanolic extracts for LT3 and HT3 and comparison between them against *Salmonella typhi, Bacillus cereus, Staphylococcus aureus* and *Vibrio cholerae*

The MIC and MBC test of the methanolic extracts of LT3 and HT3 was done by following table 4.11. After 24 hours incubation of the spreaded MHA plates showed that local ginger was effective on the selected test organisms- *Salmonella typhi, Bacillus cereus, Staphylococcus aureus* and *Vibrio cholerae*, when the amount of extracts were 400µl or more. However the same result was not obtained from the methanolic extracts of hybrid ginger. To find out the effective concentration for the hybrid ginger, the extract amount was doubled and to make the total volume 6 ml, DMSO was added as per needed. The doubled amount of hybrid ginger showed visible bactericidal activity.

4.2 Results of antibiogram

In the study of antibiogram of four test bacteria respectively *Salmonella typhi*, *Bacillus cereus*, *Staphylococcus aureus* and *Vibrio cholera* against ethanolic and methanolic extracts of three types of local and hybrid ginger, well diffusion method was used. As positive control different types of conventional antibiotics and as negative control DMSO was used. Wells were cut using a borer and fill with 100µl extracts of ginger of its MBC. The inhibition zone was measured using a millimeter scale.

4.2.1 Ethanolic extracts

4.2.1.1 LT1 and HT1

Test organism	Name of antibiotics	Zone of inhibition(mm)		
		Antibiotic	Local	Hybrid
S. typhi	Ciprofloxacin (5)	25	10	0
B. cereus	Erythromycin (15)	22	16	15
S. aureus	Ciprofloxacin (5)	30	11	11
V. cholerae	Azithromycin (15)	32	13.5	10

Table 4.13: Zone of inhibition produced by antibiotic, ethanolic extracts of LT1 and HT1 against *Salmonella typhi*, *Bacillus cereus*, *Staphylococcus aureus* and *Vibrio cholerae*

4.2.1.2 LT2 and HT2

Test organism	Name of antibiotics	Zone of inhibition(mm)		
		Antibiotic	Local	Hybrid
S. typhi	Ciprofloxacin (5)	25	0	0
B. cereus	Erythromycin (15)	22	16.5	15
S. aureus	Ciprofloxacin (5)	30	12	11
V. cholera	Azithromycin (15)	32	12	11

Table 4.14: Zone of inhibition produced by antibiotic, ethanolic extracts of LT2 and HT2 against *Salmonella typhi, Bacillus cereus, Staphylococcus aureus* and *Vibrio cholerae*

4.2.1.3 LT3 and HT3

Test organism	Name of antibiotics	Zone of inhibition(mm)		
		Antibiotic	Local	Hybrid
S. typhi	Ciprofloxacin (5)	25	0	11
B. cereus	Erythromycin (15)	22	16	13
S. aureus	Ciprofloxacin (5)	30	11	10.5
V. cholerae	Azithromycin (15)	32	13	13

Table 4.15: Zone of inhibition produced by antibiotic,ethanolic extracts of LT3 and HT3 against *Salmonella typhi, Bacillus cereus, Staphylococcus aureus* and *Vibrio cholerae*

4.2.2 Methanolic extractions

4.2.2.1 LT1 and HT1

Test organism	Name of antibiotics	Zone of inhibition(mm)		
		Antibiotic	Local	Hybrid
S. typhi	Ciprofloxacin (5)	25	11	0
B. cereus	Erythromycin (15)	22	17.5	15
S. aureus	Ciprofloxacin (5)	30	11	10.5
V. cholera	Azithromycin (15)	32	11	11

Table 4.16: Zone of inhibition produced by antibiotic, methanolic extracts of LT1 and HT1 against *Salmonella typhi, Bacillus cereus, Staphylococcus aureus* and *Vibrio cholerae*

4.2.2.2 LT2 and HT2

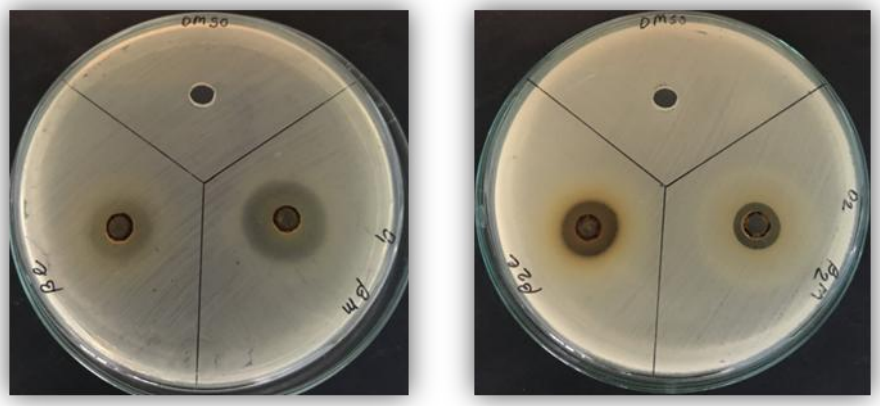
Test organism	Name of antibiotics	Zone of inhibition(mm)		
		Antibiotic	Local	Hybrid
S. typhi	Ciprofloxacin (5)	25	0	0
B. cereus	Erythromycin (15)	22	17	13
S. aureus	Ciprofloxacin (5)	30	0	11
V. cholera	Azithromycin (15)	32	8.5	11

Table 4.17: Zone of inhibition produced by antibiotic, methanolic extracts of LT2 and HT2 against *Salmonella typhi, Bacillus cereus, Staphylococcus aureus* and *Vibrio cholerae*

4.2.2.3 LT3 and HT3

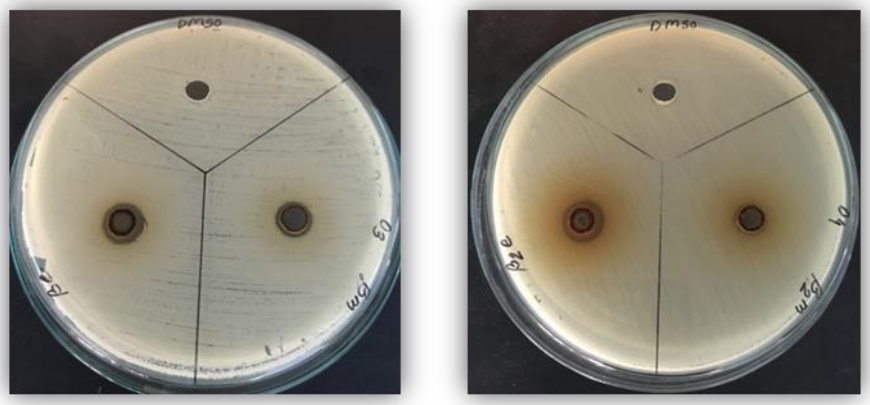
Test organism	Name of antibiotics	Zone of inhibition(mm)		
		Antibiotic	Local	Hybrid
S. typhi	Ciprofloxacin (5)	25	0	10
B. cereus	Erythromycin (15)	22	15.5	15.5
S. aureus	Ciprofloxacin (5)	30	11	9.5
V. cholera	Azithromycin (15)	32	0	11

Table 4.18: Zone of inhibition produced by antibiotic, methanolic extracts of LT3 and HT3 against *Salmonella typhi, Bacillus cereus, Staphylococcus aureus* and *Vibrio cholerae*



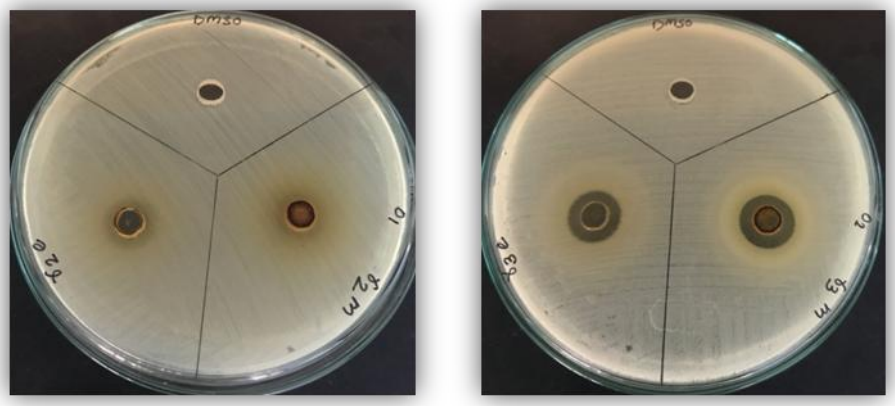
(a) (b)

Figure 4.1: Zone of inhibition produced by ethanolic and methanolic extracts of local type ginger against (a) *Salmonella typhi* and (b) *Bacillus Cereus*



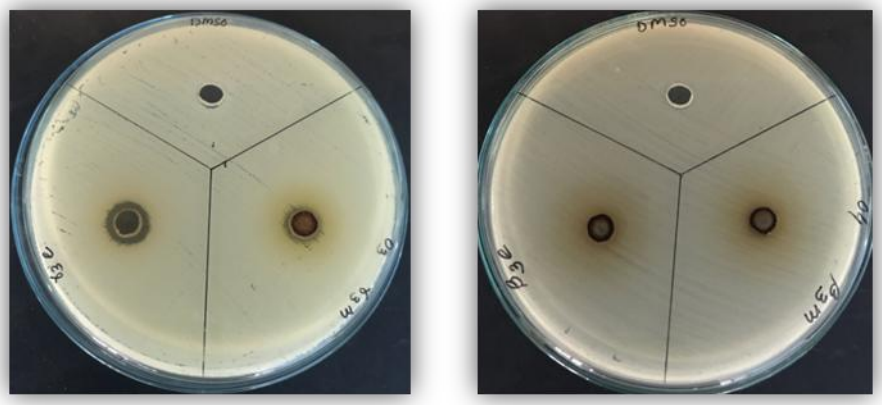
(a) (b)

Figure 4.2: Zone of inhibition produced by ethanolic and methanolic extracts of local type ginger against (a) *Staphylococcous aureus* and (b) *Vibrio cholerae*



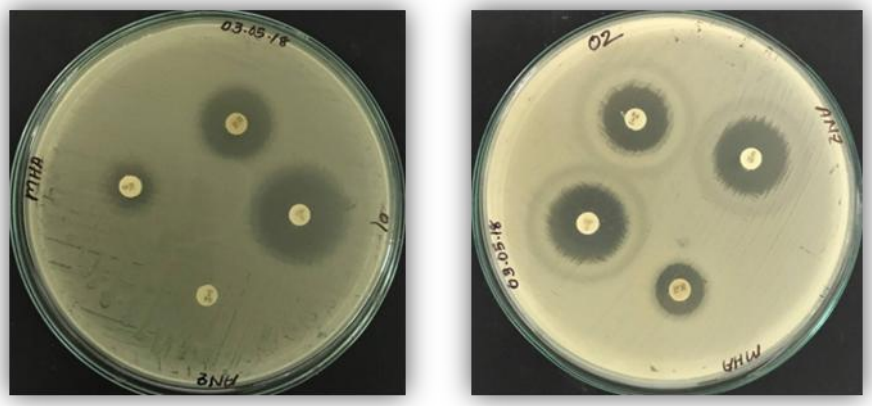
(a) (b)

Figure 4.3: Zone of inhibition produced by ethanolic and methanolic extracts of hybrid type ginger against (a) *Salmonella typhi* and (b) *Bacillus Cereus*



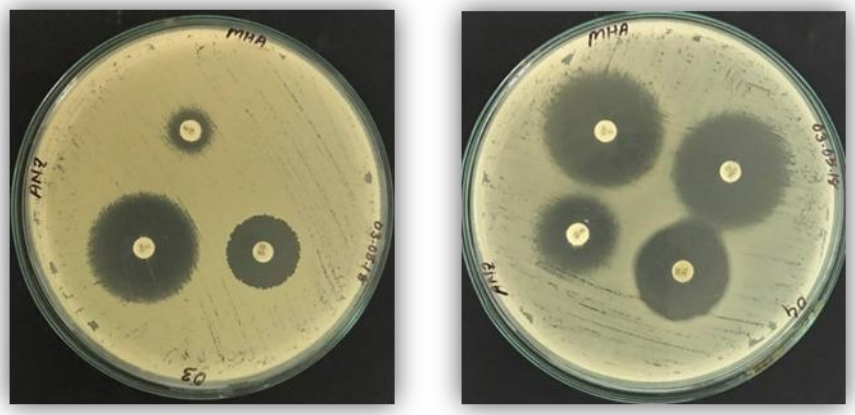
(a) (b)

Figure 4.4: Zone of inhibition produced by ethanolic and methanolic extracts of hybrid type ginger against (a) *Staphylococcus aureus* and (b) *Vibrio choleare*



(a) (b)

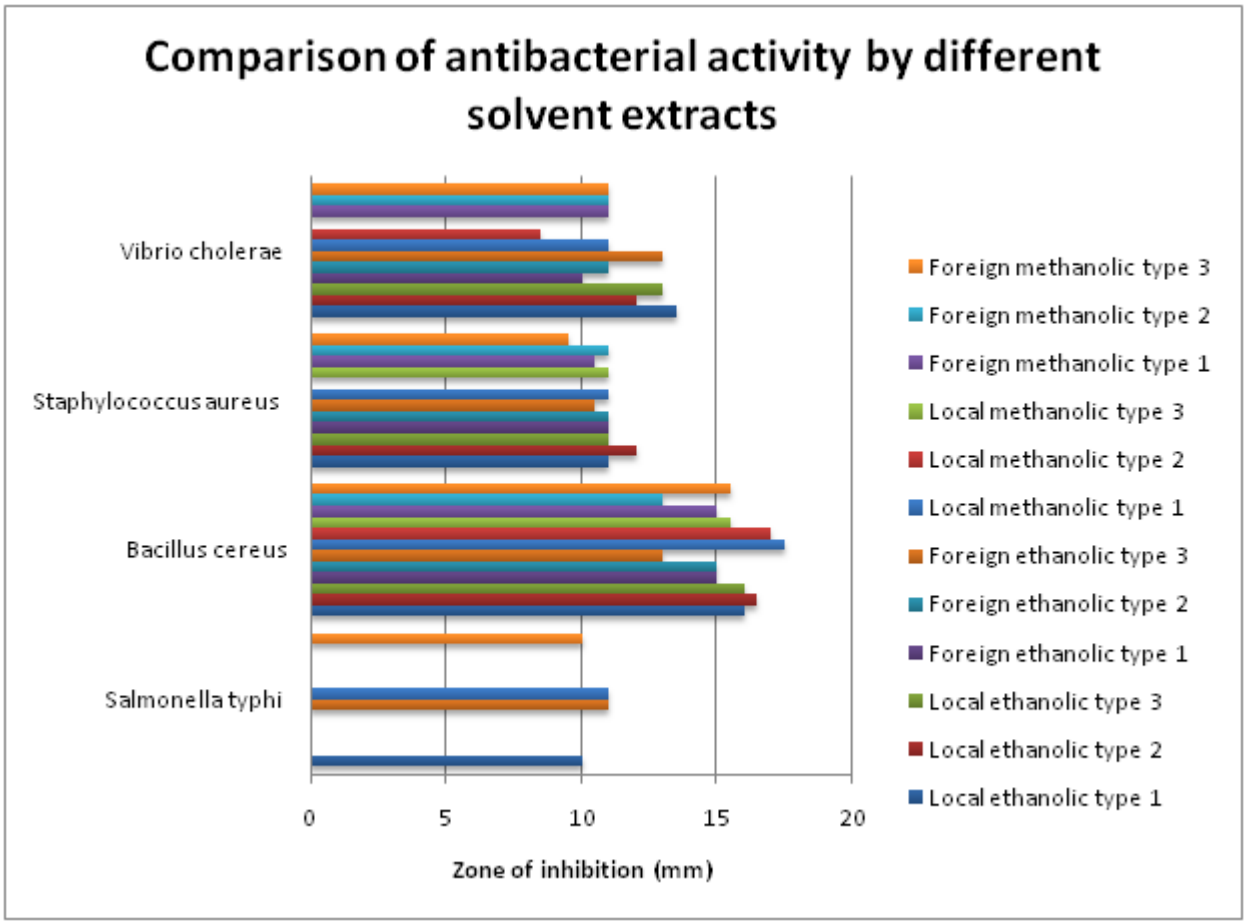
Figure 4.5: Zone of inhibition produced by selected conventional antibiotic disks against (a) *Salmonella typhi* and (b) *Bacillus cereus*



(a) (b)

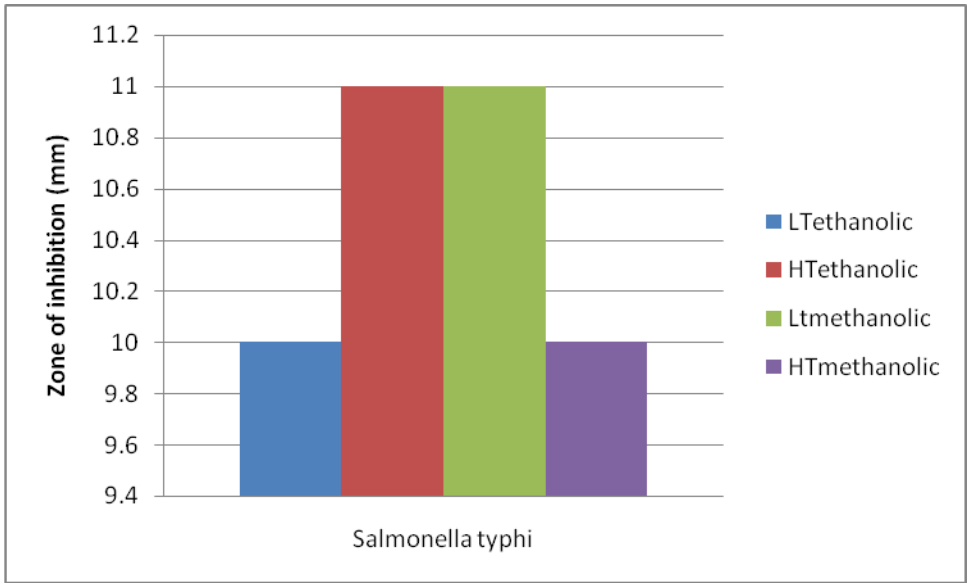
Figure 4.6: Zone of inhibition produced by selected conventional antibiotic disks against (a) *Staphylococcus aureus* and (b) *Vibrio cholerae*

4.3 Comparison of antibacterial activity by different solvent extracts

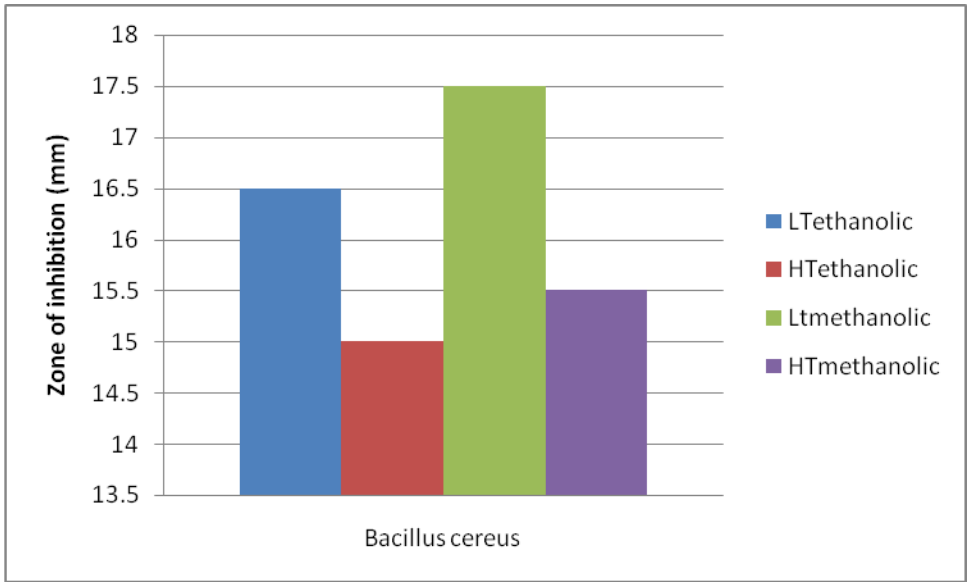


Graph 4.1: The antibacterial activities of ethanolic and methanolic extracts of local and hybrid type ginger against *Salmonella typhi, Bacillus cereus, Staphylococcus aureus* and *Vibrio cholerae*

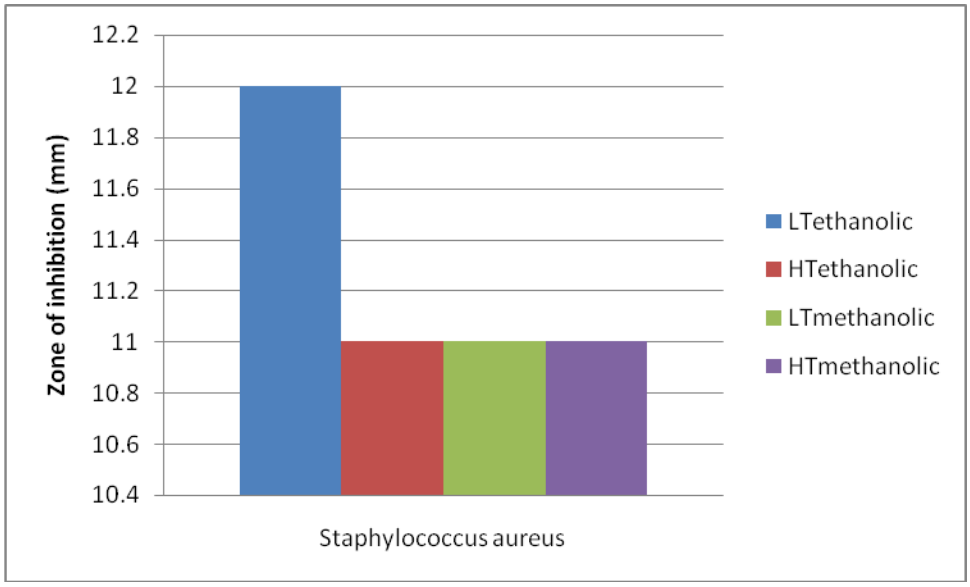
The graph above shows the antibacterial activity of both local and hybrid type ginger against *Salmonella typhi, Bacillus cereus, Staphylococcus aureus*and *Vibrio cholerae.* The highest zone is produced by methanolic extract of local type ginger which is 17.5mm against *Bacillus cereus* whereas *Salmonella typhi* showed no zone for most of the sample except three. *Staphylococcus aureus* and *Vibrio cholera* showed significant zone respectively 12mm and 13.5mm.



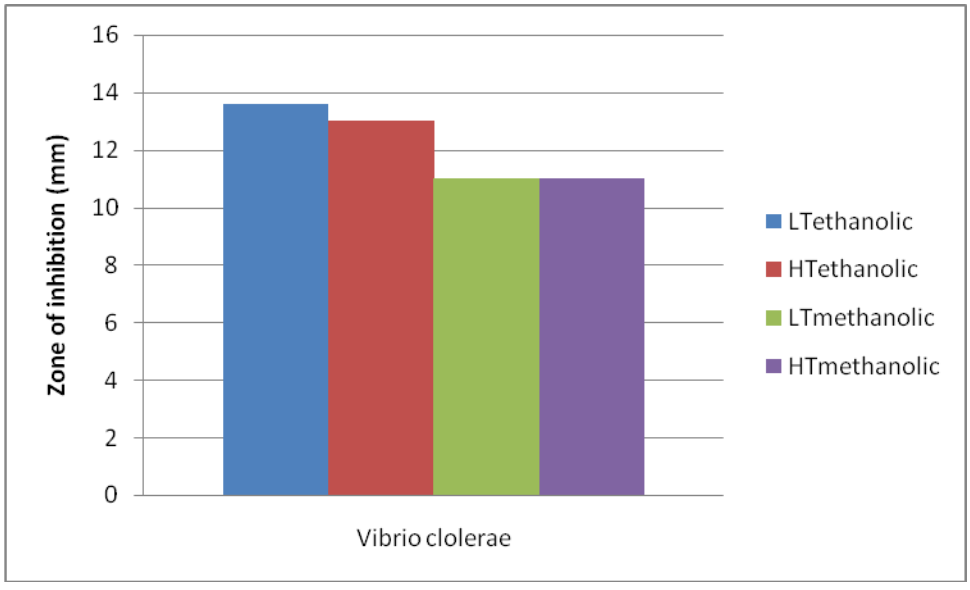
Graph 4.2: Zone of inhibition of local type and hybrid type ginger against *Salmonella typhi*



Graph 4.3: Zone of inhibition of local type and hybrid type ginger against *Bacillus cereus*



Graph 4.4: Zone of inhibition of local type and hybrid type ginger against *Staphylococcus aureus*



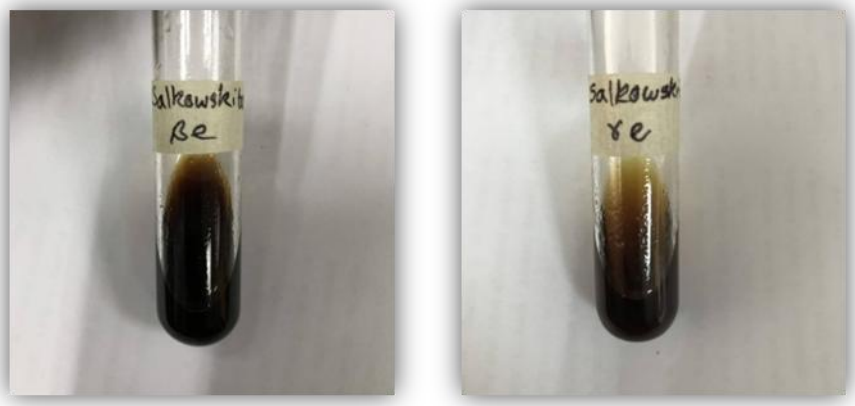
Graph 4.5: Zone of inhibition of local type and hybrid type ginger against *Vibrio cholerae*

4.4 Results of phytochemical screening

4.4.1 Ethanolic extracts

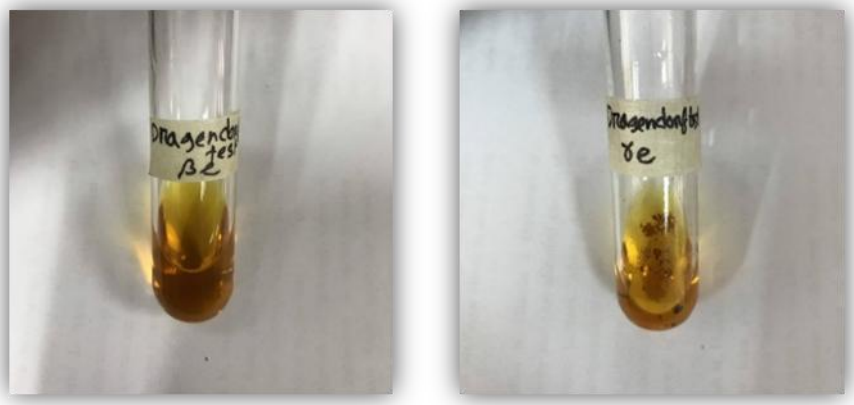
Chemical group test	Specific test	Observation	
		Local	Hybrid
Phytosterol	Salkowski's test	Positive	Positive
Alkaloid	Dragendorff's test	Positive	Positive
	Hager's test	Positive	Positive
	Wagner's test	Positive	Positive
Tannin and phenolic compounds	5% ferric chloride	Negative	Negative
	1% gelatin + 10% sodium chloride	Positive	Negative
	10% lead acetate	Positive	Negative
Protein and free amino acid	Biuret test	Positive	Negative
	Ninhydrin test	Negative	Negative
Flavonoids	Aqueous sodium hydroxide	Positive	Negative
	Conc. sulfuric acid	Positive	Negative
	Shinoda's test	Positive	Negative

Table 4.19

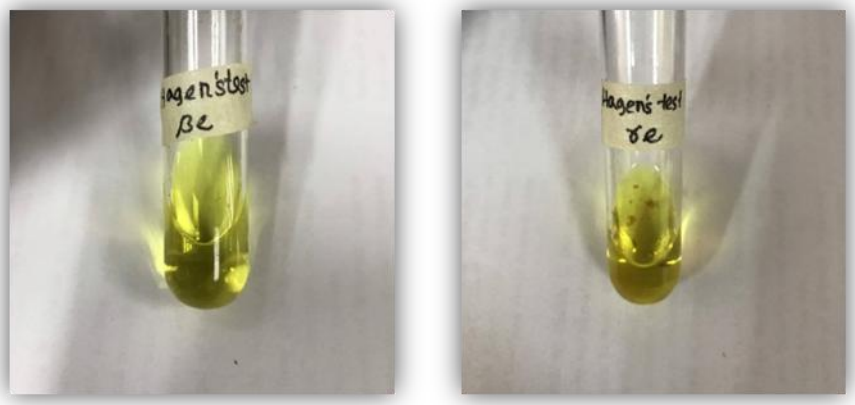


(a) (b)

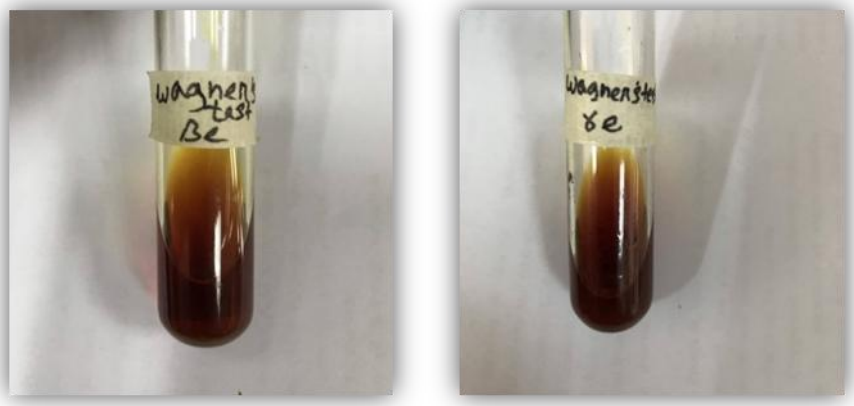
Figure 4.7: Detection of the presence of phytosterol by Salkowski test in (a) local type ginger (positive) and (b) hybrid type ginger (positive)



(a) (b)

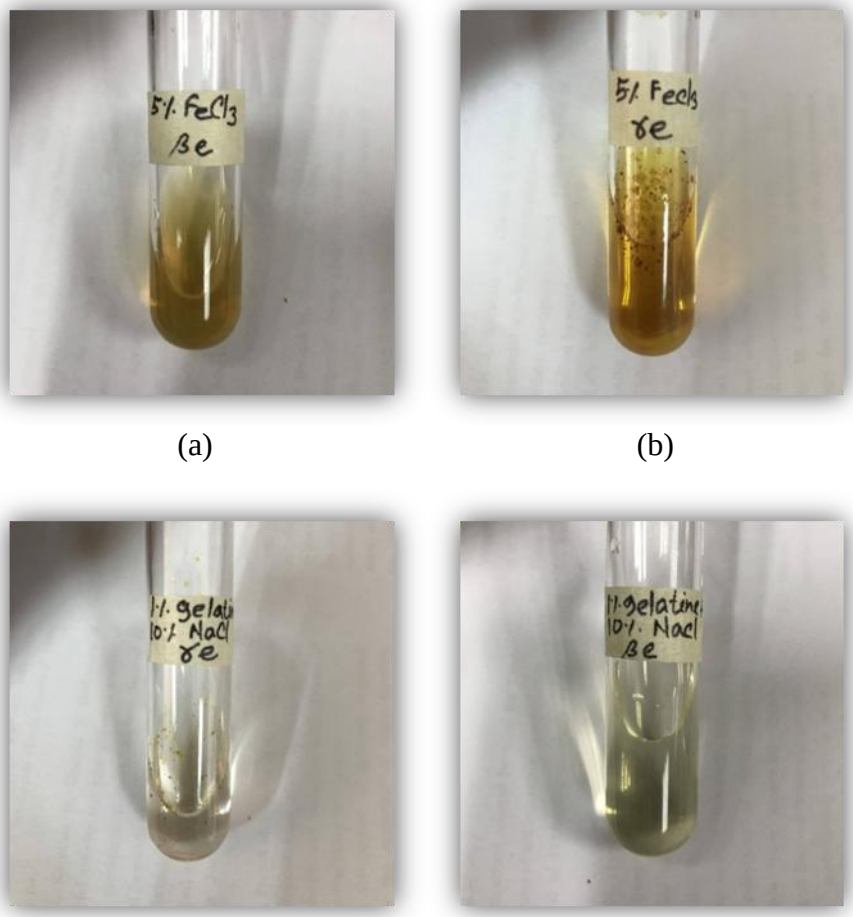


(c) (d)



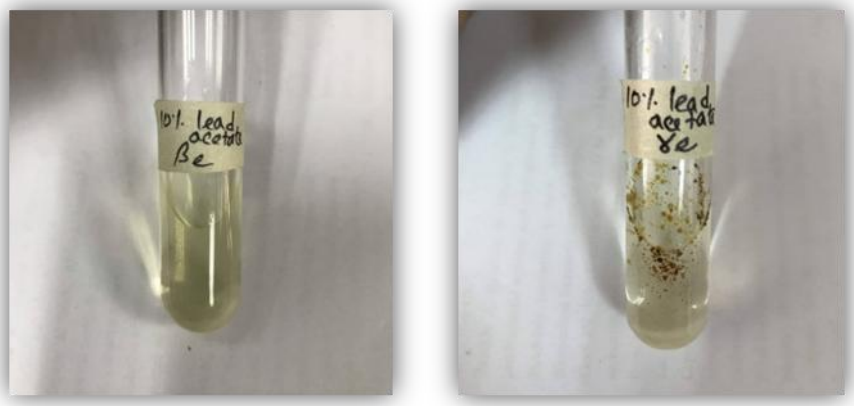
(e) (f)

Figure 4.8: Detection of the presence of alkaloids by Draggendorf's test (a) LT (positive) and (b) HT (positive), Hager's test (c) LT (positive) and (d) HT (positive), Wagner's test (e) LT (positive) and (f) HT (positive)



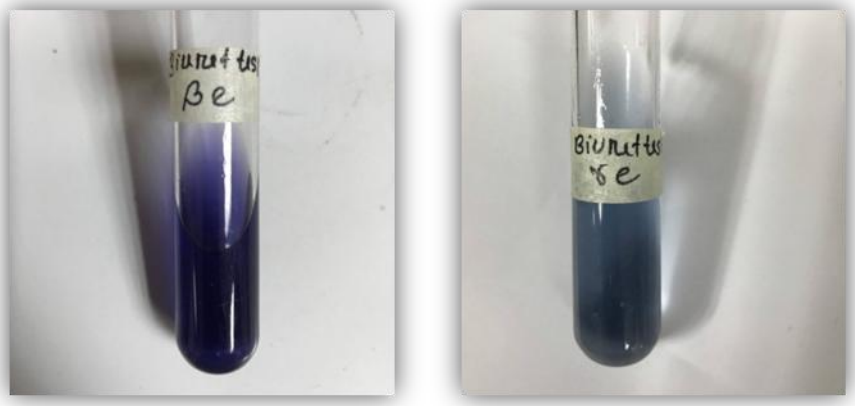
(a) (b)

(c) (d)

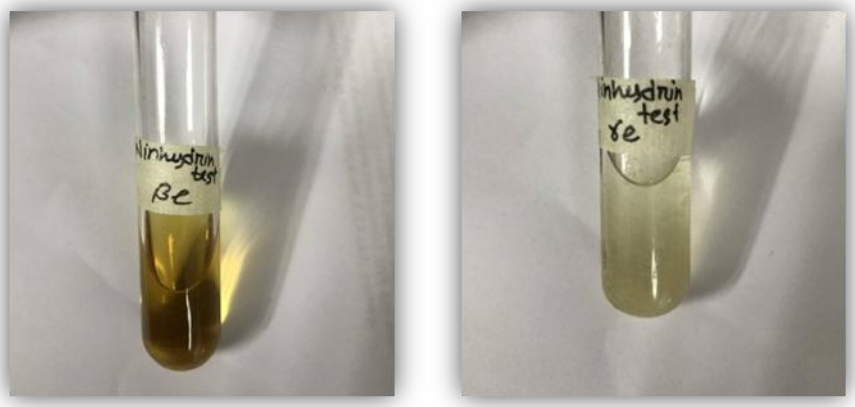


(e) (f)

Figure 4.9: Detection of the presence tannins and phenolic compounds in (a) LT (negative) and (b) HT (negative), (c) LT (positive) and (d) HT (negative), (e) LT (positive) and (f) HT (negative



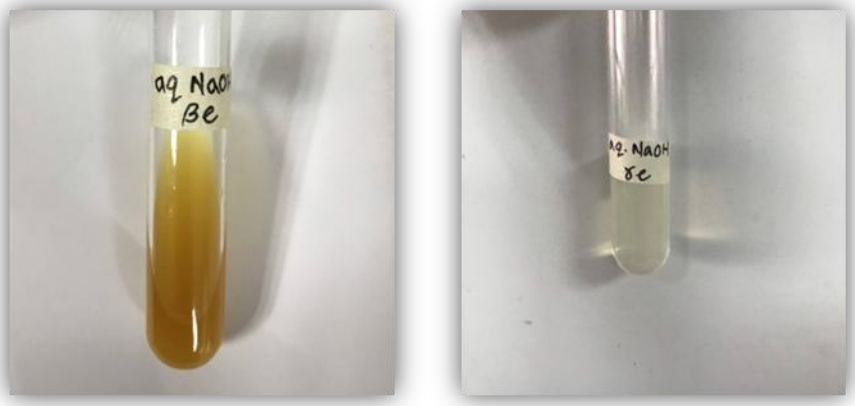
(a) (b)



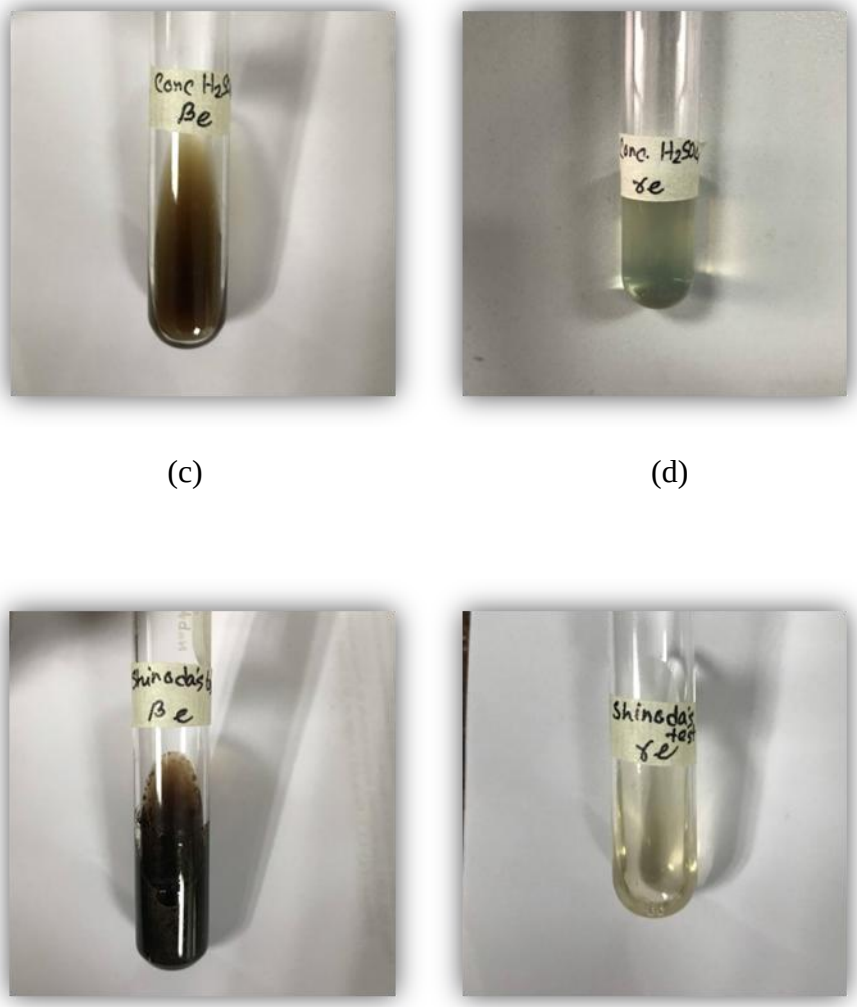
(c) (d)

Figure 4.10: Detection of the presence of protein and free amino acids by Biuret test

(a) LT (positive) and (b) HT (negative) and Ninhydrin test (c) LT (negative) and (d) HT (negative)



(a) (b)



(c) (d)

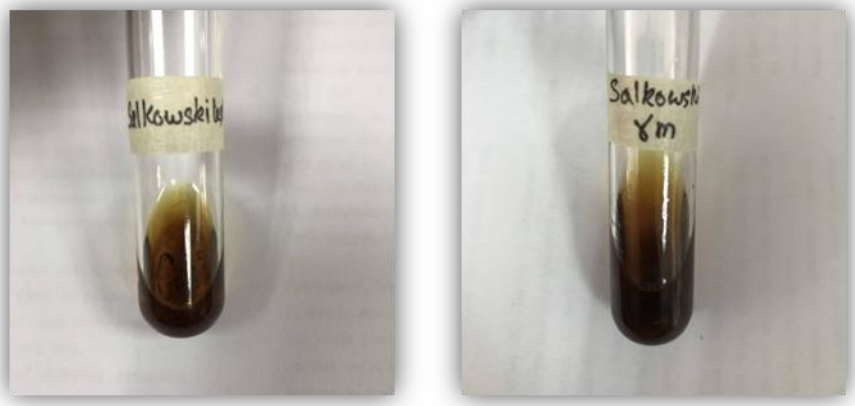
(e) (f)

Figure 4.11: Detection of the presence of flavonoid in (a) LT (positive) and (b) HT (negative), (c) LT (positive) and (d) HT (negative), (e) LT (positive) and (f) HT (negative)

4.4.2 Methanolic extracts

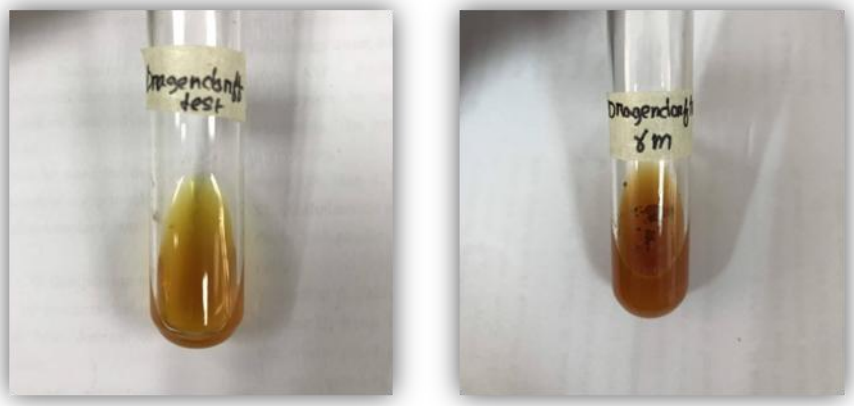
Chemical group test	Specific test	Observation	
		Local	Hybrid
Phytosterol	Salkowski's test	Positive	Positive
Alkaloid	Dragendorff's test	Positive	Positive
	Hager's test	Positive	Positive
	Wagner's test	Positive	Positive
Tannin and phenolic compounds	5% ferric chloride	Negative	Negative
	1% gelatin + 10% sodium chloride	Positive	Negative
	10% lead acetate	Positive	Positive
Protein and free amino acid	Biuret test	Positive	Negative
	Ninhydrin test	Negative	Negative
Flavonoids	Aqueous sodium hydroxide	Positive	Negative
	Conc. sulfuric acid	Positive	Negative
	Shinoda's test	Positive	Negative

Table 4.20

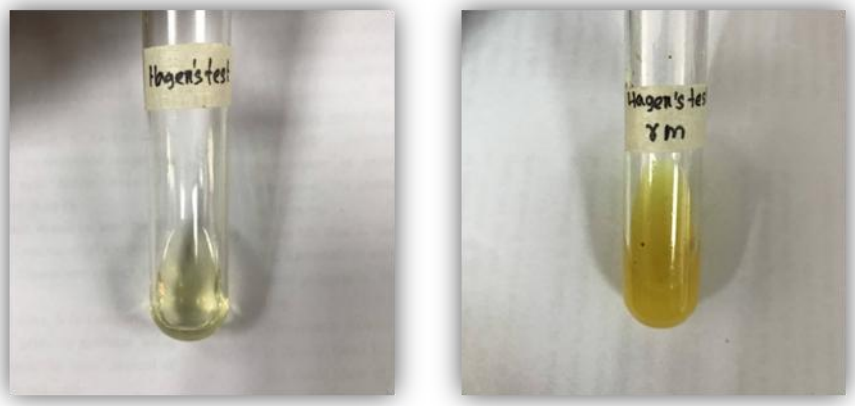


(a) (b)

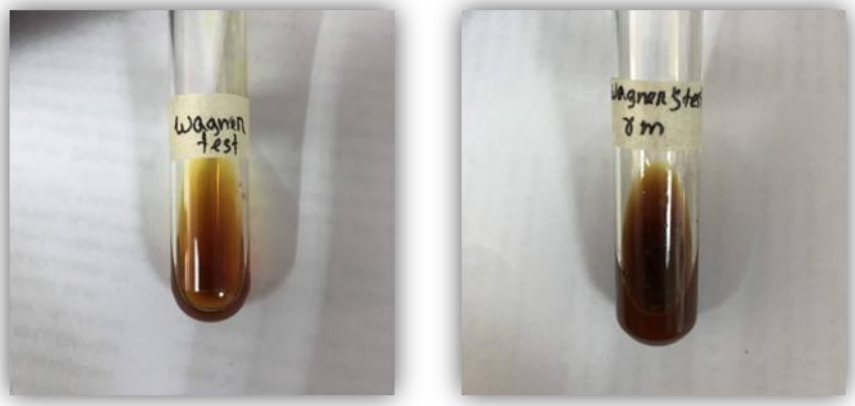
Figure 4.12: Detection of the presence of phytosterol by Salkowski test in (a) local type ginger (positive) and (b) hybrid type ginger (positive)



(a) (b)

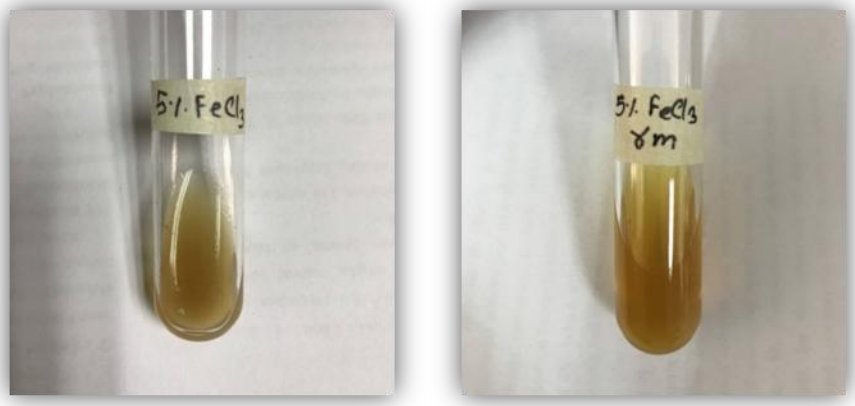


(c) (d)

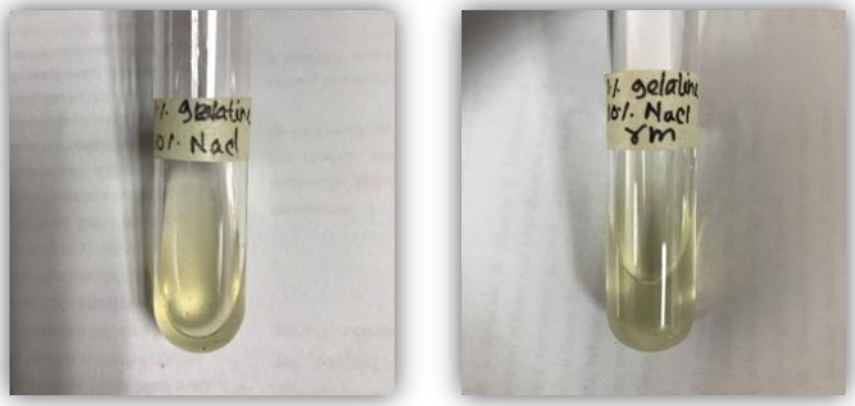


(e) (f)

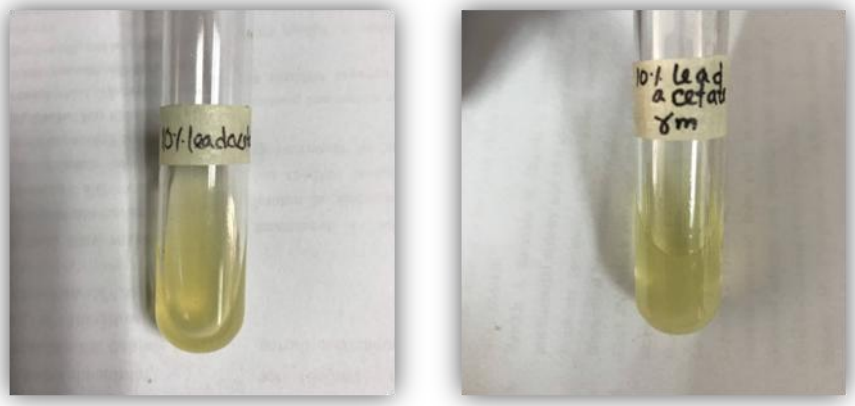
Figure 4.13: Detection of the presence of alkaloids by Draggendorf's test (a) LT (positive) and (b) HT (positive), Hager's test (c) LT (positive) and (d) HT (positive), Wagner's test (e) LT (positive) and (f) HT (positive)



(a) (b)

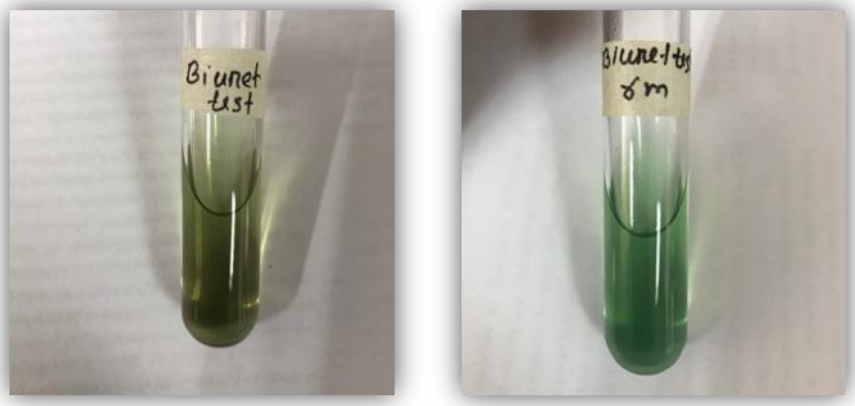


(c) (d)

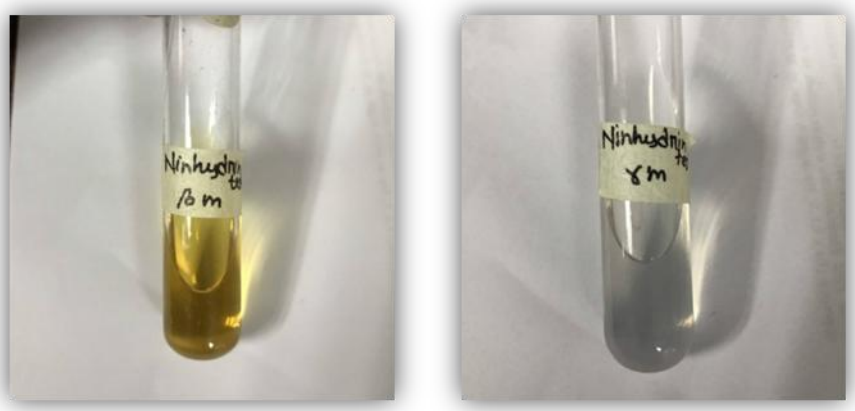


(e) (f)

Figure 4.14: : Detection of the presence tannins and phenolic compounds in (a) LT (negative) and (b) HT (negative), (c) LT (positive) and (d) HT (negative), (e) LT (positive) and (f) HT (positive)



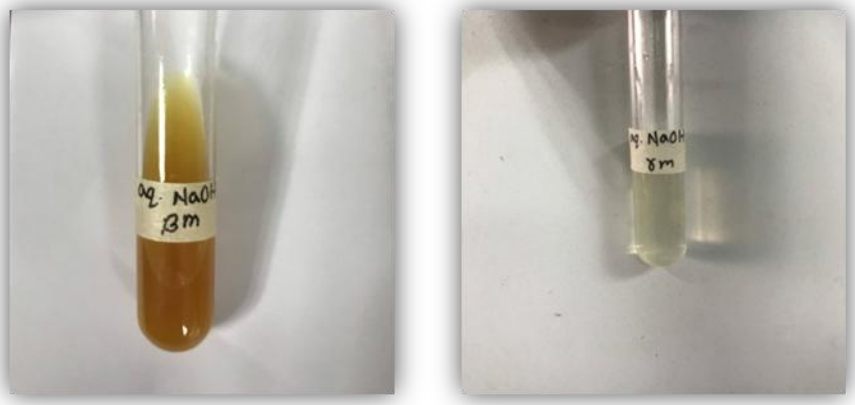
(a) (b)



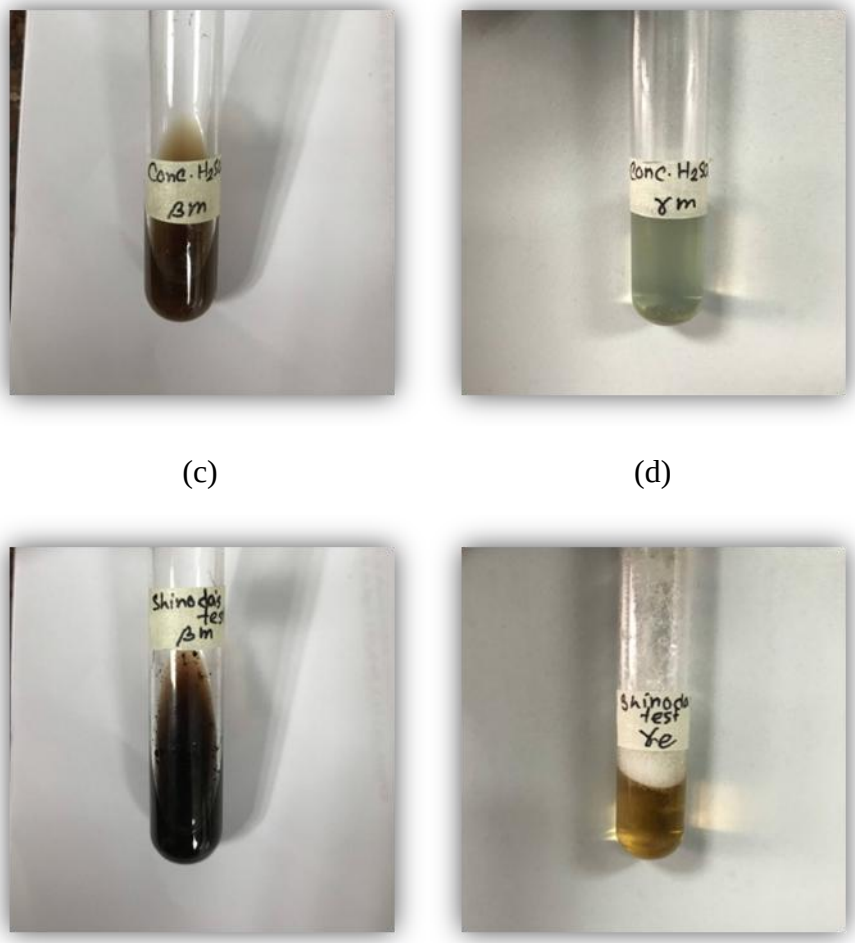
(c) (d)

Figure 4.15: Detection of the presence of protein and free amino acids by Biuret test

(a) LT (positive) and (b) HT (negative) and Ninhydrin test (c) LT (negative) and (d) HT (negative)



(a) (b)



(c) (d)

(e) (f)

Figure 4.16: Detection of the presence of flavonoid in (a) LT (positive) and (b) HT (negative), (c) LT (positive) and (d) HT (negative), (e) LT (positive) and (f) HT (negative)

Chapter five: Discussion

Medicinal plants are globally valuable sources of new drugs. There are over 1300 medicinal plants used in Europe, of which 90 % are harvested from wild resources; in the United States, about 118 of the top 150 prescription drugs are based on natural sources. Furthermore, up to 80 % of people in developing countries are totally dependent on herbal drugs for their primary healthcare, and over 25 % of prescribed medicines in developed countries are derived from wild plant species. With the increasing demand for herbal drugs, natural health products, and secondary metabolites of medicinal plants, the use of medicinal plants is growing rapidly throughout the world (Chen *et al*, 2016).

The increased usage of antibiotics has induced microorganisms to acquire resistance factors which have become a burning predicament (Abimbola *et al*., 1993). As a result there is an urgent need to find the alternative of chemotherapeutic drugs in diseases treatment particularly those of plants origin which are easily available and have considerably less side effects (Khulbe & Sati, 2009). The use of higher plants and their extracts for treating the infectious diseases has long been practiced in many parts of the world (Sofowora, 1984). The plant derived medicines may be used in many different forms including: powder, liquid or mixtures which could be raw or boiled such as, liniments, ointments and incisions (Apata, 1979).

In many countries including Bangladesh, ginger- both local and foreign; is widely used in food preparation. The purpose of this study was to prepare ethanolic and methanolic extracts multiple local and foreign ginger sample available in market and compare their antibacterial activity for four bacterial strains- *Salmonella typhi, Bacillus cereus, Staphylococcus aureus* and *Vibrio cholerae*.

From table 4.2, 4.4 and 4.6 it is evident that ethanolic extract of local ginger is effective on *Salmonella typhi* if minimum amount is 400µl(1gm/ml), on *Bacillus cereus* if minimum amount

is 400µl (1gm/ml), on *Staphylococcus aureus* if minimum amount is 600µl(1gm/ml) and on *Vibrio cholerae* if minimum amount is 200µl(1gm/ml) whereas the ethanolic extracts of foreign gingers were not effective on the selected bacteria at all compared to local ginger sample. To find the effectively of foreign ginger the amount of extract in each test tubes were doubled and it was found that ethanolic extracts of foreign ginger was effective on *Salmonella typhi* if minimum amount is 400µl(1gm/ml), on *Bacillus cereus* if minimum amount is 600µl(1gm/ml), on *Staphylococcus aureus* if minimum amount is 800µl (1gm/ml) and on *Vibrio cholerae* if minimum amount is 600µl (1gm/ml).

Moreover, from table 4.8, 4.10 and 4.12 it can be acknowledged that the methanolic extracts of local ginger is effective on *Salmonella typhi* if minimum amount is 400µl(1gm/ml), on *Bacillus cereus* if minimum amount is 600µl(1gm/ml), on *Staphylococcus aureus* if minimum amount is 800µl(1gm/ml) and on *Vibrio cholerae* if minimum amount is 800µl(1gm/ml). On the other hand methanolic extracts of foreign gingers were not effective on the selected bacteria at all compared to local ginger sample. To find the effectivity of foreign ginger the amount of extract in each test tubes were doubled and it was found that methanolic extracts of foreign ginger was effective on *Salmonella typhi* if minimum amount is 600µl (1gm/ml), on *Bacillus cereus* if minimum amount is 600µl (1gm/ml), on *Staphylococcus aureus* if minimum amount is 800µl (1gm/ml) and on *Vibrio cholerae* if minimum amount is 800µl (1gm/ml).

It has been reported (Ampting T. J *et al*) that clindamycin greatly increased the cytotoxicity of *Slamonella* sp in fecal water where as the present study provides evidence that there was no zone of inhibition against *Salmpnella typhi in vitro* by clindamycin through our suggested ginger extracts from both source showed significant results.

The results of antibacterial assays of ethanolic extracts of both local and foreign gingers are recorded in table 4.13, 4.14 and 4.15 alongside some conventional antibiotics ciprofloxacin (zone of inhibition 25mm in dia for *Salmonella typhi* and 30mm for *Staphylococcus aureus*), erythromycin (zone of inhibition 22mm for *Bacillus cereus*) and azithromycin (zone of inhibition 32 for *Vibrio cholerae*). The ethanolic extract of local ginger produced highest inhibition zones for *Salmonella typhi* 10mm, for *Bacillus cereus* 16.5mm, for *Staphylococcus aureus* 12mm and for *Vibrio cholera* 13mm. On the other side, the ethanolic extract of foreign ginger produced highest inhibition zones for *Salmonella typhi* 11mm, for *Bacillus cereus* 15mm, for *Staphylococcus aureus* 11mm and for *Vibrio cholera* 13mm.

The results of antibacterial assays of methanolic extracts of both local and foreign gingers are recorded in table 4.16, 4.17 and 4.18 alongside some conventional antibiotics ciprofloxacin (zone of inhibition 25mm for *Salmonella typhi* and 30mm for *Staphylococcus aureus*), erythromycin (zone of inhibition 22mm for *Bacillus cereus*) and azithromycin (zone of inhibition 32 for *Vibrio cholerae*). The methanolic extract of local ginger produced highest inhibition zones for *Salmonella typhi*11mm, for *Bacillus cereus* 17.5mm, for *Staphylococcus aureus*11mm and for *Vibrio cholera* 11mm. On the other side, the ethanolic extract of foreign ginger produced highest inhibition zones for *Salmonella typhi*10mm, for *Bacillus cereus* 15.5mm, for *Staphylococcus aureus*11mm and for *Vibrio cholera* 11mm.

In 2014, according to Zadeh and Kor*et al*, in the fresh ginger rhizome, the gingerols were identified as the major active components and gingerol [5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3-one is the most abundant constituent in the gingerol series. The powdered rhizome contains 3-6% fatty oil, 9% protein, 60-70% carbohydrates, 3-8% crude fiber, about 8% ash, 9-12% water and 2-3% volatile oil. The volatile oil consists of mainly mono and sesquiter–

penes; camphene, beta-phellandrene, curcumene, cineole, geranyl acetate, terphineol, terpenes, borneol, geraniol, limonene, linalool, alpha-zingiberene (30-70%), beta-sesquiphellandrene (15-20%), beta-bisabolene (10- 15%) and alpha-farmesene. In dried ginger powder, shogaol a dehydrated product of gingerol, is a predominant pungent constituent upto biosynthesis3-5. Oleoresin, which is isolated by acetone and ethanol extraction, contains 4-7.5% of dried powder, pungent substances namely gingerol, shogaol, zingerone and paradol. The oleoresin has also been found to contain zingiberol, the principal aroma contributing component as well as zingiberene, gingediol, diarylheptanoids, vitamins and phytosterols (Zadeh and Kor, 2014).

According to present study, our observation coincides with Zadeh and Kor, *et al.* the phytochemical analysis of both local hybrid ginger showed presence of phytosterol, alkaloid, tannin, phenolic compounds, proteins and flavonoids. The phytochemical screening of ethanolic extracts of local ginger showed positive results for phytosterols, alkaloids and flavonoids. As for the detection of tannin and phenolic compounds, two of three were positive whereas one of two tests was positive for the detection of protein and free amino acid. Like ethanolic extracts of local ginger, ehtanolic extracts of hybrid ginger showed positive result for phytosterol and and alkaloid detection tests. However, none of the tests were positive for tannin and phenolic compounds, protein and free amino acids and flavonoid tests.

The results of the tests of phytosterol and alkaloid detection were also positive for the methanolic extracts of both local hybrid ginger for the detection of phytosterol and alkaloid although for local sample two of three tests were positive for the detection of tannin and phenolic compound and one of two was positive for the detection of free amino acid. Flavonoid tests were positive for the methanolic extract of local ginger. As for hybrid ginger none of the tests for tannin and phenolic compound, protein and free amino acids and flavonoid were positive.

5.1 Safety Issues of Ginger

Adverse effects after ingestion of ginger are uncommon but can include mild gastrointestinal complications, such aspyrosis (heartburn or reflux) (Arfeen Z, *et al*, 1995) and eructation (belching)(Smith C*et al* , 2004). In a study of 27 healthy volunteers who were given a single oral dose of ginger (ranging from 100 mg to 2 g), minor gastrointestinal upsets were the major treatment associated toxicities (Zick SM, *et al*, 2009). Despite previous studies indicating that ginger could interfere with platelet aggregation and cause excessive bleeding, (Srivastava KC, 1985) in a randomized crossover study of 12 healthy volunteers taking 1.2 g of dried rhizome three times daily for two weeks, ginger did not affect platelet aggregation and had no effect on the pharmacokinetics or pharmacodynamics of a single 25 mg dose of warfarin taken on day 7.

Chapter six: Conclusion

The results for the antibacterial screening have shown that the methanol and ethanol extracts of ginger extracts obtained from local and hybrid source in Bangladesh have antibacterial activity. The results of the inhibition of bacterial growth have shown that the extracts are active at high concentration and inactive at very low concentrations. Thus the study may suggest that the inhibition of bacterial growth activity of the extracts is dose dependent.

The diameter of the inhibition zone obtained against *Salmonella typhi, Bacillus cereus, Vibrio cholera* and *Staphylococcus aureus* at 100% concentration by agar well diffusion method was compared to those obtained against commonly used antibiotics. Ginger extract presented higher diameter of inhibition zones than conventional antibiotics *in vitro*. Moreover extracts obtained from local source were proved to be more potent against this pathogenic organism than hybrid source extracts. The results obtained in this study showed an explanation for the relatively higher therapeutic efficacy of plant materials (spices).It is recommended for further in the future studies that should focus more on other advantages of spices especially the clinical applications in order to obtain low cost treatment and also prevention of recurrent infection.

Chapter seven: References

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APPENDIX-I

Media composition

Compositions of the media used in this study are provided below. The media were autoclaved at 121°C for 15 min at 121psi.

1. Nutrient Agar (HiMedia, India) Ingredients	**Amounts (g/L)**
Peptic digest of animal tissue	5.0
Beef extract	1.5
Sodium chloride	5.0
Yeast extract	1.5
Agar	15.0

2. Nutrient Broth (HiMedia, India) Ingredients	**Amounts (g/L)**
Peptic digest of animal tissue	5.0
Beef extract	1.5
Sodium chloride	5.0
Yeast extract	1.5

3. Mueller-Hinton Agar (HiMedia, India) Ingredients	**Amounts (g/L)**
Beef infusion	300
Casamino acids	17.5
Starch	1.5
Agar	17.0

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 WagiELEktroniczne Model: WTB 200
Refrigerator (40ºC)	Model: 0636 Samsung
Laminar flow chamber	SAARC Engineering
Rotary evaporator	Heidolph Made in Germany
Fume hood	-